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BY-PRODUCTS OF PALM OIL PRODUCTION CHAIN TO OBTAIN LIPASES BY *Yarrowia lipolytica*

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ABSTRACT

Palm oil production chain by-products are mainly composed by free fatty acids and lignin, as palm oil deodorizer distillate (PODD) and palm fiber, respectively. These agro-industrial wastes were used as lipase inducers in submerged fermentation for 96 h using an unconventional yeast in Erlenmeyer flasks. Both palm oil wastes were useful for extracellular lipase production, especially when combined, which provided the highest lipase activity (7211.61 U/L) and productivity (150.24 U/L.h) in 48 h. Therefore, palm residues seem to be a promising alternative to produce lipase by the yeast *Yarrowia lipolytica* in submerged fermentation.

Keywords: Lipase. Palm fiber. Palm oil deodorizer distillate. Waste valorization.

1 INTRODUCTION

Palm plant (*Elaeis guineensis*) belongs to the Arecaceae family and grows in humid tropical environments, withstanding a range of temperature from 22 ºC to 33 ºC. Palm oil is extract from the plant's fruit, which can generate two types of oils: crude palm oil, from the mesocarp, and palm kernel oil, from the seed. Malaysia and Indonesia are the biggest palm oil producers and registered 60 million tons of crude oil in 2018[1].

Palm oil deodorizer distillate (PODD) is a low value by-product from the refining process of palm oil, generated in the physical refining route of the deodorization path. PODD is a light brown greasy residue composed mainly by free fatty acids, with palmitic acid as major compound, followed by oleic acid and linoleic acid. Due to its high fatty acid content and the increasing need to provide alternative destinations to industrial wastes, PODD can be used as feedstock in several reactions using microorganism to generate bioproducts[2], including biodiesel, biolubricants and emulsifiers.

Palm mesocarp's fiber is the external layer from palm fruit and is rich in lignin, therefore it presents low digestibility. Palm fiber also contains cellulose and hemicellulose, besides lipids, proteins, mineral matter and this subproduct's moisture content can range from 15 to 30% (w/w). Fiber from palm oil industry was successfully used to produce lipase in solid state fermentation when in combination with palm cake[3].

Lipases are triacylglycerol hydrolases (E.C. 3.1.1.3) which catalyse hydrolysis of esters bonds in triacylglycerol and in absence or in low moisture content can assist the reverse reaction[2]. Lipase is one of the most important metabolites produced by *Yarrowia lipolytica*, a strict aerobic yeast, which can assimilate different carbon sources, including hydrophilic and hydrophobic substrates. *Y. lipolytica* is the most studied unconventional yeast, however, there is still a lack of data regarding the use of some agro-industrial wastes to produce lipase by this microorganism[4]. In this present study, we used PODD and palm fiber to induce the production of lipase by *Y. lipolytica* in submerged fermentation.

2 MATERIAL & METHODS

Palm Oil Deodorizer Distillate (PODD) and palm fiber originated from palm oil refining process was kindly provided by Companhia Refinadora da Amazônia (Grupo Agropalma, Brazil).

A wild-type strain of *Yarrowia lipolytica* cultivated at 28 ºC, 160 rpm, in 500 mL flasks with 200 mL of YPD medium (Yeast Extract 1%, Peptone 2%, Glucose 2%) for 72 h was used to inoculate lipase production medium. Lipase production by *Y. lipolytica* using PODD and palm fiber was performed in 1 L Erlenmeyer flasks with 200 mL of lipase production medium (Yeast Extract 1%, Peptone 0,64%). Palm fiber and PODD were used in production medium in four different forms: i. 1.5% (w/v) of PODD; ii. 5.0% (w/v) of fiber; iii. 1.5% of PODD (w/v) and 5.0% (w/v) of fiber; iv. absence of PODD or fiber (negative control). The media were inoculated and incubated at 28 ºC in a rotary shaker at 250 rpm for 96 h.

For lipase determination in a free-cell medium, samples were taken once a day and then centrifuged (4000 *g*) for 10 minutes. Determination of enzymatic activity of lipase was performed by hydrolysis of *p*-nitrophenyl laurate (*p*-NPL)[5]. One lipase unit (U) is defined as the amount of enzyme which releases 1 μmol of *p*-nitrophenol per minute at pH 7.0 and 37 ºC.

3 RESULTS & DISCUSSION

Lipase was produced with both palm residues: PODD and palm fiber (Figure 1). With only YP* medium, *Y. lipolytica* produced 3366.59 U/L of lipase in 24 h. However, when PODD and palm fiber are added to YP* medium the enzyme activity reaches more than double of it in 48 h (7211.61 U/L), producing an enzyme extract with higher activity. The use of the residues separately was not so effective. Diniz et al.[6] reported a lipase activity produced by *Y. lipolytica* of 2051 U/L in submerged fermentation using pomegranate residue in Erlenmeyer flasks in 16 h. Residual frying oil was also reported as a good lipase inducer, producing approximately 4700 U/L of extracellular lipase activity in 24 h of fermentation[7].

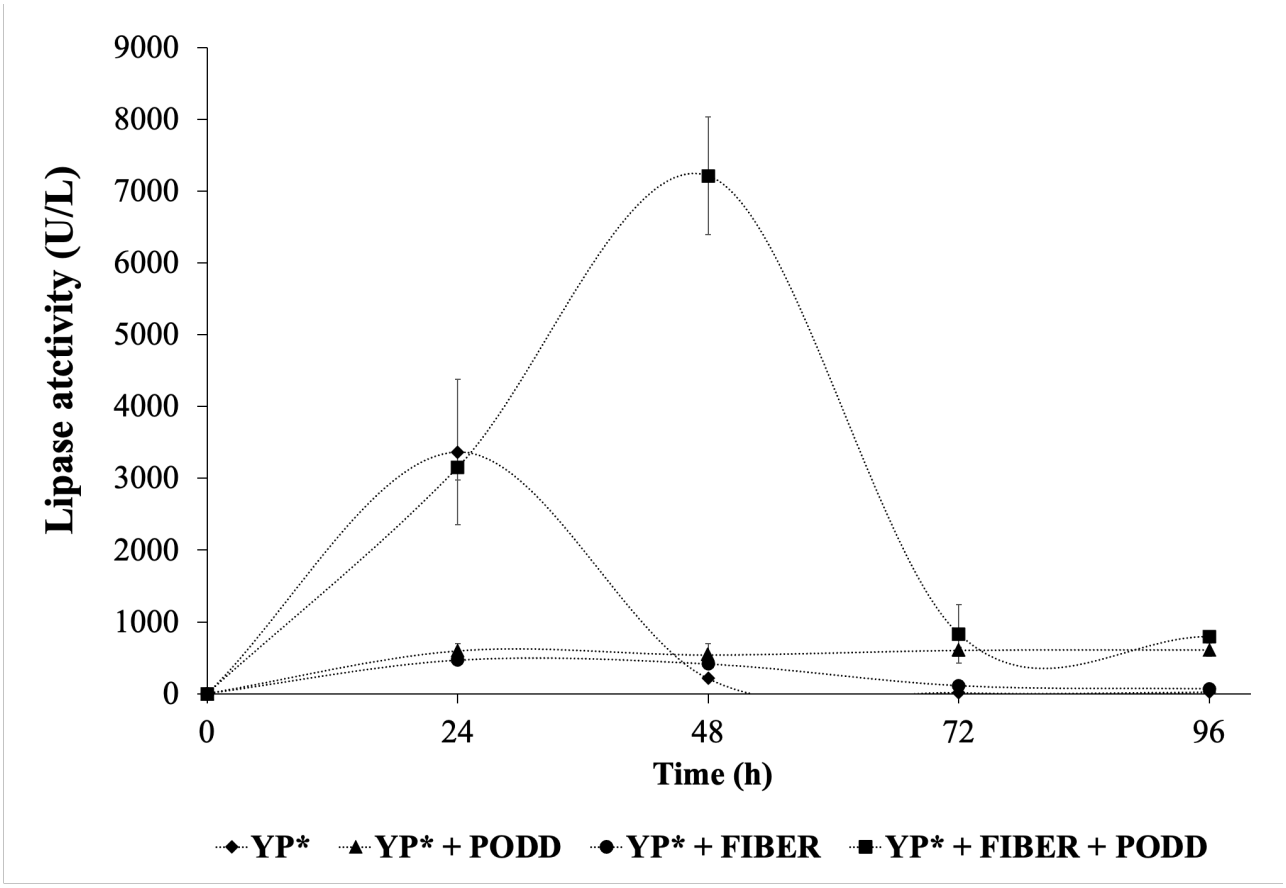

Figure 1 Lipase production during growth of *Yarrowia lipolytica* in submerged fermentation with YP*, YP*+PODD, YP* + PALM FIBER and YP* + PODD + PALM FIBER; YP* - Yeast extract and peptone medium; PODD – palm oil deodorizer distillate

In terms of productivity, the combination of PODD and fiber in YP* medium was better (150.24 U/L.h) in comparison with just YP* medium (140.27 U/L.h). For mango residues, lipase productivity reported by Pereira et al.[5] reached 170.6 U/L.h after 20 h of fermentation, which is slightly higher than the value found in this present work. PODD and palm fiber together are composed of lipids and proteins, which can be carbon and nitrogen sources for *Y. lipolytica* growth and for lipase production.

4 CONCLUSION

Palm oil residues were able to induce *Y. lipolytica* lipase production and productivity. PODD and palm fiber together was the best inducer for extracellular lipase production.

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