ISSN: 1415-7314
ISSN online: 2317-6717

ENGEVISTA
Página da revista: http://www.uff.br/engevista/seer/

Produção de lipase por *Cunninghamela echiculata* (UCP 1308) através de fermentação submersa utilizando diferentes meios de produção

Production of lipase by *Cunninghamella echinulata* UCP 1308 in through submerged fermentation in different culture media

Paloma Santa Cruz de Sales[1]

Tainã Crísia de Souza Fonseca[2]

Felipe André Pereira da Cunha Amaral[3]

Carlos Alberto Alves da Silva[4]

Resumo: As lipases são enzimas capazes de realizar reações de hidrólise em ácidos graxos, facilitando inúmeras reações hidrolíticas nas indústrias. Podem ser produzidas por animais, vegetais e micro-organismos. Várias maneiras de cultivo desta enzima têm sido desenvolvidas, modificando os tipos de micro-organismos produtores, tipos de substrato e processos de fermentação. Dentre os fungos capazes de produzir lipase, a *Cunninghamella echinulata* se destaca por se desenvolver e produzir a enzima em condições adversas. Frequentemente, uma das formas de aproveitamento de resíduos agroindustriais tem sido a produção dessa enzima que é de interesse industrial, seja para baixar os custos de produção, seja para dar utilidade a tais rejeitos. Neste trabalho foi estudada a produção de lipases a partir de cepas do fungo *Cunninghamella echinulata*, utilizando 4 meios diferentes para produção de lipase, usando as amostras, denominadas UCP 060, UCP 1299, UCP 1308 isoladas da Caatinga de Pernambuco. Os ensaios de produção ocorreram a 150 rpm, 28ºC, durante 168 h. Os resultados evidenciaram que o melhor meio testado foi o meio 4, com a amostra UCP 1308, com uma produção de 5,84 U/mL de lipase.
Palavras-chave: produção enzimática, meio de produção, fungo filamentoso; Caatinga.

[1]UNICAP – Universidade Católica de Pernambuco
[2]UNICAP – Universidade Católica de Pernambuco
[3]UNICAP – Universidade Católica de Pernambuco
[4]UNICAP – Universidade Católica de Pernambuco

ISSN: 1415-7314
ISSN online: 2317-6717

Abstract: Lipases are enzymes capable of performing the hydrolysis of fatty acids, which is very important in industries. They can be produced by animals, plants and microorganisms. Several ways of cultivating these enzymes have been developed by modifying the types of producing microorganisms, substrate types and fermentation processes. Among fungi capable of producing lipase, *Cunninghamella echinulata* stands out for developing and producing the enzyme under adverse conditions. Often, one of the ways of using agroindustrial waste has been the production of this enzyme that is of industrial interest, either to lower production costs or to give utility to such wastes. In this work the production of lipases from *Cunninghamella echinulata* strains was studied using four different media for lipase production (UCP 060, UCP 1299 and UCP 1308) isolates from the Caatinga of Pernambuco. The production assays occurred at 150 rpm, 28 °C, for 168 h. The results showed that the best medium tested was medium 4, with the UCP 1308 sample, with a production of 5.84 U/mL of lipase.
Keywords: enzyme production, media production, filamentous fungi; Caatinga.

ISSN: 1415-7314
ISSN online: 2317-6717

Introduction

Microbial enzymes are often more useful than enzymes derived from plants or animals because of the wide variety of catalytic activities available, high yields, ease of genetic manipulation, and rapid growth of microorganisms in cheap media. They are also more stable than their plant and animal enzymes and their production is more convenient and safer (Messias et al., 1999; Silva, Izabeli, Gusmão, 2014, Singh et al., 2016).

Lipolytic enzymes from microorganisms present a great value for biotechnological application, mainly due to the versatility of their properties and large-scale production facility, being one of the most used groups in the industrial sector (Miletic et al., 2012; Choudhury, Bhunia, 2015; Mehta, Bodh, Gupta, 2017). However, from a point of view of industrial application, lipases in the free form cannot be reused, and are not economically feasible in some processes due to the need for large amounts of enzyme (Houde, Kademi, Leblanc, 2004; Souza et al., 2015).

Lipases are a heterogeneous group of proteins found in numerous species of plants, microorganisms and animals and are described as triacylglycerol lipases. They act directly on esters of triglyceride carboxyl esters, mainly hydrolyzing the long chain (acyl chain with more than 10 carbon atoms), releasing fatty acids and glycerol (Messias et al., 1999; Bhutani et al., 2015).

Lipases are the most important group of biocatalysts for biotechnological applications (Benjamin, Pandey, 1998; Adrio, Demain, 2014). Lipases have been isolated from many species of plants, animals, bacteria, fungi and yeasts. Microbial enzymes are used in many industries such as dairy, food, detergent, textile, pharmaceutical, cosmetic and biodiesel industries, and in the synthesis of fine chemicals, agrochemicals and new polymeric materials (Jaeger, Eggert, 2002; Sandoval, Marty, 2007). Research on microbial lipases has increased due to its great commercial potential (Saxena, 1999, Hasan, Shah, Hameed, 2006; Nigam, 2013; Jaiswat, Preet, Tripti, 2017).

Cunninghamella is one of the most common fungi within the *Mucorales* order. Species of this genus are generally found in soil, exhibit rapidly growing colonies, ranging from white to gray, sporangiophores erect, branched, and the end of each branch forms pyriform or globose vesicles with various sporangioles. Mycelium not septate when young, becomes septate with the age of the culture (Alexopoulos, Mims, 1996., Lopes, Veiga, Moraes, 2015). The aim of this work was to select samples of *Cunninghamella echinulata* producing lipase in solid medium and the production of the enzyme by submerged fermentation in different means of production.

ISSN: 1415-7314
ISSN online: 2317-6717

Material and methods

Microorganisms and medium of maintenance

The microorganisms used in this study were three samples of *Cunninghamella echinulata*, isolated from the Caatinga of Pernambuco and named UCP 060, UCP 1299 and UCP 1308. They were previously cataloged at the Cultures Collection of the Catholic University of Pernambuco (UNICAP) - Nucleus of Research in Environmental Sciences and Biotechnology (NPCIAMB). The cultures were kept in medium Sabouraud Dextrose Agar (SDA), with the following composition: dextrose (40 g/L), peptone (10 g/L), agar (20 g/L), distilled water 1000 mL pH 5.5, and supplemented with olive oil 0.1%, during 96 hours at 28 °C.

Selection of lipase-producing samples on solid medium

To detect the presence of lipase enzyme in solid medium, the methodology described by Hankin and Anagnostakis (1975), using medium for detection of lipolytic activity (g/L): Glucose (40 g); Peptone (10 g); Agar (20 g); Tween 20 (10 mL); pH 5.5. The culture medium was dispensed into Petri dishes and, after solidification, a hole of 0.8 cm in diameter was made in the center of the plates. Spore suspensions were prepared with the three samples of *Cunninghamella echinulata* (UCP 060, UCP 1299 and UCP 1308) and were inoculated 100 µL of the suspension in the hole. Plates were incubated at different temperatures (28 °C, 37 °C and 45 °C) for 96 hours with daily monitoring. All experiments were performed in triplicate. The formation of characteristic halo around the colony growth showed the production of lipase.

Preparation of inoculum

The number of sporangioles / mL in suspension was counted in a Neubauer chamber. 25 mL of the 10^7 U/mL of spore suspension was inoculated in Sabouraud broth medium.

Production media

Four lipase producing media were used, with different compositions:

Medium 1 (g/L): Glucose (1.0), yeast extract (0.5), peptone (2.0), $NaNO_3$ (0.1), KH_2PO_4 (0.1), $MgSO_4.7H_2O$ (0.05), olive oil (1.0), pH 6.5;

Medium 2 (g/L): Olive oil (0.30), peptone (70), $NaNO_3$ (1.0), KH_2PO_4 (1.0), $MgSO_4.7H_2O$ (0.05), pH 7.0;

Medium 3 (g/L): Peptone (1.0), NaCl (0.5), $CaCl_2$ $2H_2O$ (0.1), Tween 20 (1.0), pH 6.0;

ISSN: 1415-7314
ISSN online: 2317-6717

Medium 4 (g/L): Glycose (0.1), $MgSO_4.7H_2O$ (0.2), K_2HPO_4 (0.7), yeast extract (0.4), olive oil (0.20), pH 6.5.

Lipase production was performed in an orbital shaker using 250 mL Erlenmeyers, with a working volume of 125 mL (w%:v), 150 rpm, 37 ºC for 144 hours, with daily monitoring. All assays were performed in triplicate.

Determination of microbial biomass

Biomass was determined after the completion of the assays of production. The mycelial mass was filtered on filter paper and the retained material was transferred to previously labeled and weighed flasks. Subsequently they were destined to the lyophilizer for later quantification of the biomass. The supernatant named enzyme extract was used for the determination of pH and enzymatic activity.

Determination of pH

All samples were submitted to the potentiometer for pH determination.

Enzyme Detection

The enzymatic activity was determined by the methodology described by Soares et al. (1999). It was prepared a reaction containing 5 mL of an emulsion (100 mL olive oil + 7% arabic gum), plus 2 mL of phosphate buffer (0.1 M), pH 8.0 and 1 mL of the fermented sample. The mixture was placed under stirring of 82 rpm at 37°C for 10 minutes.

The reaction was stopped by adding 10 mL of an acetone-ethanol-water mixture (1:1:1), where the free fatty acids present in the mixture were released. The mixture was titrated with a solution of KOH (0.4M) in the presence of the phenolphthalein indicator.

Enzyme activity was determined by the following relationship: one unit of lipolytic activity (U/mL) will be defined as the amount of crude enzyme that released 1 µg/mL of fatty acid per minute. The results obtained were calculated using the following equation:

$$AE\ (U/mL) = \frac{(Va - Vb) \times N \times 1000}{t \times Vc}$$

Where:

AE is the lipolytic activity (U/mL);
Va is the volume of sample titrated (mL);
Vb is the volume of the sample used in the reaction blank (mL);
N is the molarity of the KOH solution (N);
t is the reaction time in minutes;

ISSN: 1415-7314
ISSN online: 2317-6717

Vc is the volume of the sample used in the reaction (mL).

Results and discusion

Enzymatic screening of *Cunninghamella echinulata* producing-lipase in solid medium is demonstrated in table 1 shows the results obtained in the assays for the detection of enzymatic activity in solid medium. It is verified that at 45 °C were not detected the presence of the characteristic halos of the enzyme tested.

Samples	pH	Temperature (ºC) 28	37	45
UCP 1299	5	1,5 cm	2,0 cm	-
	6	3,0 cm	3,5 cm	-
	7	2,0cm	3,0 cm	-
UCP 1308	5	4,5cm	2,0 cm	-
	6	4,0 cm	2,2 cm	-
	7	3,5 cm	3,0 cm	-
UCP 060	5	1,5 cm	1,6 cm	-
	6	1,5 cm	2,0 cm	-
	7	1,5 cm	1,2 cm	-

Table 1- Assay for the detection of lipolytic activity in solid medium using different samples of *Cunninghamella echinulata*.

(-) The presence of the enzyme tested was not detected

For the temperature of 37 °C and pH 5.0, 6.0 and 7.0 it was detected the halo formation between 1.2 and 3.0 cm in the three samples tested. The best result was obtained by the sample denominated UCP 1308 with the temperature of 28 °C and pH 5.0, that presented the largest halo (4.5 cm).

Papagianni (2014), AbdeL-AAL et al. (2016) performed experiments involving the production of lipase using alternative residues and proved that the ideal pH range for enzyme production is 4.0-7.0.

The production of lipases by microorganisms can be directly influenced by different factors, such as carbon source, dissolved oxygen concentration, temperature and pH of the medium, and aeration conditions (Cammarota, Freire, 2006; Lima, 2014).

According to Orlandelli et al. (2012), Momsia (2015) the success in obtaining a fungal product requires a detailed knowledge of the growth characteristics and physiology of the

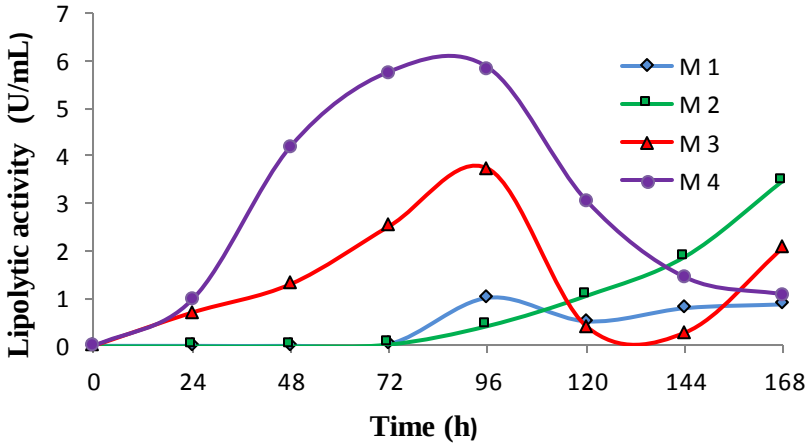
ISSN: 1415-7314
ISSN online: 2317-6717

producing strain, being each fungus unique in its anatomical, morphological and physiological development.

After the identification of the sample that presented the highest enzymatic production in solid medium, lipase production assays were performed in liquid medium, using four different media by submerged fermentation. Samples were collected every 24 hours for 168 h for determination of growth curve, biomass, pH and lipolytic activity.

Bento, et al. (2014), Rodrigues, et al. (2015) described the presence of mineral elements (phosphorus, sulfur, potassium, calcium, magnesium, sodium, iron and chlorine) and a small number of elements that perform important role as constituents of enzymes and coenzymes. They are generally required in the media of enzymes production by microorganisms.

Figure 1 shows the growth curve of the UCP 1308 sample. It is verified that the highest growth obtained was detected in the medium 4.

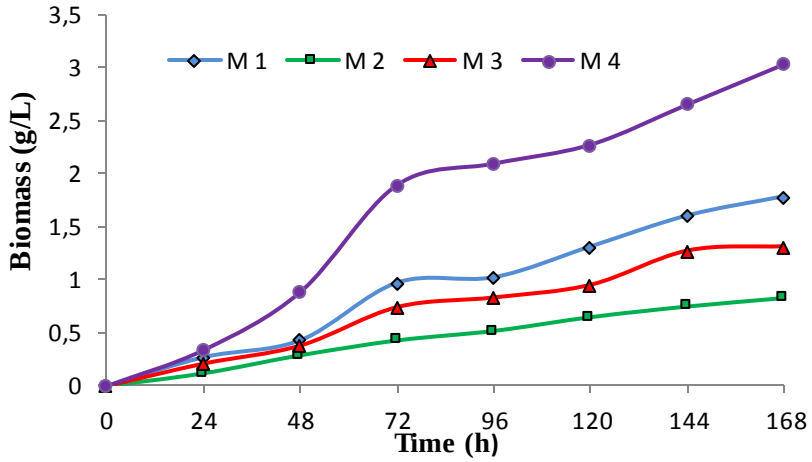


*M=Medium 1; Medium 2; Medium 3; Medium 4.

Figure 1– Lipolytic activity of the UCP 1308 sample in different production media.

In figure 2 are described values of biomass (g/L) obtained in four different media of lipase production, during 168 hours. It is found that media 1 and 4 produced a larger amount of biomass during the production processes. The sample produced higher amount of biomass in medium 4 with 168 hours of growth (3.04 g/L).

ISSN: 1415-7314
ISSN online: 2317-6717



*M=Medium 1; Medium 2; Medium 3; Medium 4

Figure 2- Biomass growth of the sample UCP 1308 in different media.

The pH values obtained during the four lipase production assays with the UCP 1308 sample are described in Table 2.

It is found that the initial pH values of the four media were maintained at 5.5, and after 72 hours there was a variation. At the end of the production process media 1 and 2 were in the alkaline pH range, medium 3 was near neutrality, and medium 4 remained in the acidic range.

Table 2 - Determination of the pH values in the tested media of the sample UCP 1308.

Medium	pH	0h	24h	48h	72h	96h	120h	144h
1	5.5	-*	-	5.3	5.4	6.6	7.5	8.1
2	5.5	-	-	7.0	6.6	8.4	8.6	8.4
3	5.5	-	-	6.5	8.3	6.4	6.7	6.6
4	5.5	-	-	6.3	6.1	5.7	5.2	4.4

*(-) There was no production of lipase

The table 3 shows the values obtained in the determination of the activity of the lipase produced in different media using the sample UCP 1308. The highest activity values were obtained in medium 4 with 72 h (5.76 U/mL) and 96 h (5.84 U/mL).

ISSN: 1415-7314
ISSN online: 2317-6717

Table 3 - Determination of the enzymatic activity of lipase in different media of the sample UCP 1308.

	Lipolytic activity (U/ml)							
Medium	0h	24h	48h	72h	96h	120h	144h	168h
1	-*	-	-	0,04	1,02	0,52	0,80	0,88
2	-	-	-	0,04	0,02	1,06	1,88	3,48
3	-	-	-	2,52	3,72	0,04	0,08	2,08
4	-	-	-	5,76	5,84	3,04	1,44	1,08

* (-) There were no lipolytic activity

Momsia (2013) described that in their studies the highest values of enzyme levels were 2.684 U/mL and 1.386 U/mL and were observed after 144 hours of culture from which a decrease in lipolytic activity was observed. In turn, it presented the lowest enzymatic activities, being the activity 0.866 U/mL) observed in the time of 72h.

Martin et al., 2007., Lee, (1999) conducted experiments related to lipase production, and found that the highest production rates were in when the fermentation had pH values in the range of 5.25 to 9.25. Lotrakul, Dharmsthiti, 1997, Briones, Serrano, Labidi, (2012) obtained in their works that the maximum enzymatic activity is reached when the optimum pH value is in the neutral range, with values between 6.5 and 7.0.

Lipase activity is commonly measured by monitoring the release of fatty acids or glycerol from triacyl glycerol. The use of solid media with inducing substrates such as vegetable oils, standard triglycerides (tributyrin, triolein), Tween 80 and colorants has been extensively described in the literature, aiming at the pre-selection of lipase-producing microorganisms (SandovaL, Marty, 2007., Rodrigues et al., 2015).

In view of the results achieved, it is highlighted the ability of the sample *Cunninghamella echinulata* UCP 1308 to hydrolyze fatty acids present in the composition of the studied media, by breaking the three-ester bonds and transforming them into the enzyme studied.

Conclusions

Cunninghamella echinulata demonstrated to be a microorganism suitable for the lipase production with a satisfactory result. The studies showed that the fungus establishes promising conditions for industrial production, considering the easy handling, easy cultivation and the short time of synthesis of the medium, promoting a decrease in production costs.

ISSN: 1415-7314
ISSN online: 2317-6717

Acknowledgements

The authors are grateful to the Coordination for the Improvement of Higher Level Education Personnel (CAPES) and the Nucleus of Research in Environmental Sciences and Biotechnology (NPCIAMB) of Catholic University of Pernambuco (UNICAP) for the infrastructure for implementing all the experimental part.

References

ABDEL,A.A. H., ISMAIL, M. A., HUSSEIN, N. A., & GOUDA, H. A. (2016). Enzyme producing capabilities of some extremophilic fungal strains isolated from different habitats of Wadi El-Natrun, Egypt. Part 1: Protease, lipase and phosphatase. *European Journal of Biological Research*, 6(2), 92-102.

ADRIO, J. L.; DEMAIN, A.L. Microbial Enzymes: Tools for Biotechnological Processes.(2014). *Biomolecules*, 4(1), 117-139.

ALEXOPOULOS, C.J.; MIMS, C.W.; BLACKWELL, M. (1996). Introductory Mycology. John Wiley, Sons, Pub., 233.

BENJAMIN S., PANDEY A. *Candida rugosa* lipases. (1998). *Molecular biology and versatility in biotechnology*, 14, 1069-1087.

BENTO, C. A. B. P., da SILVA, J. S., RODRIGUES, M. T., KASUYA, M. C. M., MANTOVANI, H. A. C. (2014). Influence of white-rot fungi on chemical composition and in vitro digestibility of lignocellulosic agro-industrial residues. *African Journal of Microbiology Research*, 8(28), 2724-2732.

BHUTANI K, K., LUNAGARIYA, N. A., PATEL,N. K., JAGTAP,S. C. (2014). Inhibitors of pancreatic lipase: state of the art clinical perspectives. *Excli Journal*, 13,897-921.

BRIONES, R., SERRANO, L., LABIDI, J. (2012).Valorization of some lignocellulosic agro-industrial residues to obtain biopolyols. *Journal of Chemical Technology and Biotechnology*, 87(2), 244-249.

CAMMAROTA, M. C., FREIRE, D. M. G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil grease content. *Bioresource Technology*, 97, 2195-2210.

CARLILE, M. J., WATKINSON, S. C. (1997).The fungi. London: Academic Press.
DOMSCH, K. H., GAMS, W., ANDERSON, T.H.(1980).Compendium of soil fungi. *Academic Press*, 1, 120.

CHOUDHURY, P.; BHUNIA, B. (2015). Industrial Application of Lipase: A Review. *Biopharm. Journal.* 1, 2: 41-47.

GOPINATH, S.C.B., HILDA, A., LAKSHMI PRIYA,T., ANNADURAI, G.(2002). Purification of lipase from *Cunninghamella verticillata* and optimization of enzyme activity using response surface methodology. *World Journal of Microbiology*,18, 449-458.

HASAN, F., SHAH, A. A., HAMEED, A. (2006). Industrial applications of microbial lipases. *Enzyme and Microbial Technology*, 39(2), 235-251.

ISSN: 1415-7314
ISSN online: 2317-6717

HOUDE, A., KADEMI, A., LEBLANC, D. (2004). Lipases and their industrial applications. *Applied Biochemistry and Biotechnology,* 118(1-3), 155-70.

JAEGER, K. E., EGGERT, T . (2002). Lipases for biotechnology, Curr. Opin. *Biotechnol*, 13, 390-397.

JAISWAT, A.; PREET, M.; TRIPTI ,B. Production and Optimization of Lipase Enzyme from Mesophiles and Thermophiles. (2017). *J. Microb. Biochem. Technol.*, 9:3, 126-131.

LEE K.M.(1999). Bistage control of pH for improving exopolysaccharide production from mycelia of Ganoderma lucidum in an air-lift fermentor. *Journal of Bioscience and Bioengineering***,** 88, 646-650.

LIMA, B. F., AMORIM, H. S., DO NASCIMENTO, A. E., DE CAMPOS TAKAKI, G. M., ALVES DA SILVA, C. A. (2014). Seleção de meios de produção de lipase por amostras de *Aspergillus* sp isoladas da caatinga de Pernambuco. *e-xacta*, *7*(1), 147-157.

LOPES, S. A.; VEIGA, I. G.; MORAES A. M. (2015). Desenvolvimento de dispositivo de quitosana e xantana para a liberação tópica ou em tecidos moles de indometacina. *Blucher Chemical Engineering Proceedings*, 1(2),13205-13212.

LOTRAKUL, M., S. DHARMSTHITI.(1997).Lipase production by *Aeromonas sobria* LP004 in a medium containing whey and soybean meal. *World Journal of Microbiology & Biotechnology*, 13, 163-166.

MARTIN, C., ALRIKSSON, B., SJÖDE, A., NILVEBRANT, N. O., & JÖNSSON, L. J. (2007). Dilute sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production. *Applied Biochemistry and Biotecnology*,137(1-12),339-352.

MARTINS, V. G., KALIL, S. J., COSTA, J. A. V. (2008). Lipases and biosurfactant production by solid state fermentation for utilization in bioremediation of vegetable oils and hydrocarbons. *Química Nova*, 31(8), 1942-1947.

MESSIAS, J. M., MIRANDA, O. A., SALGUEIRO, A. A., PIMENTEL, M. C. B., LIMA FILHO, J. L., MELO, E. H. M., & DURAN, N. (1999). Lipase production by a Brazilian strain of Penicillium citrinum using an industrial residue. *Bioresource Technology*, *69*(2), 145-147.

MEHTA, A.; BODH, U.; GUPTA, R. (2017). Fungal lipases: A review. *Journal of Biotech. Research*, 8: 58-77.

MILETIC, N., VUKOVI, Z., NASTASOVI, A., LOSS, K. (2012).Immobilization of biocatalysts for enzymatic polymerizations: Possibilities, advantages, applications. *Bioresource Technology*, 115, 126-135.

MIRANDA, O. A., SALGUEIRO, A. A., PIMENTEL, M. C. B., LIMA FILHO, J. L., MELO, E. H. M., & DURAN, N. (1999). Lipase production by a Brazilian strain of *Penicillium citrinum* using an industrial residue. *Bioresource Technology*, *69*(2), 145-147.

MOMSIA, T. (2013)A review on microbial lipase-versatile tool for industrial applications. *International journal of Life Sciences Biotechnology and Pharma Research Hyderabad*, 2(4).

NIGAM, P.S. Microbial Enzymes with Special Characteristics for Biotechnological Applications. (2013). *Biomolecules*, 3(3), 597-611.

ISSN: 1415-7314
ISSN online: 2317-6717

OLIVEIRA, G. R. B. (2015). Inibição da lipase pancreática no controle do metabolismo lipídico. *Visão Acadêmica,* 16(3), 47-52.

ORLANDELLI, R. C., SPECIAN, V., FELBER, A. C., & PAMPHILE, J. A. (2012). Enzimas de interesse industrial: produção por fungos e aplicações. *SaBios-Revista de Saúde e Biologia*, *7*(3),97-109.

PANESAR, R.; KAUR, S., PANESAR, P. S. (2015). Production of microbial pigments utilizing agro-industrial waste: a review. *Current Opinion in Food Science*, 1, 70-76.

PAPAGIANni, M. (2004). Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnology advances*, *22*(3), 189-259.

POORNA, C. A., PREMA, P. (2006). Production and partial characterization of endoxylanase by *Bacillus pumilus* using agro industrial residues. *Biochemical Engineering Journal***,** 32(2), 106-112.

RODRIGUES, C., CASSINI, S. T., ANTUNES, P. W., KELLER, R. P., & GONÇALVES, R. F. (2016). Isolation and selection of lipase-producing fungi based on lipase activity and hydrolytic potential on soybean oil and grease trap scum. *Engenharia Sanitaria e Ambiental*, 0-0.

SANDOVAL, G; MARTY, A. (2007). Screening methods for synthetic activity of lipases. *Enzyme and Microbial Technology*, 40(3), 390-393.

SAXENA, R. K; GHOSH, P. K; GUPTA, R; SHEBA DVIDSON, W; BRADOO, S; GULATI, R. (1999). Microbial lipases, potential biocatalysts for the future industry. *Curr. Sci.*,77,101-115.

SILVA, K.R.I., DURRANT L.R., MENEZES C.R. (2007). Produção de enzimas lignocelulolíticas por fungos basidiomicetos de degradação branca cultivados em bagaço de cana-de-açúcar através de fermentação semisólida. In: Workshop Internacional Brasil-Japão em biocombustível, meio-ambiente e novos produtos de biomassa, 4.

SILVA, S. S., SANTA IZABEL, T. D. S., & GUSMÃO, L. F. P. (2014). Fungos conidiais associados a substratos vegetais submersos em algumas áreas do bioma Caatinga. *Rodriguésia-Instituto de Pesquisas Jardim Botânico do Rio de Janeiro*, 65(2), 527-538.

SINGH, R.; KUMAR, M.; MITTAL, A.; MEHTA, P.K. (2016). Microbial enzymes: industrial progress in 21st century. *3 Biotech*, 6: 174, 1-15.

SOUZA, F. M., TRAVÁLIA, B. M., LIMA, Á. S., SOARES, C. M. F., & DE AQUINO SANTANA, L. C. L. (2015). Produção de lipase de *Aspergillus niger* e imobilização em membranas de poliétersulfona. *Blucher Biochemistry Proceedings*, 1(2), 267-270.