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Lipase production by microorganisms isolated from the Serra de Ouro Branco State Park

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Abstract: Lipases are hydrolases used in various sectors such as the food, pharmaceutical and chemical synthesis industries. In this study, epiphytic microorganisms were isolated from the Serra of Ouro Branco State Park (Minas Gerais, Brazil) and were subsequently evaluated for their ability to produce extracellular lipases. Among the 46 isolated strains, 25 presented positive results for lipase production in the agar plate screening assay. Two of these strains that expressed the highest diffusion halos, were genetically identified as *Serratia marcescens* and *Pseudomonas fluorescens* and catalogued in the Tropical Cultures Collection from the André Tosello Foundation/Brazil as CCT 7796 and CCT 7797, respectively. The fermentation growth kinetics indicated that the maximum extracellular lipase activities were achieved between 96 and 120h of cultivation. The highest lipolytic activity for both strains was observed at an optimum temperature and pH of 37°C and 7.0, respectively. At these conditions, the lipase activity detected in the crude enzymatic extract of both strains was close to 15.0 U/mL. We consider that these species are promising lipase producers for industrial applications.

Key words: enzyme characterization, lipolytic activity, microorganism screening, biodiversity.

INTRODUCTION

The enzymatic reactions are the basis of the metabolism of all living organisms and offer many opportunities for the industry to develop more economical and efficient biocatalytic conversions (Van Beilen & Li 2002). The enzyme technology is an interdisciplinary field recognized as an important tool for sustainable industrial development (OECD 2001). The global industrial enzymes market should reach $7.0 billion by 2023 from $5.5 billion in 2018 at a compound annual growth rate (CAGR) of 4.9% for the period 2018-2023 (BCC Research 2018). This growth is a result of an increasing demand of enzymes mainly in emerging markets (e.g.: India, China and Brazil) and an expanding bioenergy market (Novozymes 2013). Among industrial enzymes, lipolytic enzymes constitute one of the most important groups of enzymes, with particular interest for industries of detergent, modified oils and pharmaceutical sectors (Carvalho et al. 2003).

Lipases (triacylglycerolacylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. Those enzymes catalyze reactions such as hydrolysis or synthesis of triacylglycerols, transesterification, aminolysis, and lactonization, which are important to obtain high value-added products in different industrial segments, such as food processing, pharmaceutical, cosmetics and fine chemistry (Fickers et al. 2011). These enzymes do not

require co-factors and display wide specificity and high enantioselectivity to substrates (Griebeler et al. 2011). Lipases can be produced extracellularly by different microorganisms which present diverse properties of specificity, thermal and pH stability. From an economic and industrial point of view, microbial lipases are preferred over animal and plant sources (Geraldine et al. 2008), due to the high yields achieved, shorter production time, and the non-dependence of environmental conditions (Zimmer et al. 2009). Many microorganisms are known as extracellular lipase producers, including bacteria, filamentous fungi and yeasts (Treichel et al. 2010). The search for new lipase-producing microorganisms is an active field of research for future industrial applications, since new enzymes with improved stability and activity at harsh conditions, including the presence of solvents and large variations of pH and temperature, may be isolated (Rajendran et al. 2009). Therefore, it is considered that a careful selection of lipolytic microorganisms has a central role in the development of new industrial enzymes.

The Serra of Ouro Branco State Park is located in the southernmost boundary of the Serra do Espinhaço, a mountain range situated in the state of Minas Gerais, in Brazil. Its vegetation is primarily constituted by high-altitude savannas, also known as montane savannas. At lower altitudes, arborous vegetation and also meadows occupy riversides, and remaining areas of the Atlantic Forest, represented by the phytophysionomy of seasonal semi-deciduous forests. This environmental diversity conditions a rich and endemic flora (IEF 2009). The good conservation status of great part of the original biota, the richness of the endemic species and the incidence of three Brazilian biomes (Caatinga, Cerrado and Mata Atlântica) make of the Serra do Espinhaço one of the most important areas for the conservation of the Brazilian biodiversity (Leite et al. 2008). Because of the great biodiversity of that region and the increasing applicability of enzymes in biotechnology, it is reasonable to select and identify microorganisms that are able to produce lipases. Therefore, this stud aimed to isolate epiphytic microorganisms collected in the Serra of Ouro Branco State Park and select lipase-producing strains with potential industrial use.

MATERIALS AND METHODS

Microorganism collection and isolation

Ten samples (water, bryophytes, soil, termite mound material, leafs, tree stem and bromeliads) were collected from nine different locations at the Serra of Ouro Branco State Park (S20°28'51,7'' / W043°43'00,8''). Each sample was placed in a sterilized propylene beakers (1L), maintained refrigerated in an insulating box and transported to the laboratory. All samples were processed in a period no longer than 5 hours after having been collected in the park.

The microorganisms were isolated through imprinting technique in Yeast Malt Agar medium (YMA) ($g.L^{-1}$): malt extract, 3, yeast extract, 3, tryptone, 5, glycose, 10, agar, 17. Thus, the samples were placed in direct contact with the solid medium for 0h (the samples were removed just after the contact with the culture medium) or 24h. Each Petri dish was incubated at 28ºC until colony growth (Fuenteria & Valente 2004). Subsequently, each different colony was isolated in YMA solid medium and preserved at 4ºC. The phenotypic identification of the isolated microorganisms was done by the preparation of fresh microscopic slides using the Gram staining technique. For the filamentous fungi, micro-cultivation technique was employed.

Screening for lipase-producing microorganisms

The selection of lipase-producing strains was performed using two different approaches. The first consisted of cultivating the microorganisms in a solid medium containing ($g.L^{-1}$): tryptone, 10; NaCl, 5.0; $CaCl_2.2H_2O$, 0.1; Tween 80, 10; bacteriological agar, 20; pH 7.0 (Carissimi et al. 2007). The second, involved a medium constituted of ($g.L^{-1}$): tryptone, 5.0; meat extract, 3.0; NaCl, 5.0; soybean oil, 5.0 mL; bacteriological agar, 15; Rhodamine B, 1.0. After 96h of incubation at 28°C, the lipolytic activity was indicated in the first medium by the presence of an opaque disk around the colonies, and, in the second, by the presence of a fluorescent disk around the colonies, when exposed to ultraviolet radiation (230 to 280 nm) (Stopiglia et al. 2005). The enzymatic activity index (EI) was calculated as the ratio between the mean diameters of the substrates degradation halos and the average diameters of colonies (Hankin & Anagnostakis 1975). Those microorganisms showing the highest lipolytic activities (EI) in solid medium for both tests were selected growth in YMA liquid medium using Tween 80 and Rhodamine B as only carbon source, respectively.

Fermentation process and the kinetics of growth and enzyme production

Three loopfuls of each selected microorganisms were added to 250 mL Erlenmeyer flasks, each containing 100 mL of the culture medium consisted of ($g.L^{-1}$): $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 1.0; $FeSO_4.7H_2O$, 0.01; $(NH_4)_2SO_4$, 1.0; tryptone, 20.0; soybean oil, 10 $mL.L^{-1}$; initial pH of 7.0. After cultivation at 30°C and 150 rpm for 24h, the resulting culture was used as inoculum.

For the fermentation process, a 10 mL aliquot from the inoculum was added to 90 mL of fresh culture medium in a 250 mL Erlenmeyer. These flasks were incubated at 30°C and 150 rpm. The enzymatic kinetics and the cellular growth were monitored at the following time intervals: 0h, 4h, 8h, 12h, 24h, 48h, 72h, 96h and 120h. At each time interval, a 1 mL aliquot was withdrawn from the fermenting broth. The biomass was separated by centrifugation (13000 rpm/20 min) and the supernatant, without any prior treatment, was considered as the "crude enzymatic extract". In order to monitor the cellular growth, the centrifuged cells were resuspended in 1 mL of distilled water and its optical density was recorded at 600 nm using a spectrophotometer (SP-220/Biospectrum).

Determination of lipase activity

The lipase activity was determined by two approaches: the *p*-nitrophenyl palmitate (*p*NPP) method and the hydrolysis of oils.

For the *p*NPP method, solution C was first prepared by mixing one volumetric unit of solution A (90.0 mg of *p*NPP in 30.0 mL of isopropanol) and nine volumetric units of solution B (9.0 g of Triton-X-100 and 0.90 g of Arabic gum in 450 mL of a 0.09 $mol.L^{-1}$ Tris–HCl buffer at pH 8). The enzymatic reaction occurred by adding 270 µL of solution C and 30 µL of the crude enzymatic extract from each microorganism. After a 15-minute incubation period at 37°C, the *p*-nitrophenol released was monitored spectrophotometrically at 410 nm in a Sinergy/HT microplate reader (Biotek ®, United States) coupled to the software Gen5 and its concentration was determined based on standard curve. This was constructed by measuring the absorbance of a solution of *p*-nitrophenol (with serial dilution ranging from 1.0 µmol to 10.0 µmol) at 410 nm. Where One unit of lipase activity (recorded as U per mL of crude enzymatic extract), was defined as the amount of enzyme responsible for releasing 1.0 µmol of *p*-nitrophenol per minute.

The enzyme activity was also determined by the hydrolysis method. The reaction mixture for this experiment was composed of 4.0 mL of 20 mM phosphate buffer at pH 7.0, 5.0 mL of an oil emulsion (75 mL of gum arabic 7% and 25 mL of soybean oil) and 1 mL of crude enzymatic extract (fermentation time for removal of the crude enzymatic extract peak corresponded to the enzyme activity test defined *p*-NPP). The reaction took place at 30°C and 150 rpm agitation for 20 minutes and it was interrupted by the addition of 15 mL of an acetone/ethanol mixture (1:1 v/v). The released fatty acids were determined by titration with NaOH 0,051 M, using phenolphthalein as indicator (Baron 2008). One unity of lipase activity (recorded as U per mL of crude enzymatic extract) was defined as the amount of enzyme which released 1.0 µmol of fatty acid per minute.

Effect of temperature and pH on lipase activity

The effect of temperature on the lipolytic activity at pH 8.0 was verified by the *p*-NPP method at the following temperatures (°C): 25, 30, 37, 40, 45, 50, 55, 60, and 70. The optimum pH, at temperature of 37°C, was determined using the same method using different buffers to prepare solution B, which, in this case, were: sodium phosphate buffer: pH = 6.0, 6.5, 7.0, 7.5 and Tris-HCl buffer: pH = 8.0, 8.5, 9.0.

Identification of the selected microorganisms

Phylogenetic identification of the microorganisms that expressed the highest lipolytic enzyme activity was held at the Laboratory of Biotechnology and Biodiversity Environment, Federal University of Viçosa. The microorganisms were identified based on the analysis of the 16S rDNA gene and the fatty acid profile (Qasem et al. 2010).

RESULTS AND DISCUSSION

Phenotypic identification of the isolated microorganisms

Serra do Ouro Branco State Park is located on the southern border of the Serra do Espinhaço. Recognized as one of the regions with the highest floristic diversity in South America, Serra do Espinhaço has over than 30% endemic species, most of which are associated with rocky flourishing environment (Rapini et al. 2008). For this reason, and due to the rich biodiversity of the region, the bioprospection at the Serra of Ouro Branco State Park led to an assortment of 10 samples from different natural sources, from which 46 microorganisms were isolated, being 36 bacteria and 10 fungi. The bacterial microscopic analysis indicated that 75% of them were Gram-negative and the remaining 25% was Gram-positive. The isolated fungi were identified as *Aspergillus* sp., *Geotrichum*s sp., *Penicillium*s sp., *Fusarium* sp. The isolated fungi 34 (Table I), was not possible to be identified morphologically. As far as the authors' knowledge, this is the first screening of microorganisms from the Serra of Ouro Branco State Park.

Selection of microorganisms with lipolytic activities

From the 46 isolated microorganisms, 25 of them presented positive results for lipase production in, at least, one of the methods used to identify lipase production (mediums with Tween 80 or Rodamine B). On the other hand, 8 microorganisms presented halos of enzymatic degradation in the plates containing Tween 80 and Rodamine B, respectively (Table II). A microorganism is considered as a potential enzyme producer when it displays an enzymatic activity index ≥ 2.0 (Lealem & Gashe 1994). In this study, 15% of the selected microorganisms exhibited activity levels larger than 2.0 in Tween

Table I. Phenotypic characterization of the isolated microorganisms.

Microorganisms	Samples	Colony Color	Aspect	Cellular Morphology
1	Bromeliad 1	White	Shiny	Gram- / Spherical Shaped
2	Bromeliad 1	Yellow	Shiny	Gram+ / Rod-Shaped
3	Bromeliad 1	Orange	Shiny	Gram+ / Rod-Shaped
4	Bromeliad 1	White	Dim	*Aspergillus* sp.
5	Bromeliad 1	White	Dim	*Geotrichum* sp.
6	Bromeliad 1	White/Green	Dim	*Aspergillus* sp.
7	Bromeliad 2	Light Brown	Shiny	Gram– / Spherical-Shaped
8	Bromeliad 2	Light Beige	Shiny	Gram- / Spherical-Shaped
9	Bromeliad 2	White	Shiny	Gram- / Spherical-Shaped
10	Bromeliad 2	Orange	Shiny	Gram - / Spherical-Shaped
11	Bromeliad 2	Light Beige	Shiny	Gram - / Spherical-Shaped
12	Bromeliad 2	Orange	Shiny	Gram+ / Rod-Shaped
13	Bromeliad 2	Yellow	Shiny	Gram- / Spherical-Shaped
14	Bromeliad 2	Light Beige	Shiny	Gram- / Spherical-Shaped
15	Bromeliad 2	Beige	Shiny	Gram- / Spherical-Shaped
16	Bromeliad 2	Light Orange	Shiny	Gram- / Spherical-Shaped
17	Bromeliad 2	Beige	Shiny	Gram- / Rod-Shaped
18	Green Bryophite	Beige	Shiny	Gram- / Spherical-Shaped
19	Green Bryophite	White	Shiny	Gram- / Spherical-Shaped
20	Green Bryophite	White	Shiny	Gram- / Spherical-Shaped
21	Green Bryophite	Light Beige	Shiny	Gram- / Rod-Shaped
22	Leaf 1	Moss Green/ White Background	Dim	*Pencillium* sp.

Table I. Continuation

23	Leaf 1	Green	Dim	*Pencillium* sp.
24	Leaf 1	Orange	Shiny	Gram- / Rod-Shaped
25	Leaf 1	Dark Orange	Shiny	Gram+ / Spherical-Shaped
26	Leaf 1	Dark Orange	Shiny	Gram- / Spherical-Shaped
27	Tree Stem	White	Shiny	Gram- / Spherical-Shaped
28	Tree Stem	Dark Yellow	Shiny	Gram- / Rod-Shaped
29	Termite Mound Material	White	Dim	*Pencillium* sp.
30	Termite Mound Material	White	Shiny	Gram+ / Rod-Shaped
31	Termite Mound Material	White	Shiny	Gram- / Spherical-Shaped
32	Leaf 2	White	Dim	*Pencillium* sp.
33	Leaf 2	Yellow	Shiny	Gram+ / Spherical-Shaped
34	Leaf 2	White	Mesh	Non identified Filamentous Fungus
35	Leaf 2	White	Dim	*Pencillium* sp.
36	Water	Pink	Shiny	Gram- / Spherical-Shaped
37	Water	Purple	Shiny	Gram- / Rod-Shaped
38	Water	Yellow	Shiny	Gram- / Spherical-Shaped
39	Water	White	Shiny	Gram- / Rod-Shaped
40	Water	Beige	Shiny	Gram- / Spherical-Shaped
41	Black Bryophite	Orange	Shiny	Gram- / Spherical-Shaped
42	Black Bryophite	White	Shiny	Gram+ / Rod-Shaped
43	Black Bryophite	White	Shiny	Gram- / Rod-Shaped
44	Black Bryophite	Brown	Dim	*Fusarium* sp.
45	Soil	White	Shiny	Gram+ / Rod-Shaped
46	Soil	White	Shiny	Gram+ / Rod-Shaped

80, while none of them presented an enzymatic activity index ≥ 2.0 for the Rodamine B method (Table II). In an enzymatic screening study of 67 native microorganisms isolated in Central Amazon, the lipase activity detected in only 4.5% of the isolates, all of which showed lower enzyme index to 2.0 (Oliveira et al. 2006). In the work of Frantz et al. (2014), from the ninety-nine strains of filamentous fungi isolated from decaying leaves, 29.3% were lipase positive with an enzyme index ranging from 0.57 to 3.72. Meneses et al. (2016) evaluated the cellulolytic, amylolytic and lipolytic activity of 42 strains of rhizobia isolated from Vale do Curu Experimental Farm, semiarid region of Ceará, where 16.7% of the isolates expressed lipase activity with an enzymatic index in the range of 0.77 to 2.81.

A comparison between the data from Tables I and II indicates that the majority of the microorganisms that exhibited lipolytic activities in at least one of the solid media displayed Gram-negative spherical shape. The microorganisms (15 and 16) showing the highest halos (1.8 ± 0.15 and 1.94±0.31, respectively) in both substrates were conducted for further tests. These microorganisms were taxonomically identified by 16S rDNA gene sequencing as *Serratia marcescens* and *Pseudomonas fluorescens,* respectively. The strains identified were added to the Tropical Cultures Collection from the André Tosello Foundation, and catalogued as CCT 7796 to *Serratia marcescens* and CCT 7797 to *Pseudomonas fluorescens*.

Fermentation Kinetics

The time-course for bacterial growth and lipase production for the two microorganisms tested are shown in Figure 1. For both bacteria, in the initial time of cultivation, it was possible to detect lipase activity which has been added to the fermentation medium as a component of the inoculum. In the case of *S. marcescens* (Fig. 1a), stationary phase could not be reached in the first 120h of growth and lipase production increased up to 96h of bacterial growth. For *P. fluorescens* (Fig. 1a), stationary phase was reached after 48h of growth, while maximal lipase activity was observed after 120h. *Serratia marcescens* and *Pseudomonas fluorescens*, both isolated from a bromeliad sample, displayed maximum lipolytic activities of 3.85 ± 0.10 U/mL and 3.53 ± 0.24 U/mL after 96 hours and 120 hours of cultivation, respectively (Fig. 1a, b). Therefore, for *S. marcescens* (Fig. 1a), enzyme production was shown to be growth-related, with maximal productivity during exponential growth phase, whereas for *P. fluorescens* (Fig. 1b) enzymatic production was not influenced significantly by cellular growth, therefore in this case both behaviors can detected. Papon & Talon (1988) reported that most bacterial lipases are produced during the exponential growth phase and that growth conditions greatly influence enzyme production. On the other hand, Sooch & kauldhar (2013) observed that lipase production progressively increased during a slow log phase of growth of *Pseudomonas* sp. BWS-5 and reached its maximum level when the culture was found in its stationary phase of growth after 36 h of cultivation. The same authors highlighted that a maximum lipase activity from *Pseudomonas* sp. MS1057 has also been recorded in the stationary phase of growth (Kiran & Shanmughapriya 2008). These findings have been also reported in the work of Gupta et al. (2004).

The effect of temperature and pH on lipase activity

Enzyme activity is greatly influenced by many parameters, perhaps the most important being temperature and pH. The results obtained for the effect of temperature and pH on lipase production of the two selected microorganisms

Table II. Enzymatic levels of the lipases yielded by the isolated microorganisms.

Microorganisms	Enzymatic Index (EI)	
	Tween® 80	Rhodamine B
1	2.08 ± 0.10	-
2	1.28 ± 0.01	-
3	2.26 ± 0.14	-
4	-	-
5	1.38 ±0.04	1.31 ± 0.04
6	-	-
7	1.64 ± 0.07	1.63 ± 0.07
8	-	-
9	-	-
10	1.74 ± 0.07	1.45 ± 0.09
11	-	-
12	-	-
13	-	-
14	-	-
15	2.10 ± 0.11	1.80 ± 0.15
16	1.59 ± 0.19	1.94 ± 0.31
17	1.98 ± 0.17	-
18	2.63 ± 0.33	1.31 ± 0.40
19	2.96 ± 0.62	-
20	2.76 ± 0.35	-
21	2.27 ± 0.42	-
22	-	-
23	-	-
24	1.63 ± 0.04	-
25	-	-
26	-	-
27	1.94 ± 0.21	-
28	-	-
29	-	1.24 ± 0.01
30	1.36 ± 0.01	-
31	-	-
32	-	1.43 ± 0.01
33	-	-
34	-	-
35	-	-
36	1.52 ± 0.60	-
37	1.60 ± 0.09	0.78 ± 0.32
38	-	-
39	1.61 ± 0.27	-
40	-	-
41	1.89 ± 0.49	1.58 ± 0.06
42	1.37 ± 0.16	-
43	1.40 ± 0.01	-
44	-	-
45	-	-
46	1.42 ± 0.17	-

are shown in Figures 2 and 3. It was observed that the optimum temperature for the lipases from *S. marcescens* and *P. fluorescens* was 37°C and pH 7, which resulted in activities of 3.85±0.10 and 3.09±0.56 U/mL, respectively. Previous studies demonstrated that the optimum temperature for enzymatic activity of lipase heat-resistant *Pseudomonas* spp. MC 50 ranged from 35-40°C, similar results were found in the work of Adams & Brawley (1981). Abdou (2003) observed that *S. marcescens* isolated from raw milk was found to produce extracellular lipase. The studied enzyme showed higher stable at pH 8 and an optimum temperature was at 37°C. Whereas in the work of Prasad (2013), using *S. marcescens* isolated from industrial effluent, an optimal activity of 6.102 U/mL was obtained after 45 hour at 30°C at pH 7 in a media supplemented with olive oil, glucose and starch.

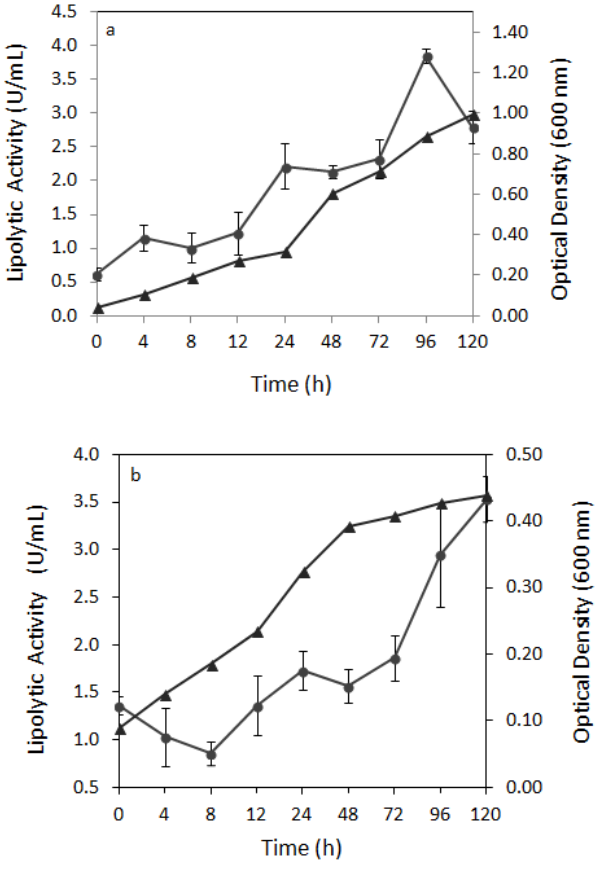

Figure 1. Lipolytic activity (▲) and bacterial growth (●) for the strains *S. marcescens* (a) and *P. fluorescens* (b). Incubated at 30°C and 150 rpm for the period of 120 hours.

Figure 3 shows the pH effect on lipase activities for the pH range of 6.0 to 9.0 and temperature of 25 to 70°C. The observed results revealed a typical bell shape curves with a maximum lipase activity at neutral pH values for the enzymes of both microorganisms. At an optimum pH 7, the lipase activities were 14.41±0.30 and 14.96±0.23 U/mL for *S. marcescens* and *P. fluorescens,* respectively.

Prasad (2013) obtained a lipolytic activity of *S. marcescens* of 8.595 U/mL at pH 7 after 45 hour incubation, using gingelly oil as substrate. On the other hand, Zaki & Saeed (2012), observed that maximum lipase activity of *Serratia marcescens* N3 appeared with gingelly oil (122 U/mL) followed by olive oil (112 U/mL), coconut (104 U/mL), sesame (93 U/mL), soybean (92 U/mL), and sunflower (82 U/mL), at pH 8 and 35°C for 48h of cultivation.

Sooch & kauldhar (2013) achieved a maximum enzyme production of 298 IU/mL with the use of simple medium at pH 6.5 after 30h of incubation at 37°C. On the other hand, Prasad (2014) observed a maximum lipase activity ranging from 6.5 to 8.2 U/mL expressed by the isolate *Pseudomonas aeruginosa* at pH 7 and 35°C after 45 hours of incubation. These results indicated that different species of *Serratia and Pseudomonas* exhibited different maximum lipase activity at specific pH and temperature.

Interesting behavior was shown by both microorganisms where a second peak was observed at a temperature of 55°C, indicating the presence of esterase enzyme (Figure 3). Ramnath et al. (2017) explained that lipases (belonging to lipolytic family I) from *Psedomonas* sp and gram-positive bacteria can present higher activities and stability at alkaline pH values and at temperatures ranging from 20 to 50°C, with maximum activity at 30°C over a 1h incubation

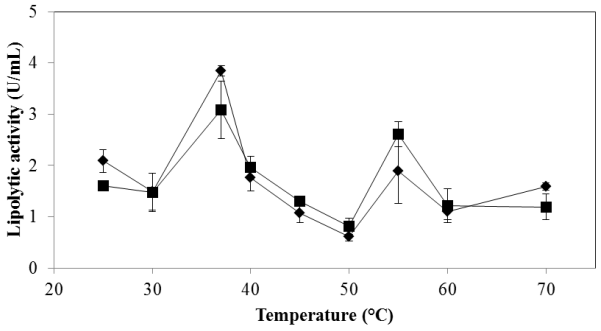

Figure 2. Optimum temperature for the enzymes of the strains *S. marcescens* (♦) and *P. fluorescens* (■), based on the hydrolysis of *p*-nitrophenyl palmitate (*p*NPP). The reactions were carried out at a pH of 8.0.

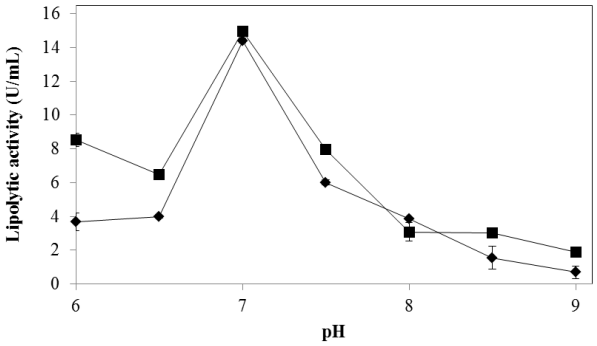

Figure 3. Optimum pH for the enzymes of the strains *S. marcescens* (♦) and *P. fluorescens* (■), based on the hydrolysis of *p*-nitrophenyl palmitate (*p*NPP). The reactions were carried out at a temperature of 37°C.

period (Glogauer et al. 2011). On the other hand, Esterases (Lipolytic family VI) with a low molecular mass of 23 to 26 kDa, such as *P. fluorescens* AK102 presented an optimum enzymatic activity at cultivation conditions of pH between 8 and 10 and temperature of 55°C (Kojima et al. 1994).

According to Chahinian & Sarda (2009) Carboxylesterases include two groups of enzymes, namely non-specific esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3). Esterases hydrolyse water-soluble short acyl chain esters and are inactive against water-insoluble long chain triacylglycerols which, in turn, are specifically hydrolyzed by lipases.

CONCLUSION

Many researchers have utilized olive oil, soybean oil, sunflower oil, palm oil and corn oil for lipase production by several microorganisms. Because of its high availability, low cost and potential applications at industrial scale in Brazil, soybean oil was chosen to perform the studies. After defining the fermentation kinetics, the optimum temperature and pH for the lipase enzymatic activity was studied. It was demonstrated that activities of the crude enzymatic extracts for the strains *S. marcescens* and *P. fluorescens* were of 2.94±0.14 U/mL and 3.47±0.16 U/mL, respectively.

The authors concluded that two microorganisms isolated at the Serra of Ouro Branco State Park (Minas Gerais, Brazil), and selected based on the highest lipolytic activity were identified as belonging to the species *S. marcescens* and *P. fluorescens*. These microorganisms produced a lipase with optimal activity at 37°C and pH 7.0. At these conditions, the enzymatic activity was in the range of 14 to 15 U/mL. According to the obtained results the species *S. marcescens* and *P. fluorescens* showed to be promising lipase producers for industrial applications. Future works will be focused on the purification of the crude lipolytic extract.

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