Induction of *Penicillium expansum* lipase in low-cost medium containing sunflower husk

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ABSTRACT

The greater use of lipases in the industrial sector is directly associated with the reduction of production costs. Sunflower Husk (SH), a low-cost by-product, proved to be an efficient inducer of lipase in *P. expansum*. After 72 h of cultivation in submerged fermentation, there was 261.1 U of lipase per g of biomass, with a specific activity of 37.50 U/mg. No statistically significant difference was found in lipase activity when sunflower oil was used in the cultivation medium, in the same or higher concentration compared to SH. The results reaffirm the potential of sunflower hulls for inducing lipase expression, that is a product with high added value.

Keywords: Lipase. Submerge Fermentation. Solid-State Fermentation. Sunflower Husk. *Penicillium expansum*.

1 INTRODUCTION

Lipases (triacyl glycerol ester hydrolases, EC 3.1.1.3) are enzymes known as carboxylesterases that catalyze chemical reactions from a triacylglycerol structure, releasing mono- or diglycerides, free fatty acids and glycerol [1,2]. They can also catalyze reverse reactions (synthesis) in low concentrations of water, such as esterification, transesterification and interesterification.

The greater use of lipase in various sectors is directly linked to the reduction in production costs. Ways to reduce costs include the selection of new producer microorganisms and low-cost culture media [3].

When processing sunflower seeds, one of the main residues produced is the husks. Sunflower husk (SH) accounts for about 21-30% of the total seed weight [4]. Due to the lipid content present in the peels, this biomass has the potential to be used as a source of lipase production by microorganisms. However, it has a low amount of protein, which acts as an organic source of nitrogen, necessary for microorganism growth.

In this context, this work proposed a study on the use of sunflower husk, added with wheat bran (WB), as a substrate and inducing source for the production of lipase by the fungus *Penicillium expansum*, and the characterization of the lipase produced, aiming to understand its properties.

2 MATERIAL & METHODS

Submerged Fermentation and Solid-State Fermentation in culture medium with biomass. *P. expansum* was grown under Submerged Fermentation (SF), in Erlenmeyer flasks containing 100 mL of the liquid culture medium: 1.5 g/L of KH_2PO_4, 1.0 g/L of NO_3NH_4, 0.5 g/L of $MgSO_4$, 0.2 g/L of $CaCl_2$ and 2.5 g/L yeast extract in Tris-HCl buffer, pH 7, 100 mM and 2 g of biomass (SH + WB) in the following proportions: 100 % SH; 75 % SH + 25 % WB; 50 % SH + 50 % WB; 25 % SH + 75 % WB and 100 % WB. The flasks were maintained in an orbital shaker, at 28 °C and 150 rpm, for 168 h. Aliquots were removed at 24-hour intervals, centrifuged at 10,000 *g* for 10 min and the supernatant was considered the crude enzymatic extract.

For Solid-State Fermentation (SSF) cultivation, the Erlenmeyer flasks contained 5 g of biomass (SH + WB), previously dried to remove moisture, in the proportions described previously, and 7 g of mineral solution with yeast extract, as described previously. The flasks were autoclaved at 121 °C, 1 atm, for 15 min, cooled and inoculated with 10 discs of agar medium containing the fungal mycelium. The flasks were stored in a B.O.D (Biological Oxygen Demand) chamber at 28 °C, during a time period from 72 h to 168 h. After these cultivation times, the enzymes were recovered by adding 50 ml Tris-HCl buffer, pH 7, 100 mM to each flask and shaking for 1 h, at 150 rpm and 28 °C. After centrifugation for 10 min at 10,000 *g,* the supernatant was considered the crude enzymatic extract.

Submerged Fermentation in culture medium with biomass supplemented with Oil. The fungus was grown as described in item **2.1,** for 72 h, at 28 °C and 150 rpm, in liquid medium containing 2 g of SH (control), or SH supplemented with 0.5, 1.0, 1.5 and 2.0 g of sunflower oil. The total content of lipids in the culture media were 0.13 g, 0.63 g, 1.13 g, 1.63 g, and 2.13 g, respectively. Lipids present in sunflower hulls were quantified using the Soxhlet method.[5].

Enzymatic and Protein Assay. Lipase assay was performed in 96-well plate, using 50 µL of appropriately diluted enzyme and 50 µl of a substrate solution (9:1 of aqueous solution of gum arabic 0.1% w/v and Triton-X-100 0.4% w/v + *p*-nitrophenyl palmitate solution in 20 mM isopropanol). The mixture was incubated at 50 °C for 15 min in a water bath, and the reaction was stopped by adding 100 µL of 0.5 M Na_2CO_3 solution. The absorbance was taken at 410 nm. One unit of enzymatic activity (U)

corresponded to 1 µmol of *p*-nitrophenol released per minute of reaction per mL of enzyme solution. Protein determination was carried out according to Bradford method [6] using bovine serum albumin (BSA) as a standard protein.

Statistical Analysis. The statistical analyses of ANOVA and Tukey test were carried out using the Minitab program.

3 RESULTS & DISCUSSION

The total lipolytic activity, quantified in the crude extract, was considerably lower when *P. expansum* was grown under SSF (**Fig 1A**), compared to SF (**Fig 1B**). Possibly, the smaller amount of free water in SSF, negatively influenced the expression of the enzyme and its catalytic potential. The highest specific activities were detected in the culture medium containing 100% SH, during 48 - 96 h of cultivation (**Fig 2**). The specific activity values, detected in the culture medium with 100 % SH, did not differ significantly at different cultivation times according ANOVA and Tukey statistical tests, with *p-value* < 0.05 (**Table 1)**. These results showed that the highest total enzymatic activity (522,2 U) and specific activity (37,50 U/mg) were detected when the fungus was grown in medium containing 100 % SH, in the cultivation time of 72 h.

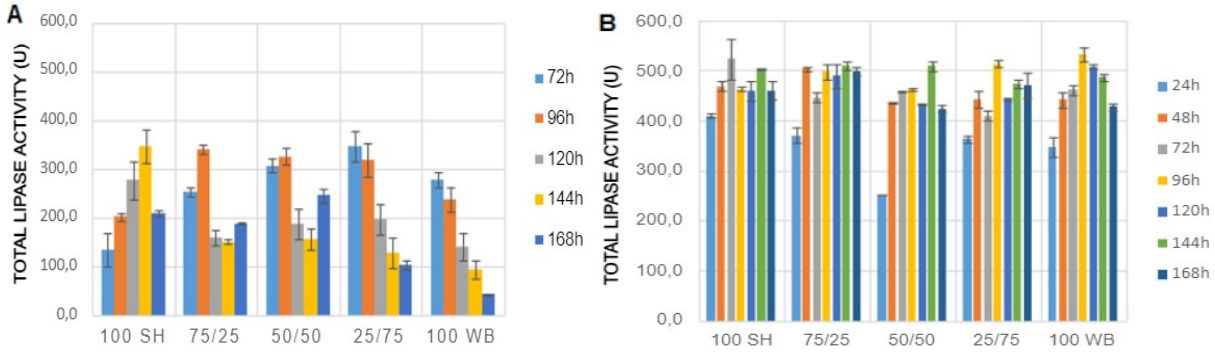

Figure 1 Total lipase activity detected in the crude extract of *P. expansum* grown under SSF (A) and SF (B) at different times, in medium containing sunflower husk (SH) and wheat bran (WB) at different proportions.

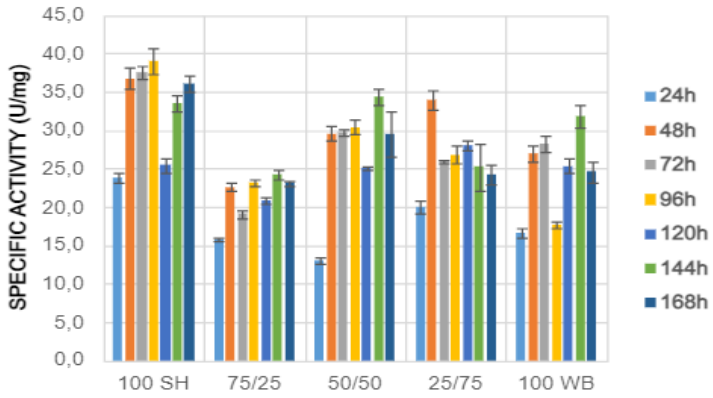

Figure 2 Specific activity detected in the crude extract of *P. expansum* grown under SF at different times, in medium containing sunflower husk (SH) and wheat bran (WB) at different proportions.

Table 1 Means of specific activity values detected in the crude extract of *P. expansum* cultivated under SF, in medium containing 100 % SH. Means followed by the same letters do not differ significantly using the Tukey test p-value < 0.05.

Cultivation Time (h)	24	48	72	96	120	144	168
Means U/mg of protein	23,81 ^{c}	36,73 ^{ab}	37,50 ^{ab}	38,97 ^{a}	25,43 ^{c}	33,54 ^{ab}	36,04 ^{ab}

The lipase activity values did not show a statistically significant difference, using de ANOVA test at 5 % probability, when the fungus was grown under submerged fermentation in medium containing sunflower husk or this biomass supplemented with sunflower oil from 0.5 g to 1.5 g (**Figure 3)**. No enzymatic activity was detected in the culture media containing SH supplemented with 2.0 g of oil or with more than 0.13 g of oil. This effect could be due the low diffusion of oxygen during fungal growth in medium containing high oil concentration. The enzymatic activity values detected in the cultivation media containing only 100 % SH, SH plus 0.5, 1.0, and 1.5 g of oil or 0.13 g of oil did not show a significant difference between them, according to the ANOVA tests at 5 % probability. This result indicate that the 100% SH medium is more economically viable.

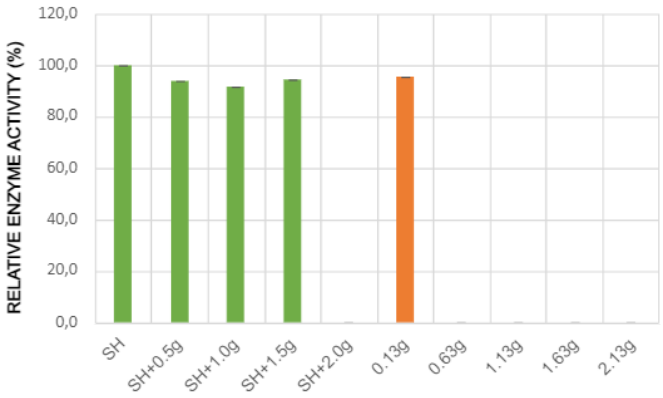

Figure 3 Relative activity detected in the crude extract of *P. expansum* grown under SF for 72 h, in medium containing sunflower husk (SH) and this biomass supplemented with 0.5- 2.0 g of sunflower oil, or containing 0.13 – 2.13 g of sunflower oil.

4 CONCLUSION

In both forms of *P. expansum* cultivation, sunflower husk, an agro-industrial byproduct, proved to be efficient in inducing lipase activity, suggesting that this biomass could be a viable alternative for lipase production. The higher total enzymatic activity (522,2 U) and specific activity (37,50 U/mg) detected in the crude extract, when the fungus was grown under submerged fermentation with 100 % SH for 72 h, compared to the activity produced under solid-state fermentation, demonstrates that the percentage of free water is an important factor for lipolytic activity, since lipases act through the interface between a polar and a nonpolar medium, in a mechanism known as interfacial activation[7]. Induction of lipase activity using sunflower oil or sunflower husk showed similar results, however, as the oil is a refined product with high added value, its use as an inducer makes the process more expensive. In this way, sunflower husk reinforces itself as an efficient and low-cost inducer, reducing costs for lipase production by *P. expansum*.

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