ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 2, pp: (52-61), Month: April - June 2017, Available at: www.researchpublish.com

Characterisation of a Yeast Lipase (*Candida parapsilosis*) from Vegetable Oil Polluted Soil

[1]Popoola, B.M, [2]Onilude, A.A

Department of Microbiology, University of Ibadan, Ibadan, Nigeria

Abstract: **Vegetable oil spills are becoming frequent and are potentially more challenging than petroleum hydrocarbon spills. Yeast lipases occupy a place of prominence among biocatalysts and have potential for rent remediation of vegetable oil spills. There is a need for extensive characterization of lipase for the treatment of vegetable oil-polluted sites. This work was carried out to preliminarily characterise the lipase of *Cadida parapsilosis*.**

Candida parapsilosis **was screened for lipase production using standard methods. Temperature, pH, ion concentration ($NaNO_3$ and $MgSO_4$), enzyme concentration, nitrogen concentration, substrate concentration, time course and agitation speed were optimised for the lipase activity as well as growth.**

Crude enzyme of *Candida parapsilosis* had the highest lipase activity and growth of 0.4 U/mL and 1.477 mg/mL respectively but when production was optimised higher activity 0.5 U/mL was seen in the use of glucose as substrate, use of ions like $(NH_4)_2SO_4$ and Nacl and at agitation speed of 120rpm. Different enzyme and substrate concentration which had optimum enzyme activities for *Candida parapsilosis* (0.5 U/mL), were 0.5 mL and 1.0 % respectively. Ion concentration ($NaNO_3$ and $MgSO_4$) had optimum growth (1.761mg/mL) at 0.05 %.

The enzyme has good potential for the hydrolysis of vegetable oils, which is an important factor in environmental cleanup of vegetable oil spill site.

Keywords: **Lipase production, vegetable oil spill, characterisation.**

1. INTRODUCTION

Lipases (triacylglycerol acylhydrolases) are a group of enzymes, which have the ability to hydrolyse triacylglycerols at an oil-water interface to release free fatty acids and glycerol. Lipases are present in microorganisms, plants and animals (Jisheng *et al*., 2005).

Lipases catalyse a wide range of reactions, including hydrolysis, inter-esterification, alcoholysis, acidolysis, esterification and aminolysis (Joseph *et al*., 2008). This hydrolytic reaction is reversible. In the presence of organic solvents, the enzymes are effective catalysts for various inter-esterification and trans-esterification reactions.

Further, microbial lipases show regiospecificity and chiral selectivity (Gupta *et al*., 2003). Especially microbial lipases have different enzymological properties and substrate specificities. Many species of bacteria, yeast and molds are found to produce lipases (Liu *et al*., 2008).

Their biotechnological potential is relying on their ability to catalyse not only the hydrolysis of a given triglyceride, but also its synthesis from glycerol and fatty acids. Therefore, microbial lipases have many industrial applications (Jaeger *et al*., 1999). The temperature stability of lipases is the most important characteristic for industrial use (Choo *et al.*, 1997). Lipase catalysed reactions are widely used in the manufacturing of fats and oils, detergents and degreasing formulations, food processing, the synthesis of fire chemical and pharmaceuticals, paper manufacture, and production of cosmetics.

Lipases are also used to acceleate the degradation of fatty wastes and polyurethane (Jisheng *et al*., 2005).

Among microbial lipases extensive reviews have been written on bacterial lipases (Jaeger *et al*. 1999; Arpigny and Jaeger., 1999). Yeats lipases have received a raw deal despite the fact that *Candida rugosa* is the most frequently used organism for lipase synthesis. Benjamin and Pandey (1998) have written a review exclusive on *Candida rugosa* lipase. The information on numerous other yeast lipases is scattered, hence the availability of this lipase with specific characteristics is still a limiting factor. Therefore, in this study, an attempt was made to assess the bio-potentials of the yeast enzyme *Candida parapsilosis* under laboratory conditions. By studying the effect of the environment on the growth and lipolytic enzyme production by the isolate and also characterising the lipolytic enzyme of the yeast isolate. The characteristics of this lipase were checked in order to understand enzyme functions better and enhance enzyme production by applying suitable substrate as well as process parameters optimisation.

2. MATERIALS AND METHODS

2.1 Growth studies and Production of the Enzymes:

2.1.1 Growth Media:

Isolates were grown in a complex basal medium whose composition was a modification of the medium of Tsujisaka *et al*. (1973) with glucose omitted. This medium contained 5% peptone, 0.1% $NaNO_3$ and 0.1% $MgSO_4$, adjusted to the desired pH, before sterilisation. Sterile olive oil (Goya) was added as carbon source.

2.1.2 Lipase Production:

2.1.2.1 Preparation of Inoculum:

A loopfull of the pure culture of yeast were grown for three days in potato dextrose broth.

2.1.2.2 Production Procedure:

One milliliter from the above preparation was inoculated into 65.0ml of sterile medium in 250ml Erlenmeyer flasks and incubated at room temperature ($27^oC \pm 2^oC$) from 24hrs to many days until the maximum lipase production was recorded. The medium was centrifuged at 30,000 g for 15min using Himac High-speed Refrigerated Centrifuge (Hitachi model CR21GII).The supernatant of the centrifuged culture broth was then decanted leaving the cells at the bottom. The cell-free extract acted as the crude lipase enzyme.

2.1.3 Growth of the Isolates:

Growth of the Isolates in the growth medium was examined spectrophotometrically using a Jenway 640 UV/VIS spectrophotometer at 540nm, absorbances were measured against blank (Gojkovic, 2009).

2.1.4 Lipase Assay:

Lipase activity was measured by a modification of the assay of Parry *et al*. (1966) using as substrate a 10% Olive oil-gum arabic solution emulsified by sonication for 2mins at 25watts output according to Linfield *et al*. (1985). One milliliter of cell-free fermentation broth prepared by centrifugation as describe above was added to 5ml of emulsion and incubated at room temperature for 1h with rapid stirring. Ethanol was added to stop the reaction and the free fatty acids produced were quantified by titration to pH 9.5 against 0.1N NaOH using a radiometer titration system. Blanks with 1ml of fermentation broth were employed in each experiment. Blanks ran with sterile or actual uninoculated broths were the same within experimental error. Samples were run in duplicate.

A unit of lipase activity was defined as the amount of sodium hydroxide (NaOH) used in the titration to bring the reaction mixture to a pH of 9.5 per min under the defined assay conditions. Alternatively, it is considered as the release of one micromole of free fatty acid (FFA)/min at room temperature.

2.2 Optimisation of Production Conditions:

2.2.1 Effect of pH on Lipase Production:

This was carried out using a modified method of Tsujisaka *et al*. (1973). Growth medium was prepared in 0.2M phosphate buffer and (0.1M citric acid mixed with 0.2M Na_2HPO_4) citrate phosphate buffer of varying pH (5.5, 6.0, 6.5,

7.0, 7.5, 8.0, 8.5). The lipase activity and the growth in the culture supernatant were determined using appropriate procedure.

2.2.2 Effect of Temperature on Lipase Production:

The effect of temperature on lipase activity was determined using the above method. The organism was cultivated in the growth medium at different temperatures, which ranged from 20^{o}C, 27^{o}C, 40^{o}C, 50^{o}C, 60^{o}C to 70^{o}C for 24hours. The lipase activity and growth in the culture supernatant were determined.

2.2.3 Effect of Aeration on Lipase Production:

After inoculating the organisms into the growth medium, the flasks were continuously shaken at 27^{o}C for 24hours at varied revolutions per minute (80, 100, 120 and 140) using orbital shaker Stuart SSLI. The lipase activity and growth in the culture supernatant were then determined.

2.2.4 Time Course of Lipase Production:

The organisms were cultivated in the growth medium for different periods that ranged from 24hours to 72hours. Samples were removed periodically and growth and lipase activity in the culture supernatant were determined.

2.2.5 Effect of Different Nitrogen Sources on Lipase Production:

The main nitrogen source in the growth medium was replaced by other nitrogen sources such as casein, urea and yeast extract at the same concentration (5 %). The lipase activity and growth in the culture supernatant were then determined.

2.2.6 Effect of Different Substrates on Lipase Production:

To determine the suitable substrate (carbon source) for the production of lipase by the organisms, substrates such as; glycerol, soy oil, olive oil and a simple sugar (glucose) were used. They were individually tested by replacing the substrate present in the growth medium at the concentration of 2%. Thereafter, the lipase activities as well as growth in the culture supernatant were determined.

2.2.7 Effect of Metal ion on Lipase Production:

The growth medium in which the organisms were cultivated had their metal ions varied. $ZnSO_4$, $FeSO_4$, $(NH_4)_2SO_4$, KNO_3, $AgNO_3$, $CaNO_3$, Na_2CO_3 and NaCl were used in equimolar concentration (0.1mM) instead of $MgSO_4$ and $NaNO_3$. The lipase activity and growth in the culture supernatant were then determined.

2.2.8 Furthermore, the effect of different concentrations of the substrate, nitrogen, anion and cation, crude enzyme and varied temperature on growth and lipolytic activity of *Candida parapsilosis* were also studied according to the assay method described above.

3. RESULT

Figure 3.1 shows the effect of pH on growth and lipase production of *Candida parapsilosis*, maximum growth and lipase production was recorded act pH 8.5 and pH 8.0 respectively. In the case of the effect on temperature as shown in figure 3.2, as temperature increase, growth and lipase production decrease. Agitation did not support lipase production in *Candida parapsilosis* as shown in figure 3.3. Growth increased for *Candida parapsilosis* with 1.8mg/ml but lipase production decreased to 0.0 U/mL at 72hours of incubation as shown in figure 3.4.

Figure 3.5 shows the effect of the different nitrogen sources on growth and lipase production by *Candida parapsilosis*, urea did not support growth (0.004 mg/mL) however yeast extract supported growth but not lipase production (0.0 U/mL). Figure 3.6 shows the effect of the different carbon sources on growth and lipase production of *Candida parapsilosis*, glucose enhanced lipase production (0.5 U/mL) whereas it was inhibited by glycerol. Figures 3.7 and 3.8 shows the effect of metal ions on growth and lipase production of *Candida parapsilosis*, in the case of effect of cations on growth and lipase production of *Candida parapsilosis* both growth and lipase activity was inhibited by $AgNO_3$, but $(NH_4)_2$ SO4 supported both, 1.757 mg/mL and 0.5 U/mL respectively, this is as shown in figure 3.8.

Temperature 27^{o}C had the maximum growth and lipase activity of *Candida parapsilosis* as shown in Table 3.1. Table 3.2, shows the effect of different concentration of substrate (Olive Oil) on growth and lipolytic activity of *Candida*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 2, pp: (52-61), Month: April - June 2017, Available at: www.researchpublish.com

parapsilosis. At 1.0 % lipase activity was highest while growth was at 1.5 %. Table 3.3 shows the effect of different concentration of anion and cation on growth and lipolytic activity of *Candida parapsilosis* with maximum growth at 0.05 %. Effect of different concentration of crude enzyme on growth and lipolytic activity of *Candida parapsilosis* as shown in Table 3.4, concentration in which growth and lipolytic activity was maximum is 0.5 ml.

Table 3.5, shows the effect of different concentration of nitrogen source (peptone) on growth and lipolytic activity of *Candida parapsilosis* maximum growth was at 3.0 g.

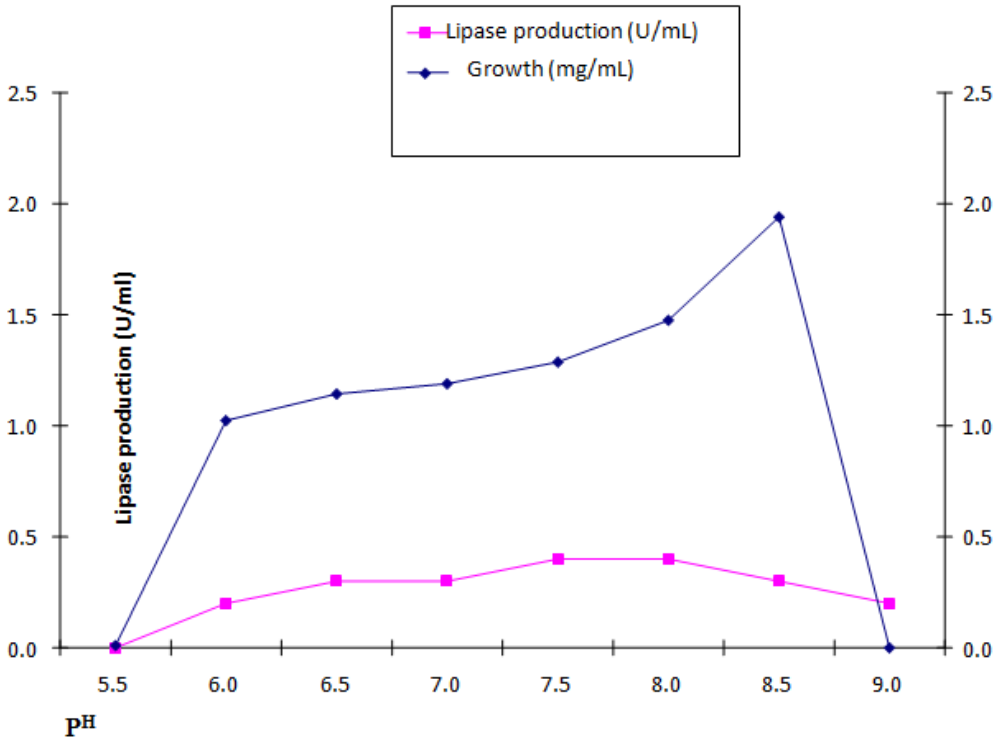

Fig 3.1: Effect of pH on growth and lipase production of *Candida parapsilosis*

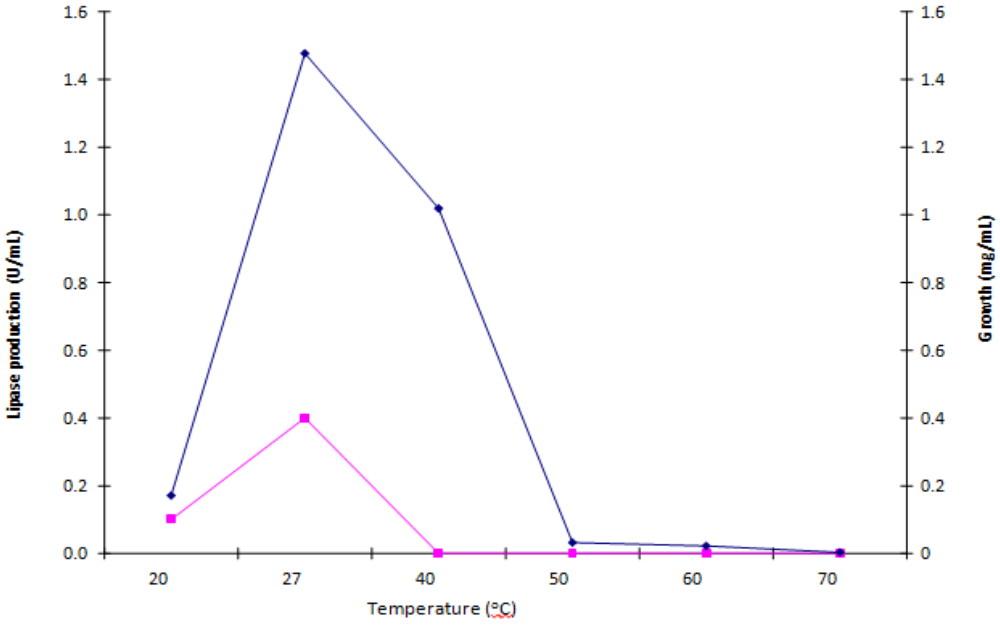

Fig 3.2: Effect of temperature on growth and lipase production by *Candida parapsilosis*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 2, pp: (52-61), Month: April - June 2017, Available at: www.researchpublish.com

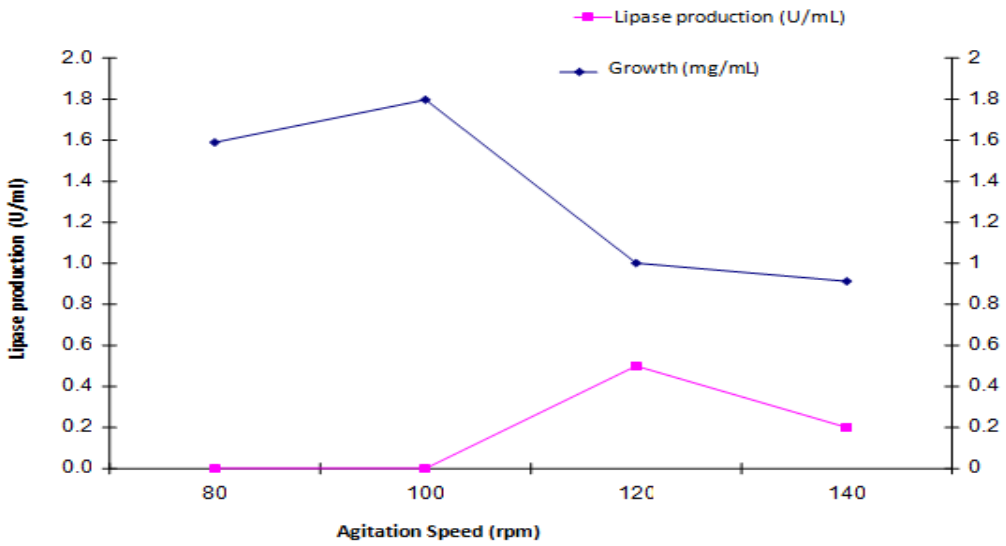

Fig 3.3: Effect of agitation on growth and lipase production by *Candida parapsilosis*

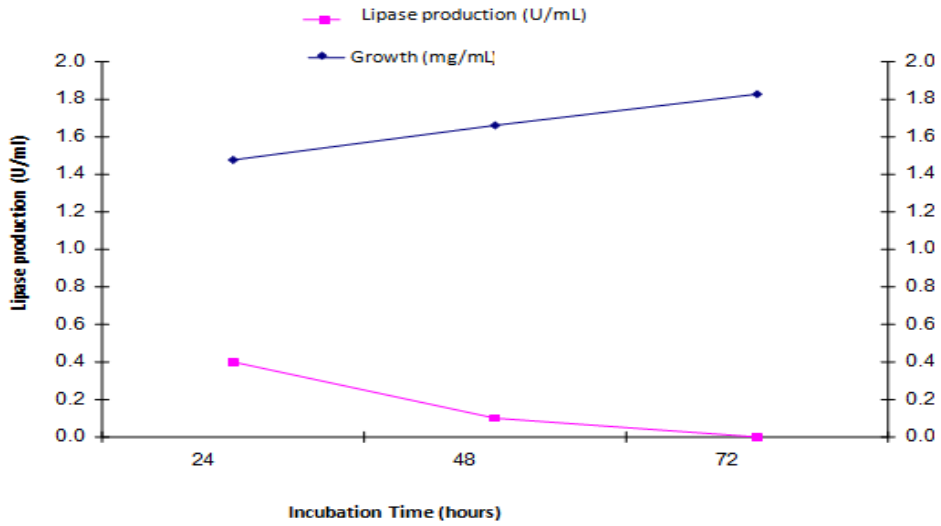

Fig 3.4: Effect of Incubation time on growth and lipase production by *Candida parapsilosis*

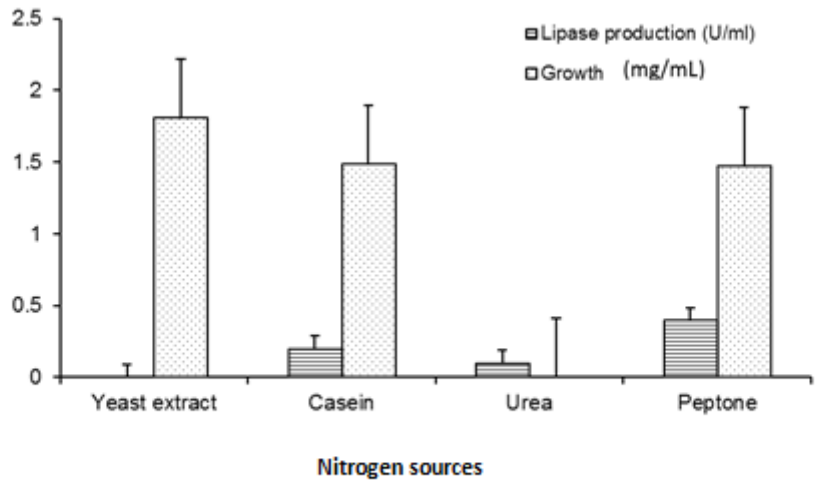

Fig 3.5: Effect of 5% different nitrogen sources on growth and lipase production by *Candida parapsilosis*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 2, pp: (52-61), Month: April - June 2017, Available at: www.researchpublish.com

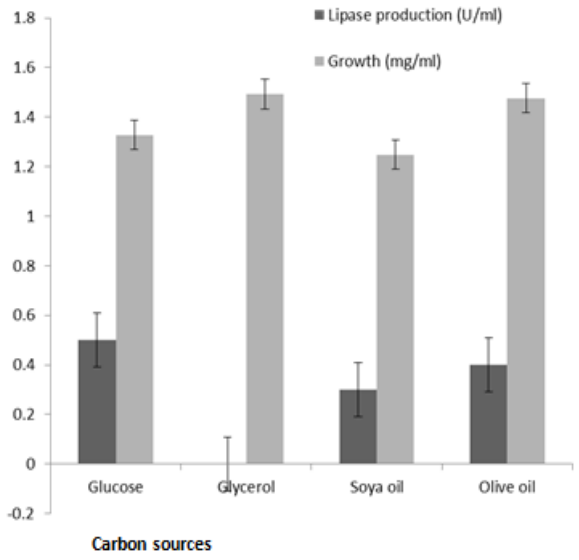

Fig 3.6: Effect of 2% different carbon sources on growth and lipase production by *Candida parapsilosis*

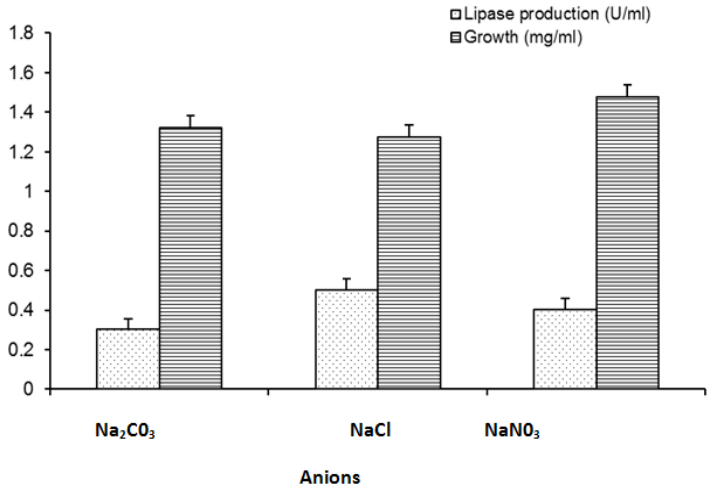

Fig 3.7: Effect of 0.1% different anions on growth and lipase production by *Candida parapsilosis*

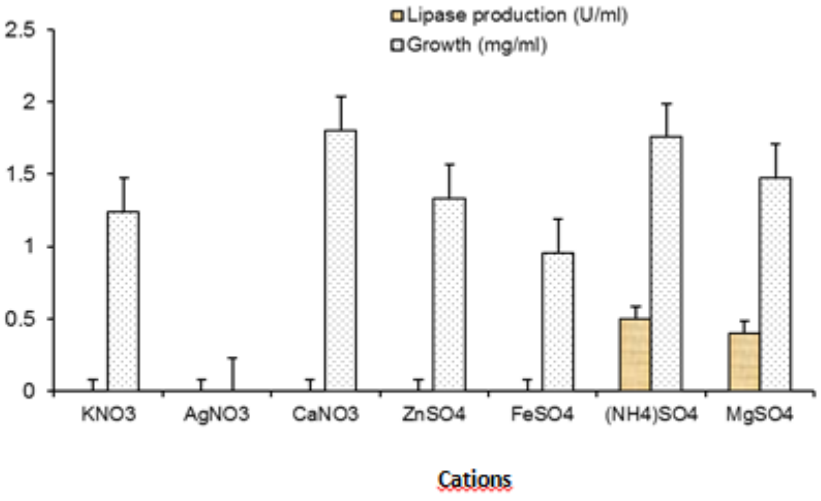

Fig 3.8: Effect of 0.1% different cations on growth and lipase production by *Candida parapsilosis*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 2, pp: (52-61), Month: April - June 2017, Available at: www.researchpublish.com

TABLE 3.1: EFFECT OF TEMPERATURE ON GROWTH AND LIPOLYTIC ACTIVITY OF *Candida parapsilosis*

PARAMETERS	Different Temperature Investigation						
	20°C	25°C	27°C	30°C	35°C	37°C	40°C
Growth (mg/mL)	0.171±0.0005	1.067±0.0002	1.477±0.00015	1.300±0.005	1.068±0.00025	1.052±0.00005	1.019±0.0005
Lipolytic activity (u/mL)	0.100±0.005	0.100±0.005	0.400±0.005	0.100±0.005	0.000±0.000	0.000±0.000	0.000±0.000

Each value is a mean of duplicate determinations ± standard error

TABLE 3.2: EFFECT OF DIFFERENT CONCENTRATION OF SUBSTRATE (OLIVE OIL) ON GROWTH AND LIPOLYTIC ACTIVITY OF *Candida parapsilosis*

PARAMETERS	Different Concentration of Olive Oil				
	0.5%	1.0%	1.5%	2.0%	2.5%
Growth (mg/mL)	1.735±0.00005	1.738±0.0002	1.756±0.0002	1.500±0.005	1.358±0.00025
Lipolytic activity (u/mL)	0.000±0.000	0.500±0.0005	0.400±0.0005	0.400±0.005	0.000±0.000

Each value is a mean of duplicate determinations ± standard error

TABLE 3.3: EFFECT OF DIFFERENT CONCENTRATION OF ANION AND CATION ON GROWTH AND LIPOLYTIC ACTIVITY OF *Candida parapsilosis*

PARAMETERS	Different Concentration of Mg SO_4 and $NaNO_3$ Tested			
	0.05%	0.1%	0.15%	0.2%
Growth (mg/mL)	1.761±0.00005	1.477±0.00015	1.474±0.00005	1.461±0.00015
Lipolytic activity (u/mL)	0.00±0.00	0.400±0.0005	0.100±0.0005	0.000±0.000

Each value is a mean of duplicate determinations ± standard error

TABLE 3.4: EFFECT OF DIFFERENT CONCENTRATION OF CRUDE ENZYME ON GROWTH AND LIPOLYTIC ACTIVITY OF *Candida parapsilosis*

PARAMETERS	Different Concentration of Crude Enzyme			
	0.5ml	1.0ml	1.5ml	2.0ml
Growth (mg/mL)	1.523±0.00005	1.477±0.00015	1.435±0.00005	1.360±0.00005
Lipolytic activity (u/mL)	0.500±0.0005	0.400±0.0005	0.200±0.0005	0.100±0.0005

Each value is a mean of duplicate determinations ± standard error

TABLE 3.5: EFFECT OF DIFFERENT CONCENTRATION OF NITROGEN SOURCE (PEPTONE) ON GROWTH AND LIPOLYTIC ACTIVITY OF *Candida parapsilosis*

PARAMETERS	Different Temperature Investigation					
	1.0g	2.0g	3.0g	4.0g	5.0g	6.0g
Growth (mg/mL)	0.968±0.00025	1.336±0.0002	1.638±0.00015	1.606±0.00005	1.477±0.00025	1.029±0.00005
Lipolytic activity (u/mL)	0.10±0.005	0.20±0.0005	0.20±0.0005	0.30±0.0005	0.40±0.0045	0.00±0.00

Each value is a mean of duplicate determinations ± standard error

4. DISCUSSION AND CONCLUSION

Increase in microbial growth does not necessarily mean increase in lipolytic activity as generally observed from this study. On the contrary, Becker *et al*. (1999), Keenan and Sabelnikov (2000) used microbial growth parameters to measure lipids degradation. However, according to Kramer (1971) and as seen in this study (physiological study of *Candida parapsilosis)* an increase on biomass concentration may not produce an increase in lipid matter hydrolysis, because lipase production is not a function of cell growth or concentration.

The obtained yeast lipase was generally observed to work best in alkaline pH (7.5). In low and high medium pH tested, the lipase activity was less. As confirmed with the result of *Candida parapsilosis* in this study, it has been well-documented that the optimum pH range of yeast lipases is generally between pH 5 and 8, with a few exceptions acting at low pH optima of 2.0 (Oishi *et al.*, 1999; Kakugawa *et al.*, 2001; Vakhlu and Kour, 2006).

In this study the yeast had its optimum growth temperature and optimum lipase activity at 27^{o}C. Temperature changes give rise to cleavage of hydrogen bonds between substrate and enzyme active sites. Optimum temperature value promotes binding potential of enzyme and substrate. However, in this study, increase in temperature above known optimum tends most likely to denature the enzymes, thereby reducing the enzyme activity.

In this study agitation supported growth better than lipase production on Ebrahimpour *et al*. (2008) showed that shallow layer (Static culture) where aeration is moderate produced much more lipase than shake cultures (high aeration). On the contrary Hala *et al*. (2010) reported that at 150 rpm, *Fusarium Oxyspirum* produced highest lipase activity compared with the static culture and even at 100rpm and 200rpm.

As observed in this research, maximum lipase activity for enzyme studied was obtained after 24hours of incubation, indicating that lipase was necessary for the first stages of growth. At longer incubation periods, and for all the isolates, the lipase activity decreased which might be due to the deplation of nutrients, accumulation of toxic end products, the change in pH of the of the medium, or loss of moisture. Other investigators have reported a different incubation period for optimal lipase production. Maximum lipase activity was achieved after 48hours of incubation by UI-Haq *et al*. (2002) with *Rhizopus oryzae*. In another study the maximum lipase activity by *Aspergillus niger* occurred after five days of incubation (Mahadik *et al*., 2002). Benjamin and Pandey (1997) obtained maximum production of lipase by *Candida rugosa* after three days of incubation.

Peptone stimulated lipase production better than other nitrogen sources experimented with in this study. This is in agreement with the work of Tembhurkar *et al*. (2012).

In the case of substrate hydrolysis, almost all the substrates tested were hydrolysed by the tested organism. Most of the substrates have long carbon chains (Olive oil has C18:1) which may take time to dissolve. Since lipases hydrolyse esters in emulsion and usually water - insolude substrates, the organisms take up the substrate at different concentration, form and time. Typically, triglycerides composed of long-chain fatty acids, whereas esterases preferentially hydrolyse “simple” esters and usually only triglycerides bearing fatty acids shorter than six carbon chains (C6). Thus, these results strongly suggest that the enzyme used in this study show lipase activity. The findings of Pogaku *et al*. (2010), olive oil supported good growth and increased lipase activity significantly. Olive oil in this case acted as an inducer of lipase production; hence lipase production has been shown to be induced remarkably in the presence of lipidic carbon sources like oils and fatty acids. Glucose was found effective in stimulating lipase production. Prabhakar *et al*. (2002) obtained maximum production of enzyme with glucose among various carbon sources studied.

The activity of lipase in synchrony with growth was found to be best at 0.1% concentration of $MgSO_4$ and $NaNO_3$ for the yeast enzyme. Islam *et al*. (2009) also found that higher concentration of Ca^{2+} decreased lipase activities. These metal ions may increase the binding affinity of enzyme to the substrate (Ece YaPasan, 2008). The enzyme of *Candida parapsilosis* had optimum lipase activity at substrate concentration of 1 %. Similar results have been observed by Sugihara *et al*. (1991) who reported lipase production from *Bacullus sp* in the presence of 1 % olive oil in culture medium. Several other scholars have observed different percentages of olive oil in the culture medium for lipase production by different enzymes. An example is Rajesh *et al*. (2010) who note lipase production by *Trichoderma reesei* at 2 % concentration.

The catalytic activities of the lipases in the current study was enhanced in the presence of Mg^{2+} and $NH3^{+}$, but in habited by Fe^{2+}, Zn^{2+}, Hg^{2+} and Ca^{2+}. Similar results have been reported by Chakraborty and Paul (2008). It can be explained by the fact that few metal ions have ability to interfere with the bonds between amino acid side chains of the enzyme which results in the denaturation of the active site or alter the activity by stabilising and destabilising the conformation of enzyme (Ebrahimpour *et al*., 2011).

Lipase production is influenced by the type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature and the dissolved oxygen concentration (Elibol and Ozer, 2001). The enzyme has good potential for hydrolysis of vegetable oils which is an important factor in environmental clean up of vegetable oil spill site.

REFERENCES

[1] Arpigny, J.L and Jaeger, K. 1999. Bacteria lipolytic enzyme: Classification and properties. *Biochemical Journal* 343(1): 177-183.

[2] Becker, P., Koster, D., Popov, M.N., Marboss Ian, S., Antrtanikian, G. and Markl, H. 1999. The biodegradation of olive oil and treatment of lipid rich wool, waste. Water under aerobic thermophilic condition. *Water Resources.* 133: 653-660.

[3] Benjamin, S. and Pandey, A 1997. Coconut cake: A potent substitute for production of lipase by *Candida rugosa* in solid state fermentation. *Acta Biotechnology.* 17*:* 241-251.

[4] Benjamin, S. and Pandey, A 1998. *Candida rugosa* lipases: *Molecular biology and versatility in biotechnology.* 14(12): 1069-1087.

[5] Chakraborty, K. and Raj, P.R. 2008. An extra-cellular alkaline metallolipase from *Bacillus licheniformis* MTCC 6824*:* Purification and biochemical characterization, *Food Chemistry* 109: 727–736.

[6] Choo, D., Kurihara, T., Suzuki, T., Soda, K. and Esaki, N. 1997. A cold-adapted lipase opf an Alaskan phychotrophy, *Pseudomonas* sp. Strain BII-I: Gene cloning and enzyme purification and characterization. *American Society for Microbiology.* 64(2): 486-491.

[7] Ebrahimpour, A., Rahman, R., EanChing, D. and Salleh, A. 2008. A modeling study by respond surface methodology and artificial neural network on culture parameters optimization for themostable lipase from a newly isolated thermophic *Geobacillus sp*. strain ARM. BMC *Biotechnology* 8: 96-111.

[8] Ebrahimpour, A, Rahman, R., Basri, M. and Salleh, A. 2011. Highlevel expression and characterization of a novel thermostable, organic solvent tolerant, 1, 3-regioselective lipase from *Geobacillus sp.* Strain ARM. *Bioresource Technology.* 102:6972-6981.

[9] Ece YaPasan. 2008. Partial purification and characterization of lipase enzyme from a *Pseudomonas strain*. A Thesis submitted to the graduate school of Engineering and Science of Izmir institute of Technology. xi + 49pp.

[10] Elibol, M. and Ozer, D. 2001. Influence of oxygen transfer on lipase production by *Rhizopus arrhizus. Process Biochemistry* 36: 325-329.

[11] Gojković, Ž. 2009. Studium přípravků do odlučovačů tuků. Diplomová práce, Vysokéučení technické v Brně, Fakulta chemická, 59.

[12] Gupta, R., Rathi, P., Gupta, N. and Brodoo, S. 2003. Lipase assay for conventional and molecular screening: *An overview Biotechnology Applied Biochemistry.* 37: 63-71.

[13] Hala Mohamed Rifaat., Adel Ahmed El-Mahalawy., Hassan Ar El-Menofy and Samah Abdulla Dunia. 2010. Production, optimization and partial purification of lipase from *Fusarium oxysporum*. *Journal of Applied Sciences in Environmental; tation.* 5(1): 39-53.

[14] Islam, M.A., Parveen, F., Hossain, K., Khatun, S., Karim, Md.R., Kim, N., Absar, N. and Haque, Md.S. 2009. Purification and biochemical characterization of lipase from the Dorsal part of *Grrhinus reba*. *Thai Journal of Agricultural Science.* 42(2): 71-80.

[15] Jaeger, K-E., Dijkstra, B.W. and Reetz, M.T. 1999. Bacterial biocatalysts: molecular biology, three-dimensional structures and biotechnological applications of lipases. *Annual Review Microbiol.* 53: 315-351.

[16] Jisheng Ma, ZZ., Wang, B., Kong, X., Wang, Y., Caos and Feng Yan 2005. Over expression and characterization of a lipase from *Bacillus subtilis. Science direct.* 45:22-29.

[17] Joseph, B., Ramteke, P.W. and Thomas, G. 2008. Cold active microbial lipases. Some hot issues and recent developments. *Biotechnology Advances. 26:* 457-470.

[18] Kakugawa, K., Shubayashi, M., Suzuki, O. and Miyakawa, T. 2001. Purification and characterization of lipase from the glycolipid – producing yeast *kurtzmonomyces* sp. 1-11, *Bioscience. Biotechnology Biochemistry.* 66: 978-985.

[19] Keenan, D. and Sabelnikov, A. 2000. Biological augmentation eliminates grease and oil in bakery wastewater. *Water Environmental Resources.* 72: 141-146.

[20] Kramer, R.G. 1971. Hydrolysis of lipids in waste water. *Journal of the Sanitary Engineering Division*. 97 (5): 731-744.

[21] Linfield, W.M., Serota, S. and Sivieri, L. 1985. Lipid-lipase interaction, a new method for assay of lipase activity. *Journal of American Oil Chemistry Society.* 62: 1152-1154.

[22] Liu, Z., Chi., Z., Wang, L. and Li, J. 2008. Production purification and characterization of an extracellular lipase from *Aureobasidium pullulans* HN2-3 with potential application for the hydrolysis of edible oils. *Biochemical Engineering Journal.* 40: 445-451

[23] Mahadik, N.D., Puntambekar, U.S., Bastawde, K.B., Khire, J.M. and Gokhale, D.V. 2002. Production of acidic lipase by *Aspergilus niger* in solid state fermentation. *Proceedings of Biochemistry.* 38: 715-7.

[24] Oishi, H., Morimoto, T., Watenabe, Y. and Tamai, Y. 1999. Purification and characterization of phospholipase B from *Kluyveromyces* lactis and cloning of *Phospholipase B* gene, *Bioscience, Biotechnology and Biochemistry*. 63(1):83-90

[25] Parry, R.M. Jr., Chandan, R.C. and Shahan, K.M. 1966. Rapid and sensitive assay for milk lipase. *Journal of Dairy Science.* 49: 356-360.

[26] Pandey, A., Benjamin, S., Soccol, C.R., Nigam, P., Krieyer, N. and Sccoi, U.T. 1999. The realm of microbial lipases in biotechnology. *Biotechnology Applied Biochemistry.* 29: 119-131.

[27] Pogaku, P., Suresh, A., Srinivas, P. and Reddy, R S 2010. Optimization of lipase production by *staphyloccus sp*, Lp 12. *African Journal of Biotechnology*. 9: 882-886.

[28] Prabhakar, T., Anilkumar, K and Ellaiah, P. 2002. The effect of cultural conditions on the production of lipase by fungi. *Journal of scientific and industrial research.* 61:123-127

[29] Rajesh, E.M., Arthe, R., Rajendran, R., Balakumar, C., Pradeepa, N. and Anitha, S. 2010. Investigation of lipase production by *Trichoderma reesei* and optimization of production parameters. *Electronics Journal of Environmental Agricultural and Food Chemistry* 1579-4377.

[30] Sugihara, A., Tani, T. and Tominaga, Y. 1991. Purification and characterization of a novel themostable lipase Chirumamillia, R.R., Marchant, R., Ramachandran, V.N., Ward, O.P. and Nigan P. 2002. Understanding lipase stereoselectivity. *World of Journal Microbiology Biotechnology.* 18: 81-97.

[31] Tembhurkar, V.R., Kulkarni, M.B. and Peshwe, S.A. 2012. Optimization of lipase production by *Pseudomonas* spp. In Submerged batch process in shake flask culture. *Science Research Reporter*. 2(1): 46-50.

[32] Tsujisaka, Y., Iwaii, M., Fukumoto, J. and Okamoto, Y. 1973. Induced formation of lipase by *Geotrichum candidum* link. *Agriculture Biological Chemistry.* 37: 837-842.

[33] UI-Haq, I., Idrees, S. and Rajoka, M.I. 2002. Production of lipase by *Rhizopus oligosporus* by solid-state fermentation. *Proceedings of Biochemistry.* 37: 637-641.

[34] Vakhlu, J. and Kour, A. 2006. Yeast lipases: enzyme purification, biochemical properties and gene cloning, *European Journal of Biotechnology.* 9: 1-7.