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Potential capacity of laccases produced by *Phlebia brevispora BAFC 633* in the degradation of chlorpyrifos

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INTRODUCTION

Chlorpyrifos is a pesticide that, applied in significant quantities, produces a negative impact on environmental quality. The monitoring of this pollutant is becoming a mandatory parameter to assess the performance of agricultural practices. In this sense, it has been shown that the ligninolytic enzymes produced by white rot fungi have the ability to degrade / mineralize toxic substances; both structurally diverse xenobiotic and persistent pollutants in the environment, as well as toxic compounds of a phenolic nature and low molecular weight substances. Within these enzymes, laccases have a predominant role; these belong to the protein family of multi-copper oxidases. Although its catalytic action consists of the oxidation of p-diphenols in the presence of oxygen, the specificity of the substrate that can be oxidized is quite wide and varies with the source of the enzyme. This non-specificity character allows it to have important biotechnological applications.

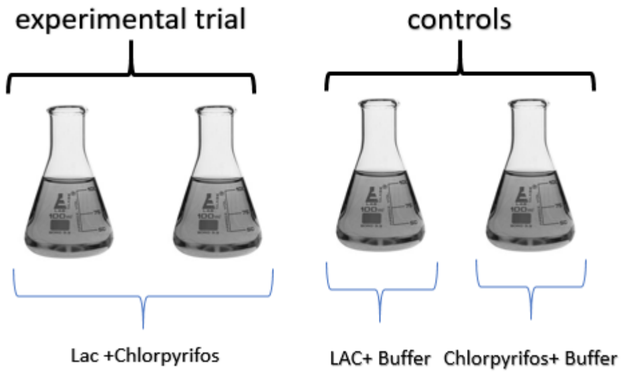
OBJECTIVE

The objective of the work was to evaluate the potential capacity of the laccases produced by the white rot fungus *Phlebia brevispora BAFC 633* to degrade the pesticide chlorpyrifos.

METHODOLOGY

P. brevispora was activated in MEA medium (12.7 g/L malt extract and 20 g/L agar) in a Petri dish for 6 days at 28°C. The enzymatic production was carried out under submerged fermentation in 4 erlenmeyers of 250 mL: To obtain the inoculum, three blocks (Ø 5 mm) of young mycelium were cut and cultivated in ME medium (12.7 g/L of malt extract and 5 g/L of soluble corn extract) with the addition of sulfate of 0.5 mM copper for 10 days at 28°C.

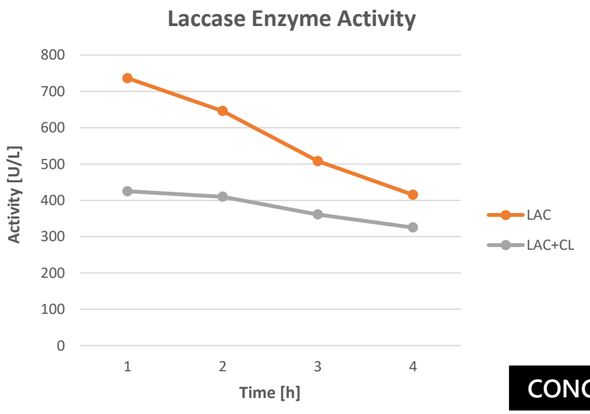
Laccase activity was carried out using the kinetic technique with 2,6 dimethoxyphenol (DMP) 5 mM as substrate in 0.1 mM sodium acetate buffer at pH 3.6. The change in absorbance was monitored at 469 nm in a spectrophotometer.

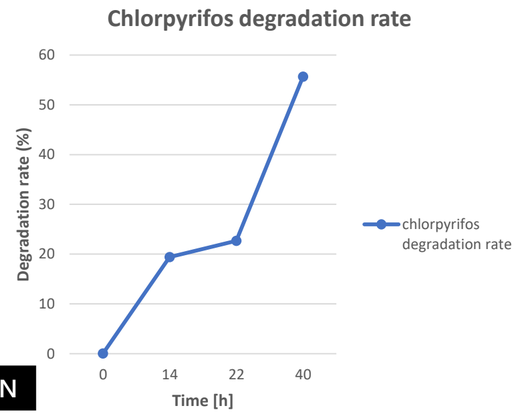


The assay was carried out in 100 mL erlenmeyers containing 35 mL of enzymatic extract with laccase 1 U/mL and chlorpyrifos 16 mg/L reaching the final volume in sodium acetate buffer pH 3.6 0.05 M, at 110 rpm and 28ºC, covered with aluminum foil to protect them from light. The samples consisted of 1.5 mL taken at 0, 14, 22 and 40 hours after starting the experiment. The assay was carried out in duplicate.

RESULTS AND DISCUSSION

The initial laccase activity of the control corresponding to the enzymatic extract at the beginning of the assay (t = 0) was 736 U/L (σ 175), decreasing during the subsequent times until reaching 415 U L (σ 6) at 40 h. The activity of the extract in the presence of chlorpyrifos remained constant during the different times with an average value of 381 U/L (σ 28). The degradation rate obtained in the experimental treatments of the pesticide with enzymatic extract was 19.4% at 14 h, 22.66% at 22 h and a maximum of 55.65% at 40 h of the assay corresponding to 7.12 mg/L of chlorpyrifos at the end of the assay.





CONCLUSION

From the results obtained, it can be seen that *P. brevispora BAFC 633* has a promising degradation capacity of the pesticide chlorpyrifos, a characteristic that could be used in specific biotechnological applications such as its optimization in the use as a specific biosensor for this contaminant.