

Research Paper

Production and characterization of an extracellular lipase from *Candida guilliermondii*

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Abstract

Extracellular lipases from the endophytic yeast *Candida guilliermondii* isolated from castor leaves (*Ricinus communis* L.) were produced using low-cost raw materials such as agro-industrial residues and applying them in the esterification of oleic acid for evaluating their potential use in biodiesel production. After partial purification using ammonium sulfate, the enzyme was characterized and presented higher activity (26.8 ± 1.5 U mL⁻¹) in the presence of 5 mmol L⁻¹ NaCl at 30 °C and pH 6.5. The production through submerged fermentation was formerly performed in 150 mL erlenmeyer flasks and, once the enzyme production was verified, assays in a 14 L bioreactor were conducted, obtaining 18 ± 1.4 U mL⁻¹. The produced enzyme was applied in the oleic acid esterification under different solvents: hexane, cyclohexane or cyclohexanone) and different acid:alcohol molar ratios. Higher ester conversion rate (81%) was obtained using hexane and the molar ratio of 1:9 was the best conditions using methanol. The results suggest the potential for development of endophytic yeast in the production of biocatalyst through submerged fermentation using agroindustrial residues as culture medium.

Key words: extracellular lipase, endophytic yeast, esterification, submerged fermentation, bioreactor.

Introduction

Lipase (triacylglycerol hydrolase EC 3.1.1.3) are hydrolytic enzymes capable of cleaving the ester bond of triacylglycerol, and catalyze ester synthesis *in vitro* by shifting equilibrium of reaction (Contesini *et al.*, 2010; Fernandes *et al.*, 2007; Li and Zong, 2010; Mohamed *et al.*, 2011). Some advantages of using these enzymes in the cleavage and synthesis of esters bonds are: they do not require co-factors, they have great stability and activity in organic media, they work in a wide variety of substrates and they exhibit high specificity and enantioselectivity (Busamara *et al.*, 2010; Hernandez-Rodrigues *et al.*, 2009). These enzymes can be obtained from several organisms; however, microorganisms are most promising for this purpose (Fernandes *et al.*, 2007; Salihu *et al.*, 2012). Among the microorganisms, yeasts have been used widely for the

production of these enzymes, with special emphasis on the genus *Candida* sp. (De María *et al.*, 2006; Salihu *et al.*, 2011; Sharma *et al.*, 2001).

In this case, it is necessary to provide to the microorganism suitable conditions that allow their growth and induce the production of the metabolite of interest. Culture media used for this purpose are usually expensive, which results in a high cost for enzyme production. Nevertheless, the use of agro-industrial residues as substrate for fermentation processes and support are a trend in recent publications, a fact that leads to reduction of production cost of these biocatalysts and can thus make them economically competitive against chemical catalysts (Damasso *et al.*, 2008; Salihu *et al.*, 2011). In previous work, our research group demonstrated the feasibility of the production of microbial lipases in shake flasks by applying a low cost fer-

mentation media, obtaining yields of up to 25 U mL⁻¹ of lipase activity (Oliveira *et al.*, 2012).

Within the broad application field of lipases, synthesis of biodiesel is attracting great interest (Gog *et al.*, 2012; Hasan *et al.*, 2006). The biodiesel is a biofuel produced from renewable sources consisting of alkyl esters of fatty acids obtained from the esterification or transesterification of various types of lipids, and its use is increasing due to the scarcity of fossil fuels used for diesel obtainment (Gog *et al.*, 2012; Tan *et al.*, 2012). Besides, the biodiesel presents interesting features such as their possibility of use in diesel engines without any adjustments and also the reduction of some gases that contribute to the greenhouse effect (Bajaj *et al.*, 2010; Lai *et al.*, 2011).

In the traditional biodiesel production, for economic reasons, homogeneous catalysts are commonly used providing high product conversion. The enzymes generally exhibit slower reactions, but have major advantages over the prior process, such as high product purity, easy separation of the catalyst from the reaction medium, mild reaction temperatures, and easier glycerol separation from biodiesel (Diaz *et al.*, 2006; Iftikhar *et al.*, 2010; Tran *et al.*, 2012).

Endophytic bacteria and yeast in plants are alternative resources for isolating new microorganisms producing useful enzymes (Molina *et al.*, 2012; Fang *et al.*, 2006). However, despite the potential for industrial application, few studies have been realized on extracellular lipases from endophytic yeast (Oliveira *et al.*, 2013; Oliveira *et al.*, 2012; Panuthai *et al.*, 2012; Venkatesagowda *et al.*, 2012; Costa-Silva *et al.*, 2011; Torres *et al.*, 2003).

In this paper, we report the production of lipases using an endophytic yeast *Candida guilliermondii* isolated from leaves from castor bean plant (*Ricinus communis*, L.). The enzyme was partially purified and its lipolytic activity measured in terms of effect of pH, temperature and different ions. The production of (m)ethyl oleate under different solvents and acid:alcohol molar ratios were also investigated.

Materials and Methods

Cultivation and identification of lipase-production yeast

The enzyme production was performed by submerged fermentation (SmF) employing an endophytic yeast strain isolated from the castor bean leaves (*Ricinus communis* L.), kindly provided by Roseli Mello, researcher from Tuiuti University of Paraná. The yeast *Candida guilliermondii* was phenotypically characterized by standard morphological and biochemical tests as carbon and nitrogen assimilation, and ability to ferment carbohydrates, as described by Guimarães (2006).

The yeast was grown in medium containing brewer's yeast (20 g.L⁻¹) and sucrose (30 g.L⁻¹) for 120 h, and after this period the cell concentration was determined by Neu-

bauer chamber counting. The fermentation was conducted in 250 mL Erlenmeyer flasks containing 150 mL of fermentative medium. This medium was composed of ammonium sulfate 15 g.L⁻¹, manganese sulfate 0.15 g.L⁻¹, brewer's yeast 10 g.L⁻¹, Tween 80 8 g.L⁻¹, glycerol 15 g.L⁻¹ and soybean oil 30 g.L⁻¹, dissolved in phosphate buffer 25 mmol L⁻¹ at pH 6.5. After sterilization, the yeast was added to the fermentative medium at a final concentration of 10⁷ cells.mL⁻¹. The fermentation was maintained in a shaker at 30 °C at 180 rpm for 72 h.

Lipase assay

The lipase assay was determined by the pNPP (p-nitrophenyl palmitate) hydrolysis method, described by Ghori *et al.* (2011) adapted to this work. A 100 µL sample of the fermented medium was incubated for 5 min at 30 °C with 800 µL of 0.25% PVA [poly(vinyl alcohol)] solution in pH 6.5 phosphate buffer and 100 µL of 8 mmol L⁻¹ pNPP solution in isopropanol. After the incubation time, the reaction was stopped by 500 µL of 3 mol L⁻¹ HCl solution, then the mixture was centrifuged and 500 µL of the supernatant was added to 1 mL of 2 mol L⁻¹ NaOH solution. The absorbance was measured in a spectrophotometer under 410 nm. One unit of enzymatic activity (U) was defined as the release of 1 µmol of pNP per minute.

Lipase characterization

The conditions for lipolytic activity evaluation were the same as describe above, varying only the parameters to be assessed. The optimal pH was determined using pNPP as the substrate in buffer solutions of pH values ranging 5.0-5.5 (acetate buffer), 6.0-7.5 (phosphate buffer) and 8.0-8.5 (Tris - HCl buffer). The optimal temperature for lipolytic activity was determined using pNPP as a substrate at different temperatures (20, 30, 37, 45 and 55 °C). The influence of ions on enzyme activity was observed by adding ions (Fe³⁺, Mg²⁺, Mn²⁺, K⁺, Na⁺, Ca²⁺, Zn²⁺) or EDTA (1 and 5 mmol L⁻¹) to the lipase assay. The results of the lipase characterization experiments were expressed as Residual Lipolytic Activity [RLA (%)], in which the value obtained in the analysis [LA_{assay}] was divided by the maximum activity determined in the optimal condition of pH and temperature tested [LA_{control}], as follows:

$$RLA(\%) = \frac{LA_{assay}}{LA_{control}}$$

Simple bioreactor batch fermentation

Larger scale submerged fermentation (Figure 1) was performed after the lipase characterization study. The fermentation medium was similar to that described for the lipase production in Erlenmeyer flasks (2.1.). The bioreactor consisted of a simple glass reactor of 20 L in which was added 14 L of fermentative medium. The bioreactor and the fermentative medium were sterilized by autoclaving previ-

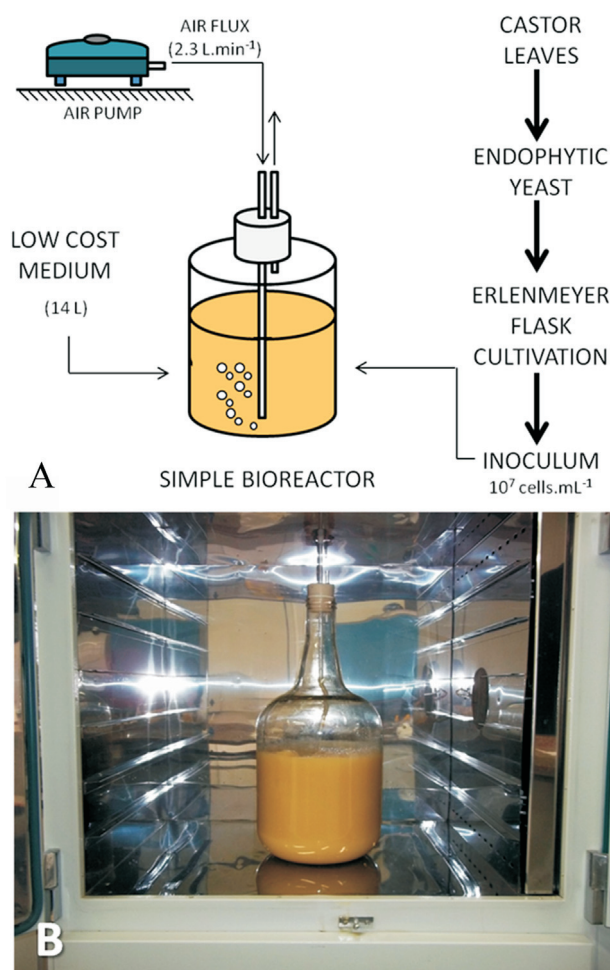


Figure 1 - Diagram (A) and picture (B) of simple bioreactor batch SmF to grown endophytic yeast to produce extracellular lipase.

ously to the addition of the yeast in a final concentration of 10^7 cells.mL⁻¹. This system was kept in an incubator at 30 °C for 72 h. A silicone tube previously sterilized was inserted to the bottom of the bioreactor and connected to an air compressor providing 2.3 L of air min⁻¹. There was no mechanical procedure to agitate the system besides the agitation provided by the air injection and no pH control was performed during the experiment as well. The lipase production in this bioreactor was repeated three times.

Partial purification of the enzyme

After fermentation, the enzyme was partially purified by solid ammonium sulfate precipitation. The precipitation was performed on an ice bath using the saturation levels of 50, 60, 70 and 80%. In the ranges of 50 to 70% irrelevant lipolytic activities were recovered, so these salt concentrations were discarded. The enzyme precipitated in the range of saturation of 80% showed higher lipolytic activity and, therefore, was used in this study. The precipitant was suspended with a minimal amount of distilled water and then

dialyzed in distilled water for 48 h at 4 °C. The dialyzed material was freeze-dried.

Assessment of syntheses of (m)ethyl oleate via esterification of oleic acid

Syntheses of methyl and ethyl oleate were performed in 125 mL Erlenmeyer flasks. To each flask were added the equivalent of 2000 Units of lyophilized enzyme, 50 mmol L⁻¹ oleic acid, 200 mmol L⁻¹ ethanol or methanol and 15 mL of organic solvent. Three organic solvents were evaluated to perform the synthesis: hexane, cyclohexane and cyclohexanone. The reactions were incubated in a shaker at 30 °C and 180 rpm for 72 h. The acid:alcohol proportions 1:6; 1:9 and 1:12 were also investigated in experiments after determination of the organic solvent with the best performance.

Ester conversion determination

The methodology described by Lowry-Tinsley (1976) was carried out to determine the fatty acids conversion into esters. This method was used previously and has great precision in the quantification of fatty acids (Oliveira *et al.*, 2012; Fernandes *et al.*, 2007; Baron *et al.*, 2005). The technique is based on the bond between free fatty acids and copper II ions under organic media, which allows the measurement of the percentage of fatty acids converted to ester. A volume of 0.2 mL of reaction medium was added to 2.4 mL of toluene and 0.5 mL of pyridine and copper acetate (5%). The reactions were vortexed for 30 s and the organic phase read in spectrophotometer at 715 nm. Thus, the determination of ester conversion rate [C (%)] was calculated based on the following equation:

$$C(\%) = \left(1 - \frac{OA_f}{OA_i} \right) \times 100$$

where OA_f correspond to final concentration of oleic acid after the reaction and OA_i correspond to initial concentration of oleic acid previously the esterification reaction (50 mmol L⁻¹).

Statistical analysis

All experiments were carried out independently in triplicate. All measurements were taken in triplicate. Steady-state conditions were reached after 30 min before starting all the experiments. The accuracy limit was calculated as being twice the standard deviation of these measurements with a confidence level of 95%.

Results and Discussion

Enzyme production

The lipolytic activity observed in the fermented medium after growth of yeast in Erlenmeyer flasks under agitation reached values of 20 ± 2.4 U mL⁻¹. After verifying that the yeast was able to secrete enough quantity of lipases

in Erlenmeyer flasks, production on a simple bioreactor (14 L) were conducted in order to evaluate the ability of the biocatalyst production on a larger scale. The lipolytic activity obtained in these experiments were $18 \pm 1.4 \text{ U mL}^{-1}$, results quite similar to that obtained in shake flasks, demonstrating that the enzyme production on a large scale may indeed be feasible.

The price of the biocatalyst is one of the limiting factors for its use in the synthesis of esters, since the prices of chemical catalysts are much more affordable. To lower the cost of production of enzymes some procedures can be adopted, such as the use of waste and low cost substrates for its production (Salihu *et al.*, 2012). In this work, it was used low cost sources of nutrients such as glycerol, which is a residue of biodiesel production, the residuum from beer yeast and ammonium sulfate. Another substrate that has been modified in the cultivation medium was olive oil. This oil is commonly used as an inducer for the lipase secretion but, in this case, was replaced by soybean oil that, besides the lower cost, it has high availability in Brazil, which becomes relevant for a large scale production. For formulating the composition of medium used in this study, traditional raw materials such as glucose, yeast extract and olive oil (U.S. dollar 3 L^{-1} , costs calculated based on prices in Brazil - August 2013) were replaced by glycerol, brewer's yeast and soybean oil, resulting in a 48% reduction in the cost of materials.

The replacement of high cost components in the culture medium by substances of lower value can even result in increased productivity as the results published by Damasso *et al.* (2011), in which the substitution of olive oil by residues from oil processing corn to produce fungal lipases during SSF resulted in 30% increase in enzyme production.

Another aspect that should be evaluated is the production of the biocatalyst on a larger scale in order to supply a large demand. The production in bioreactors is still poorly explored, and this is a major step for further industrial processing (Sharma *et al.*, 2001). Microorganisms are very sensitive to the environment to which they are exposed, and simply increasing the volume of the same fermentation medium does not mean a successful large-scale production. Several factors must be appropriate to the system, in particular the ideal aeration and agitation. In this case, it was observed that the variation of the conditions for microorganism cultivation provided by using shake flasks or a simple bioreactor resulted in the achievement of similar lipolytic activity.

Bussamara *et al.* (2010) produced lipases in yeast bioreactor with the same operational volume used in this study (14 L), but with pH control and higher agitation provided by shovels and rotors. They obtained about 1.2 U mL^{-1} , approximately 7% of the production observed in this article.

On the other hand, the use of pH control in small bioreactors (3 L) by Iftikhar *et al.* (2010) resulted in an in-

crease of 124% compared to shake flasks. Therefore, more studies are needed to verify the dependence of control mechanisms, such as pH and agitation on the production of enzymes in bioreactors. The ability of this yeast to produce enzymes in simple bioreactor and without control, as well as the use of culture medium composed of low-cost materials suggests the potential of this endophytic yeast to be used for production of large-scale biocatalysts with competitive prices.

Lipase characterization

After partial purification of the enzyme through solid ammonium sulfate precipitation, the lipolytic activity of dialyzed material was evaluated under controlled conditions of different temperature, pH and ions to determine the best conditions for enzymatic catalysis. The optimum pH value was 6.5 (Figure 2) and the optimum temperature was $30 \text{ }^\circ\text{C}$ (Figure 3). These two optimum values coincide with the optimal parameters for yeast growth. Most microbial enzymes have characteristics such as optimum pH and temperatures of their micro-secreting organisms. Yeast have a range of optimum growth at pH slightly acidic and temperatures between $28\text{--}30 \text{ }^\circ\text{C}$, providing these features to their apparatuses metabolic (Contesini *et al.*, 2010; Salihu *et al.*, 2011; Sharma *et al.*, 2001). It can be observed that the enzyme loses 70% of its activity when incubated at $55 \text{ }^\circ\text{C}$. However, the low thermal stability can be overcome by enzyme immobilization. Cui *et al.* (2013) enhanced thermal stability for the immobilization preparations compared with that for free preparations when working with *Yarrowia lipolytica* lipase Lip2 by immobilization on polyethyleneimine-coated polyurethane foam. Forsyth and Patwardhan (2012) also reported increased thermal stability of *Candida antarctica* lipase B after immobilization on bioinspired silica.

The lipolytic activity of the partially purified enzyme was assayed in the presence of different concentrations of mono, di and trivalent ions and the results are shown in Table 1. Experiments conducted in the presence of monovalent ions (Na^+ and K^+) showed that $5 \text{ mmol L}^{-1} \text{ Na}^+$ caused an increase in enzyme activity (49%) and K^+ caused no change in lipolytic activity measured. Effects of monovalent ions have also been investigated previously in lipase produced by *Pseudozyma hubeiensis* (Bussamara *et al.*, 2010), who reported that $20 \text{ mmol L}^{-1} \text{ K}^+$ resulted in increased activity. As seen in previously published work (Ghori *et al.*, 2011; Kamini *et al.*, 2000), most microbial lipases are not metal-dependent and thus suffer little change in its activity in the presence of divalent ions at low concentrations. This same phenomenon has been observed in this experiment to determine the lipolytic activity under the presence of Zn^{2+} , Mg^{2+} , Mn^{2+} or Ca^{2+} , which the ion concentrations of 1 and 5 mmol L^{-1} barely have affected the observed results. Experiments performed in the presence of $5 \text{ mmol L}^{-1} \text{ Fe}^{3+}$ showed an inhibitory effect of approximately 44% in lipolytic activ-

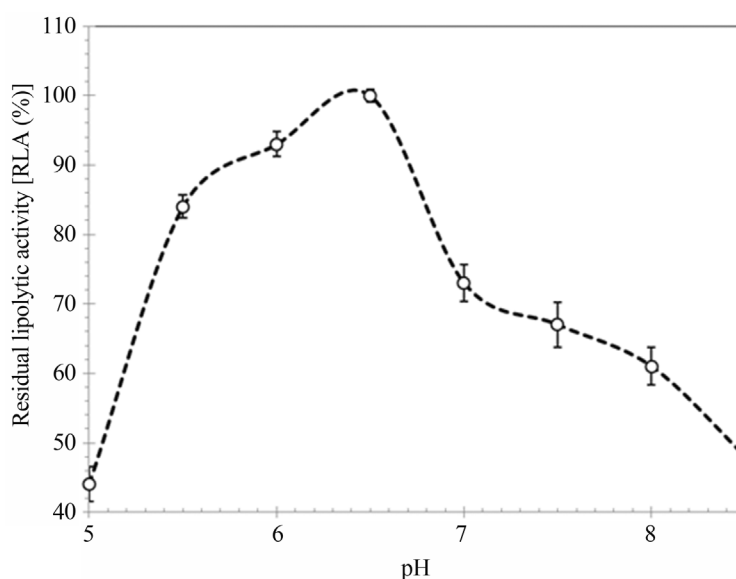


Figure 2 - Determination of optimal pH for extracellular lipase obtained from submerged fermentation of endophytic yeast *Candida guilliermondii*. 100% Residual lipolytic activity correspond to 18 U mL^{-1} determined in optimal conditions (pH 6.5 and 30°C). The results expressed correspond to average of three independent experiments conducted and analyses made in triplicate. The length of each error bar is twice the standard deviation of the activity measured.

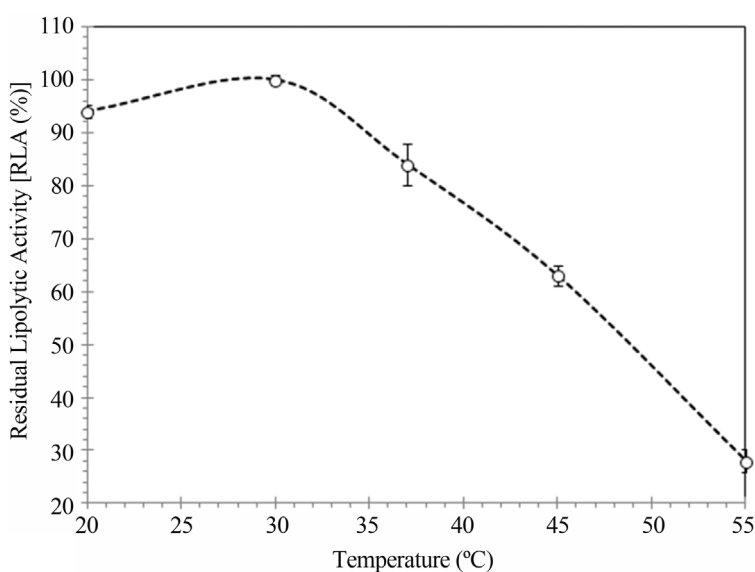


Figure 3 - Determination of the optimum temperature for extracellular lipase obtained from submerged fermentation of endophytic yeast *Candida guilliermondii*. 100% Residual lipolytic activity correspond to 18 U mL^{-1} determined in optimal conditions (pH 6.5 and 30°C). The results expressed correspond to average of three independent experiments conducted and analyses made in triplicate. The length of each error bar is twice the standard deviation of the activity measured.

ity. Furthermore, the addition of EDTA (1 and 5 mmol L^{-1}) did not alter the results.

Syntheses of (m)ethyl oleate via esterification of oleic acid

After the characterization of the lipolytic activity under different conditions, the partially purified enzyme obtained from *Candida guilliermondii* was evaluated for its potential as a catalyst for synthesis of biodiesel. In this studies, oleic

acid was used as substrate for the synthesis of (m)ethyl oleate evaluating the effect of different non-polar solvents and different molar ratios of acid: alcohol.

Effect of non-polar solvent

In enzymatic reactions catalyzed by lipases, the presence of water promotes the hydrolysis reaction. In this sense, nonpolar organic solvents are commonly used in biocatalysis using lipases because the reaction equilibrium

Table 1 - Effect of ions on lipase activity of *Candida guilliermondii*.

Ions	Residual Lipolytic Activity [RLA (%)]	
	1 mmol.L ⁻¹	5 mmol.L ⁻¹
Control*	100 ± 1.9	100 ± 1.9
KCl	101.3 ± 2.3	99.3 ± 4.7
NaCl	105.6 ± 4.7	148.9 ± 8.4
ZnCl ₂	101.2 ± 10.4	96.3 ± 4.4
FeCl ₃	91.1 ± 5.5	66.2 ± 6.9
MgCl ₂	100 ± 1.5	100 ± 10.2
CaCl ₂	97.1 ± 7.9	92.1 ± 1.6
MnCl ₂	100 ± 2.5	105.8 ± 3.4
EDTA	100.5 ± 1.8	97.8 ± 3.5

* $LA_{control}$ = Lipase activity determined in optimal conditions (pH 6.5 and 30 °C) without addition of ions or EDTA correspondent to 18 U mL⁻¹. The results expressed correspond to average of three independent experiments conducted and analyses made in triplicate. The error corresponds to twice the standard deviation of the measured data.

moving towards the synthetic route (Gog *et al.*, 2012, Faber, 2011, Fernandes *et al.*, 2007).

The action of organic solvents in enzyme catalysis occurs primarily at three levels: a) by direct interaction with the enzyme, changing its native conformation by rupture of hydrogen bonds and hydrophobic interactions, b) by interaction with substrates and products reaction, affecting their solubility and diffusion or directly participating in the reaction, c) by interaction with the water layer hydration of the enzyme and its removal (Hernández-Rodríguez *et al.*, 2009). The most important factor for the balance between stabilization and inactivation of enzymes due to the presence of the organic phase consists of solvent polarity. A reliable measure of polarity is the logarithm of the partition

coefficient (log P) of a compound in the two phases of a mixture of hydrophobic liquid (n-octanol) and a hydrophilic liquid (water). The higher the value of log P, the more hydrophobic is the solvent (Laane *et al.*, 1987). Generally, the enzyme activity is relatively low in hydrophilic solvents (log P < 2) because the solvents can denature the enzymes by penetrating into the hydrophobic core of the protein modifying the natural structure. This effect is moderate in solvents with log P between 2 and 4 and it is high in non-polar solvents, where log P > 4 (Faber, 2011).

Thus, the ester conversion rate for the synthesis reaction of (m)ethyl oleate were assessed the effects of two non-polar solvents [hexane (log P = 3.5) and cyclohexane (log P = 3.2)] and a slightly water-miscible solvent [cyclohexanone (log P = 0.81)] and the results of these analyzes are shown in Figure 4.

For systems consisting of hexane and cyclohexane, methyl ester conversions were similar: 68 and 64%, respectively. When cyclohexanone was used, the conversion to esters of oleic acid was reduced to less than 30%. Such a solvent, though having the same carbon number of the other solvents tested, has a different characteristic conferred by grouping ketone, a partial solubility in water. Enzymes are polar molecules, so they require a protective layer of water to maintain their three-dimensional conformation. Solvents with a certain polarity remove these water molecules, essential for enzyme function, thereby modifying its three-dimensional structure and, causing denaturation of the biocatalyst (Hernández-Rodríguez *et al.*, 2009; Pandey *et al.*, 1999). Aragão *et al.* (2009) performed the synthesis of isoamyl butyrate ester using lipase and found that in systems containing acetone as solvent the reaction did not occur. In this case, this high polarity solvent removed

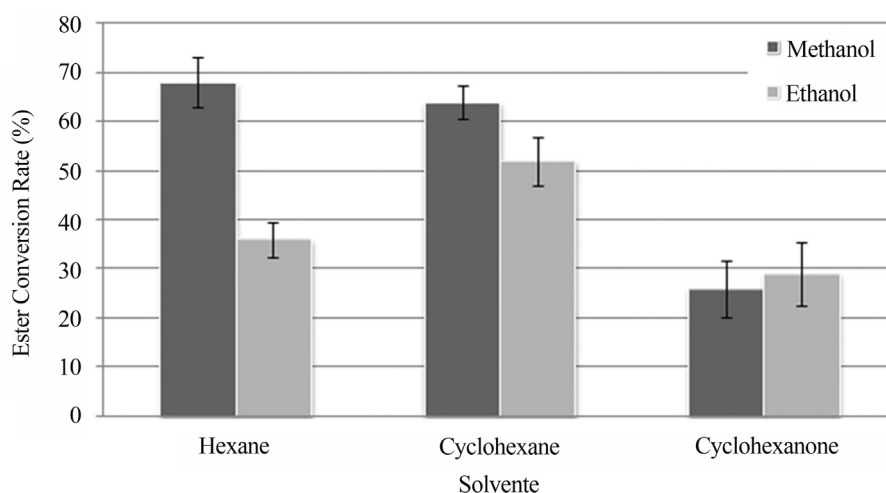


Figure 4 - Effect of solvents in ester conversion rate [C (%)] to reactions catalyzed by lipase isolated from *Candida guilliermondii*. The experiments were carried out in the presence of different solvents (hexane, cyclohexane and cyclohexanone) using oleic acid and methanol or ethanol as substrates with 1:9 molar ratio. The results expressed correspond to average of three independent experiments conducted and analyses made in triplicate. The length of each error bar is twice the standard deviation of the activity measured.

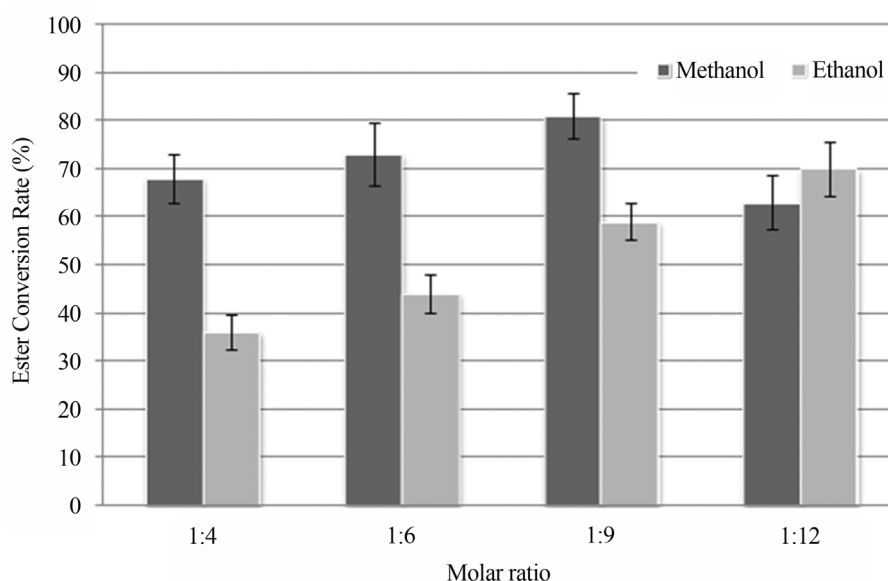


Figure 5 - Effect of acid:alcohol molar ration in ester conversion rate [C (%)] to reactions catalyzed by lipase isolated from *Candida guilliermondii*. The experiments were carried out in the presence hexane as solvent using oleic acid and methanol or ethanol as substrates, with 1:4, 1:6, 1:9 or 1:12 molar ratio. The results expressed correspond to average of three independent experiments conducted and analyses made in triplicate. The length of each error bar is twice the standard deviation of the activity measured.

water molecules responsible for hydration of enzyme in its native conformation, thereby causing its denaturation.

Effect of alcohol and different acid:alcohol molar ratios

To evaluate the effect of the proportion of oleic acid in relation to alcohol in the ester synthesis reactions, hexane was chosen as solvent due to its better performance after the evaluation of solvents (68% ester conversion rate). The use of methanol as substrate resulted in higher yields for ester conversion rates compared to ethanol when a molar ratio of 1:6 and 1:9 were performed. The highest yield of reaction (81% conversion rate) was obtained with a molar ratio of 1:9, as shown in Figure. 5. The conversion rate can be increased by 43% by enzyme immobilizing on activated silica, as previously published (Oliveira *et al.*, 2012). When tested for the esterification of fatty acids isolated from microalgae *Scenedesmus* sp. and under the same reaction conditions proposed for the esterification of oleic acid, *Candida guilliermondii* lipase showed conversion rate of 46% after 12 h.

The percentage of esterification using ethanol has been increased due to the increase of the molar ratio, showing that the reaction can achieve higher levels. However, the use of large amounts of ethanol would make the process expensive and ethanol is most difficult to be recovered from the reaction medium (Gog *et al.*, 2012; Hernández-Rodríguez *et al.*, 2009). Yet, the study of increase ester conversion rates using ethanol as in the enzymatic esterification reactions must be encouraged because of the renewability characteristics in using this alcohol detrimental to the use of methanol.

Methanol generally has superior performance to ethanol for the synthesis of esters by chemical means due to its greater reactivity, and because of this alcohol being easier to be recovered from the reaction medium, which leads to a cheaper process (Gog *et al.*, 2012; Guan *et al.*, 2010). The enzymatic synthesis using methanol varies for each enzyme, some of which are completely denatured in the presence of this alcohol due to its higher polarity (Tan *et al.*, 2010). On the other hand, the enzymes of this study show tolerance to this alcohol and, in this case, the most suitable for this reaction.

Conclusions

It was shown that partially purified enzyme containing lipolytic activity, produced by submerged fermentation in a lower cost cultivation medium by endophytic yeast *Candida guilliermondii*, can be used as a catalyst for the production of methyl oleate using methanol as substrate. The esterification of oleic acid with ethanol is also possible, although higher molar ration were required. Moreover, the outcomes contribute to the development of technologies to increase the scale of lipase production in an economically attractive way by using agroindustrial low-value products as culture medium and bioreactor without automation.

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Nomenclature and Abbreviation

C (%): ester conversion rate (%)

EDTA: ethylenediamine tetraacetic acid

LA_{assay} : lipolytic activity in the analysis ($U\ mL^{-1}$)

$LA_{control}$: lipolytic activity in optimal condition of pH and temperature ($U\ mL^{-1}$)

log P: logarithm of the partition coefficient

OA_f : final concentration of oleic acid ($mmol\ L^{-1}$)

OA_i : initial concentration of oleic acid ($mmol\ L^{-1}$)

pNP: p-nitrophenol

pNPP: p-nitrophenyl palmitate

PVA: poly(vinyl alcohol)]

RLA : residual lipolytic activity (%)

SSF: solid state fermentation

SmF: submerged fermentation

U: one unit of enzymatic activity (release of $1\ \mu mol$ of pNP per minute)

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