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Screening and decolorizing potential of enzymes from spent mushroom composts of six different mushrooms

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ABSTRACT

The production and consumption of mushrooms has increased, resulting in large amount of residue from cultivation, called spent mushroom compost (SMC). This study aimed the active enzymes recovery from SMC of *Hypsizygus marmoreus*, *Flammulina velutipes*, *Pleurotus eryngii*, *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus sp*. Results indicated that it was possible to recover different types of enzymes (exocellulase, endocellulase, total cellulase, β-glucosidase, dextranase, amylase and laccase) from the SMCs, demonstrating the diversity of enzymatic activity in these compounds depending on the mushroom. It was also observed that the extract obtained from *Pleurotus sp*, a mushroom originated from the crossbreeding of two types of *Pleurotus*, had a different enzymatic profile, with a greater decolorizing capacity than other fungus tested. Thus, the results indicate that depending on the desired enzymatic activity, and therefore the final aim, a specific type of Basidiomycete SMC should be selected, since each one presents a distinct enzymatic profile.

1. Introduction

The production and consumption of mushrooms is growing every year, given their nutritional advantages, in addition to their flavor increasingly appreciated by the world population. However, in its cultivation, it is generated an agriculture residue called spent mushroom compost (SMC). One killogram of mushroom production will generate about 5 kg of SMC, producing large quantities of wastes (Lau et al., 2003). An evident way to overcome this problem and find a solution is to explore and develop new applications of SMC. Some of the investigated and possible solutions are bioremediation, animal feed, energy feedstock, crop production and enzyme recovery (Jordan and Mullen, 2007; Ko et al., 2005; Paredes et al., 2016; Phan and Sabaratnam, 2012). The use of this compound to obtain enzymes has the advantage of using a low cost product, adding value to wastes and producing high added products (Phan and Sabaratnam, 2012; Singh et al., 2003).

Enzymes are used in a large variety of processes, from biofuels (Donohoe and Resch, 2015) to treatment of dental caries (Otsuka et al., 2015). After harvesting, the spent mushroom compost will have plenty of extracellular enzymes. Some enzymes already detected in SMC: α-amylase (EC 3.2.1.1), cellulase (EC 3.2.1.4), β-glucosidase (EC 3.2.1.21), xylanase (EC 3.2.1.8) and laccase (EC 1.10.3.2) (Ko et al., 2005).

One important application of these enzymes is stain degradation. Coomassie brilliant blue R (a triphenylmethane dye) is widely used as a model compound in degradation studies (Altikatoglu and Celebi, 2011).

The present work aims to profile different types of enzymes (exocellulase, endocellulase, total cellulase, β-glucosidase, dextranase, amylase and laccase) of SMC from Basidiomycota fungi *Hypsizygus marmoreus*, *Flammulina velutipes*, *Pleurotus eryngii*, *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus sp*.

2. Materials and methods

2.1. Enzyme extraction from spent mushroom compost

Spent mushroom composts from six different types of Basidiomycota were obtained from the Urakami Group, Mogi das Cruzes, São Paulo – Brazil (*Hypsizygus marmoreus*, *Flammulina velutipes*, *Pleurotus eryngii*, *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus sp* -The last one is a crossbreding of Japanese hiratake and Western hiratake developed by a japanese company. It is called shimofuri hiratake). Samples of the composts (5 g wet weight) were mixed with 50 mL distilled water in 250 mL flasks. These mixtures were incubated at 30 °C for 1 h with shaking at 180 rpm, filtrated through gauze and then centrifuged at 10,000 × *g* at 4 °C for 10 min. The enzyme activities of all resultant supernatants were estimated as described below.

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2.2. Extracellular enzyme activities assays

Enzyme preparations were assayed for the release of reducing sugar from the following substrates dissolved in citrate-phosphate buffer (pH 5.0): 1% carboxymethyl cellulose (CMC; low viscosity, BDH), 1% Avicel, 1% dextran, 1% soluble starch and cellulose paper (Whatman, 1 cm × 6 cm ≈ 50 mg). The reaction mixture for reducing sugar assays contained 20 μL enzyme preparation and 480 μL of substrate. The reaction mixtures were incubated at 40 °C in a shaking incubator for 30 min for endoglucanase (CMC), dextranase and amylase (soluble starch). The reaction mixtures were incubated at 50 °C in a shaking incubator for 60 min for exoglucanase (Avicel) and total cellulase (cellulose paper). Reducing sugar levels in the supernatant were determined by the dinitrosalicylic acid method (Miller, 1959). Enzyme activities were calculated as μmol reducing sugar released per minute per g of compost (U/g).

β-D-glucosidase (EC 3.2.1.21) activity were assayed using 4-nitrophenol μ-o-glucoside (PNPG, Koch-Light) (adapted from Matsuura et al., 1995). Enzyme activity was calculated as μmol ρ-NP released per minute per g of compost (U/g).

Laccase (EC 1.10.3.2) activity assay was measured (Kalra et al., 2013) by reading the absorbance at 450 nm, when due to guaiacol oxidation, a reddish brown color is developed. Enzyme activity was expressed Units (U), where 1 U is the amount of enzyme required to oxidize 1 μmol of guaiacol per min.

2.3. Dye decolorization by SMC enzymes

The ability of the SMC enzymes to degrade the dye Coomassie Brilliant Blue R-250 (Altikatoglu and Celebi, 2011) was evaluated in 96-well ELISA plates containing 270 μL of water or dye solution (20 μM) and 30 μL of SMC enzymes for the first assay; and 150 μL of dye solution (20 μM), 110 μL of water or buffer and 40 μL of *Pleurotus sp* SMC enzymes for the second assay using four different concentrations (10%, 15%, 20% and 25%). The control sample consisted of substitution of the SMC enzymes by distilled water. First assay was conducted for 360 min with all extracts, and second assay was conducted for 300. Decolorization was evaluated by the change in absorbance at 570 nm and comparing the results with their respective controls.

2.4. Laccase zymogram

A 10% (w/v) acrylamide separating gel without any substrate was used for electrophoresis. Subsequent to renaturation, the buffer was replaced with citrate-phosphate buffer (pH 5) with Guaiacol 2 mM, and incubated for 30 min at 40 °C. Areas of laccase activity appeared as brown bands (Cambria et al., 2011).

3. Results and discussion

Different types of hydrolytic and oxidative enzymes were detected in SMCs. The enzymes activity profile is presented in Table 1.

All SMC presented cellulose-degrading enzymes activity, and *L. edodes* SMC showed the highest levels. There is a need for enzymatic saccharification of biomass, being essential the use of cellulose-degrading enzymes when using lignocellulosic biomass (Donohoe and Resch, 2015). In this case, the enzymes recovered from SMC of *L. edodes* could be an alternative source.

Dextranase activity was observed in all SMC, and only *H. marmoreus* SMC extract showed no amylase activity. Dextranase could be used in caries prevention since some researchers found that this enzyme inhibits dental biofilm formation and decomposes existing biofilm *in vitro* (Otsuka et al., 2015). Also, this enzyme can be used to reduce dextranase in sugarcane juice (Sulfiate et al., 2016). In addition, isomaltooligosaccharides can be synthesized from dextranes using dextranase, amylase and α-glucosidase, contributing to the synthesis of a food with possible functional properties (Kothari and Goyal, 2016). Moreover, amylase is an important enzyme used in saccharification, prior to ethanol or organic acid fermentation (Ahmed et al., 2017), and these composts could be source of this enzyme.

SMC extracts from *F. velutipes*, *P. eryngii*, *P. ostreatus* and *Pleurotus sp* presented laccase activity with higher values for *P. ostreatus* extract. This enzyme could be used in wastewater treatment and stain decoloration (Bronikowski et al., 2017). In this sense, Figs. 1 and 2 shows the results of the dye Coomassie Brilliant Blue R-250 degradation. Only for *Pleurotus sp* the decoloration was visible (Fig. 1), and the reaction is more effective using pH 5 buffer (Fig. 2). The decolorizing effect in many reactions is attributed to laccase activity. Thus, by the tests performed would be expected an action for all *Pleurotus*. However, only *Pleurotus sp* had this capability. Palmieri et al. (2000) observed that there are different laccase isoforms in *Pleurotus*, and Giardina et al. (2002) reported that some laccase isoforms are not able to oxidize guaiacol, the substrate used in the present study. Thus, possibly the *Pleurotus sp* laccase activity was underestimated in the assay performed. This result also shows that the crossbreeding performed resulted in a mushroom with different enzymatic profile from other *Pleurotus* type.

Due to decolorizing activity, we performed the laccase zymogram (Fig. 3). The laccase activity in the zymogram was similar to that presented in tube assay, with bands more evident for *P. eryngii* and *P. ostreatus* extracts. *P. eryngii* presented the formation of two bands, indicating the presence of two laccases.

4. Conclusion

SMC is a residue generated after mushrooms cultivation. Thus, the development of applications to reuse this residue is fundamental. Despite the several studies already done with SMC, this is the first work to demonstrate the diversity of enzymatic activity in these compounds depending on the mushroom type of the SMC. In conclusion, the results indicate that depending on the desired enzymatic activity, and therefore the final aim, a specific type of Basidiomycete should be selected, since each one presents a distinct enzyme profile.

Table 1
Enzyme activities (expressed as U g^{-1} of compost) in extracts from SMC.

Enzymes (U g^{-1})	*Hypsizygus marmoreus* (Bunapi)	*Flammulina velutipes* (Enoki)	*Pleurotus eryngii* (Eryngui)	*Lentinula edodes* (Shiitake)	*Pleurotus ostreatus* (Shimeji)	*Pleurotus sp* (Shimofuri hiratake)
Total cellulase	0.67 ± 0.09	0.51 ± 0.10	0.51 ± 0.12	0.71 ± 0.19	0.41 ± 0.04	0.57 ± 0.12
CMCase	17.03 ± 2.20	None	8.82 ± 0.41	39.99 ± 3.91	19.25 ± 2.89	9.45 ± 2.00
Avicelase	None	None	None	9.73 ± 0.64	None	None
β-glucosidase	7.55 ± 1.09	137.63 ± 23.82	34.07 ± 0.44	149.93 ± 6.14	82.70 ± 4.96	44.41 ± 6.60
Dextranase	8.47 ± 0.44	8.12 ± 0.41	8.60 ± 0.41	14.41 ± 0.65	8.58 ± 0.26	11.84 ± 0.60
Amylase	None	11.09 ± 0.60	8.19 ± 0.47	9.47 ± 0.24	9.07 ± 0.41	12.39 ± 1.92
Laccase	None	2.00 ± 0.07	4.31 ± 0.29	None	4.68 ± 0.03	3.25 ± 0.03