USE OF INDUCTORS TO INCREASE PRODUCTION OF ENZYME LACCASE by *Pleurotus eryngii*

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ABSTRACT

The fungal laccase produced by many ligninolytic fungi can be inducers for greater or better production. We have examined the effects of inductors, like spent coffee ground, used frying oil and blue dye about enzymatic activity of *Pleurotus eryngii* (Ple 05). The spend coffee ground and used oil show positive and signofictive effect. The inductors increase in up 2,5 fold the activity by comparison with control.

Keywords: spent coffee ground. used frying oil. blue dye. fungi.

1. INTRODUCTION

Fungal laccases (EC 1.10.3.2 benzenediol: oxygen oxidoreductase) are phenol oxidases widely studied and several industrial applications in the pulp and paper industry, textile applications, organic synthesis, and environmental, food, pharmaceutical and nanobiotechnological applications[1,2].

The laccase enzyme is produced by many ligninolytic fungi[3]. Several studies for the production of laccase using fungi basidiomycetes of genera *Trametes*, *Pleurotus, Lentinula*, *Pycnoporus*, *Phanerochaete* and *Agaricus* have been carried out, mainly involving culture media with carbon sources from agricultural residues[4]. Some studies discuss the use of inducers (phenolic or non-phenolic compounds, natural or synthetic compounds) for greater or better production of this enzyme[5].

Synthesis and secretion of laccases are strictly influenced by nutrient levels, culture conditions, developmental stage as well as the addition of a wide range of inducers to cultural media, with variations among both different fungal species and different isoforms in a same strain[5]. Compounds that have the capacity to function as inducers of laccase synthesis have been explored in a variety of fungal species. These compounds have structures that are very similar to or are analogues of lignin, and serve as cellular signals to produce specific laccases[6].

In order to expand knowledge about the induction of fungal laccases, solid state fermentation experiments were carried out with basidiomycete fungi to produce laccase, using organic and inorganic inducers, residual oil fried, spent coffee ground and dye textile in a medium of eucalyptus sawdust as carbon source.

These three inducers were chosen because dye textile has a substrate for the enzyme's action, these enzymes have the capacity to degrade dyes[7]. Residual or used frying oil is availability and is a ambiental problem and spent coffee ground was used due to its great availability and high lignin content, around 20%[6].

2. MATERIAL & METHODS

All chemicals were reagent-grade and obtained from Dinâmica (São Paulo, Brazil) or Synth (São Paulo Brazil).

The basidiomycete *Pleurotus eryngii* (PLE 05) was obtained from the Professor Maria Catarina Kasuya collection (Microbiology department - Universidade Federal de Viçosa, Viçosa, Minas Gerais - Brazil). The fungus culture was grown on medium PDA (Potato dextrose agar) at 28 °C for 10 days and stored at 4 °C.

The substrates were prepared with eucalyptus sawdust and wheat bran (9:1) in 500 mL becker, where it was added water (1:4). After homogenization, the inductors were added for preparing of different substrates (Table 1).

Table 1 Treatments with description and concentration of the inductors used and how the addition was carried out.

Treatment	Inductors	Methodology
Control	-	
Spent coffee grounds	15% and 25%	based on the amount of sawdust and bran (without water)

Blue dye	2 mL and 5 mL	added to 20 grams of substrate prepared in the Erlenmeyer flask
Used oil fried	2% and 5%	based on the amount of sawdust and bran (without water)

Solid state fermentation was carried out in a 125 mL Erlermeyer flask. 20g of prepared substrate was placed in the Erlemeyer flasks. After autoclaving (121ºC ofr 30 minutes) and cooling, two 1 cm discs of fungus from petri dish were added to each flask. Fermentations were carried out in BOD at a temperature of 28 ºC for 14 days, based on previous results (not shown). All tests were done with 4 repetitions. After this incubation period, the enzymes were extracted from these Erlenmeyer flasks.

The extraction consists of adding 40 mL of 0.1 molar, pH 5 sodium acetate buffer solution to each Erlenmeyer flask and placing them in the shaker, at a temperature of 5°C and 70 RPM for approximately 5 minutes. Then, using a filter and filter paper, what was extracted is filtered, removing as much of the enzymes as possible, using a spatula to help with this process. Afterwards, the extract is collected and placed in a falcon tube identified, for later analysis.

Guaiacol assay method for laccase assay - The laccase activity U/g (of substrate) were determined in the crude extract. The methodology reported by Kalra et al.[8] was followed with modifications. Oxidation of guaiacol to reddish brown colour developed due to oxidation by laccase is used to measure enzyme activity at 450 nm. The reaction mixture can be prepared as 1 mL Guaiacol (2mM), 3 mL Sodium acetate buffer pH 5,0 (10 mM) and 1 mL enzyme extract. The mixture was incubated at 30°C for 15 min and the absorbance was read at 450 nm blank using UV spectrophotometer. Enzyme activity was expressed as Units per grams of substrate (U), where 1 U is the amount of enzyme required to oxidize 1 µmol of guaiacol per min, divided by amount of substrate in grams. The analysis were performed in duplicates.

3. RESULTS & DISCUSSION

The found results of apparent activity enzymatic of laccase's *Pleurotus eryngii* are shown in Figure 1.

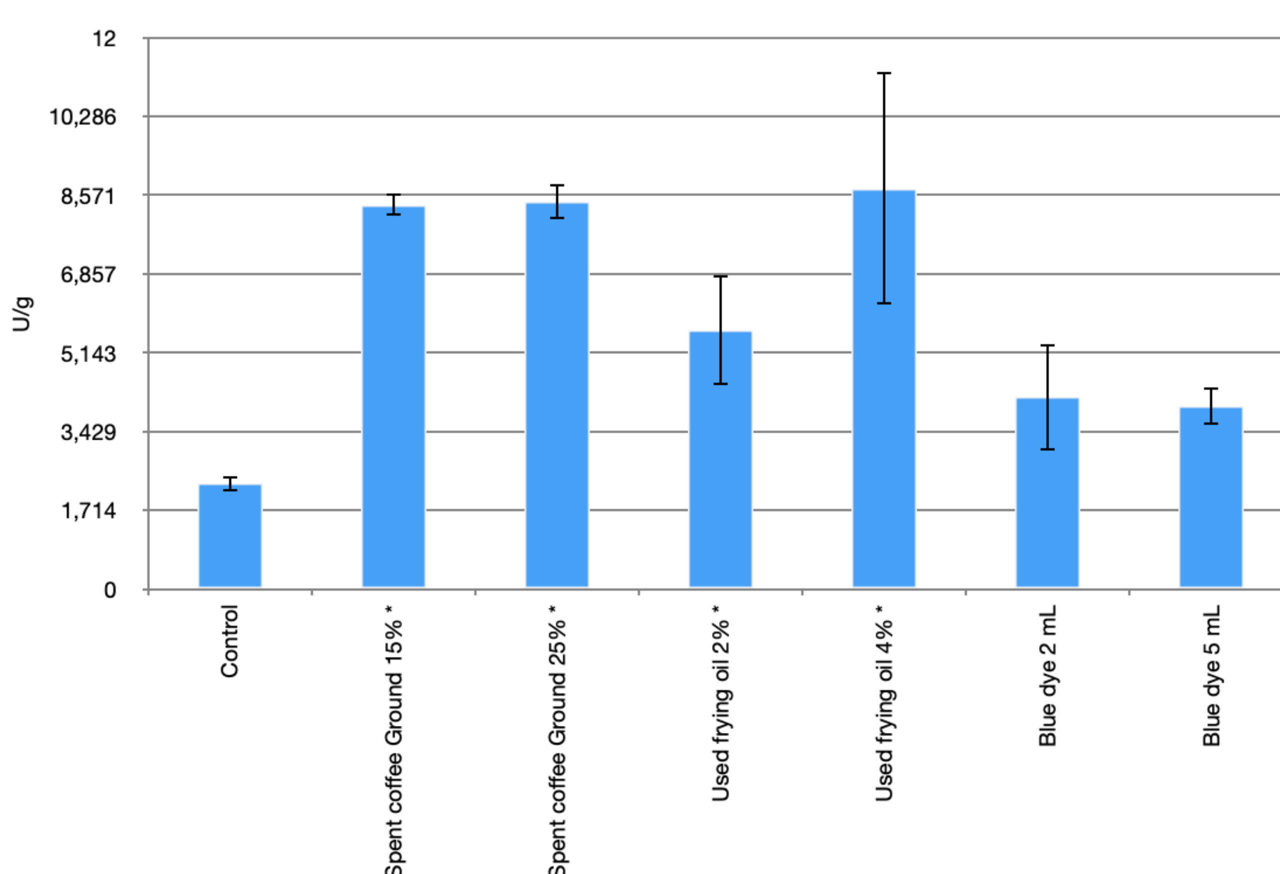

Figure 1 Results of appante activity of laccase of *Pleurotus eryngii* expressed as mean and standard deviation of enzyme activity U/g of substrate. * significant differences between the treatment and control (Dunnett test).

The values obtained for enzyme activities (apparent) ranged from 2 to 8,5 U/g. Higher values were achieved with spent coffee ground and used frying oil.

The inductors spent coffee grounds (15 and 25%) and used frying oil (5%) show significant ($p < 0,05$) upper activity enzymatic, over control (Dunnett test). Engenio *et al.*[9], in the conditions assayed, found the copper sulphate and wheat straw act as laccase inductors with *P. sanguineus.*

The significant results of laccase activity from the fungus *Pleurotus eryngii* for treatments with inducers spent coffee grounds (15 and 25%) and used frying oil (5%), represented up to 260% increase in activity in relation to the control (Table 2) .

Table 2 Results of increase over control (without indutors) em % of apparent activity enzymatic of laccase of *Pleurotus eryngii* with use of inductors

Inductor	Increase over control
Spent coffee Ground 15%	264,01%
Spent coffee Ground 25%	266,46%
Used frying oil 2%	144,78%
Used frying oil 4%	279,16%

In Engenio *et al.*[9] , by comparison with the maximum laccase activity obtained without inductor (480 U/l), the addition of copper sulphate and wheat straw to the medium yields a 1.5- fold and 2.5- fold increase in laccase activity, respectively, of *P. sanguineus*.

The results were relevant and new studies must be conducted to evaluate the interaction between inducers and seek an optimized formulation for laccase production.

4. CONCLUSION

The use of inducers, with emphasis on organic residues, showed potential as inducers to increase the apparent activity of laccase enzyme from the fungus *Pleurotus eryngii*, which implies improving the production of this enzyme in a more economical and efficient way.

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