Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2012 Global Science Books

Optimization of Culture Conditions for the Production of Lipase from *Gliomastix indicus* and its Enzymatic Properties

Ashima Kapoor* • **Shraddha Sharma** • **Shveta Prakash**

Department of Biotechnology, Meerut Institute of Engineering and Technology, Meerut 250005 (UP), India

Corresponding author: * **ashimakapoor28@rediffmail.com**

ABSTRACT

Gliomastix indicus is a novel filamentous fungus which was isolated from a wasteland soil sample collected from Tamil Nadu, India. The strain was tested for the production of extracellular lipase by a submerged fermentation technique and was found to produce 10.6 U/ml lipase activity. The culture conditions were optimized for maximum enzyme production. Parameters such as temperature, incubation period, pH, carbon and nitrogen source were studied. Maximum growth and lipase production were observed after 72 h of incubation at 30°C in culture medium with an initial pH of 7.5. Among the number of oils tested as a carbon source as well as inducer for lipase production, linseed oil at 1% (v/v) resulted in a marked increase in lipase production. Malt extract at 1% was the best nitrogen source. The optimization of various growth parameters resulted in a 2.62-fold increase in lipase activity (27.8 U/ml). Some properties of lipase which are desirable for its industrial applications were characterized. Crude lipase had broad substrate specificity, exhibiting maximal activity with linseed oil as substrate. The enzyme had an optimum temperature of 45°C, with 80% of the maximum activity retained at 45°C. Lipase from *G. indicus* also exhibited appreciable stability in different organic solvents except *n*-butanol, suggesting that it has potential to be used in the oil and detergent industries. Among the additives, Ca^{2+} stimulated lipase activity whereas Mn^{2+} and Hg^{2+} had an inhibitory effect.

Keywords: *Gliomastix indicus*, lipolytic, thermostable lipase

INTRODUCTION

Lipases (Triacylglycerol acyl hydrolases, E.C. 3.1.1.3) are a class of hydrolases which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids (FFA) on an oil-water interface. In addition, they are carboxylesterases that catalyze the hydrolysis and transesterifiction of esters (Gupta *et al.* 2011). Today lipases stand amongst the most important biocatalysts carrying out novel reactions in both aqueous and non-aqueous media. This is primarily due to their ability to utilize a wide spectrum of substrates, high stability towards extreme temperatures, pH and organic solvents, chemo-, regio- and enantio-selectivity. In addition to their role in synthetic organic chemistry, lipases also find extensive applications in pharmaceutical, food and leather industries (Gulati *et al.* 2005). In particular, lipases of microbial origin have drawn much attention for their potential use in industrial applications. This is mainly due to their versatile catalytic behaviour, availability and stability. The exponential increase in the applications of lipases in various fields, over the past few years, necessitated both qualitative and quantitative improvement in enzyme production. Quantitative enhancement requires strain improvement and medium optimization for overproduction (Immanuel *et al.* 2008). Hence, the present investigation aimed to produce lipase from a fungus, *Gliomastix indicus.* The fungal culture *G. indicus* was isolated from a wasteland soil sample collected from Tamil Nadu, India. Some findings of the morphological studies of this isolated new culture were as follows: conidia were present in three shapes, viz. spherical, oval, oblong, and having sizes 11.0 × 11.0 µm, 13.8–8.40 × 7.0–9.0 µm, 14.0–17.5 × 6.5–11.5 µm, respectively. *G. indicus* comprises the properties of a filamentous fungus. It is closely related to acremonium and the monophialidic species of *Paecilomyces*. The grey black pigments of the spores are very similar to the pigment of *Stachybotrys* (Nagalakshmi *et al.* 2009). Here it has been screened and exploited for the first time for the production of different industrial enzymes, out of which results of lipase production and characterization studies are presented here.

MATERIALS AND METHODS

Chemicals

Tributyrin, Tween-20, Tween-80 and components of fermentation medium were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). The substrate olive oil was obtained from CDH (Central Drug House Pvt. Ltd., Delhi, India). All the chemicals used in this study were of analytical grade. The different oil samples (mustard, coconut, linseed, amla, til, sunflower, ricinus and soyabean oils) were purchased from the local market.

Screening of lipolytic potential of *Gliomastix indicus*

1. Composition of growth medium

The culture was maintained on malt extract medium which consisted of (g/L): 20.0 malt extract, 0.5 K_2HPO_4, 1.0 NH_4Cl.

2. Composition of different media screened for maximum lipase production from Gliomastix indicus (g/L) (pH 7.0)

Four different types of media viz. Medium A: Malt extract liquid medium supplemented with 1% olive oil; Medium B [(modified lipase assay medium: Shukla and Gupta 2007) (in g/L): peptone 15.0, NaCl 5.0, $CaCl_2$ 1.0, olive oil (1%)]; Medium C [(Cho *et al.* 2007) (in g/L): Glucose 10.0, peptone 50.0, $NaNO_3$ 1.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, olive oil (1%)]; Medium D [(Savitha *et al.* 2007) (in g/L): yeast extract 1.0, K_2HPO_4 7.0, Na_2HPO_4 2.0,

Received: 1 June, 2011. Accepted: 22 February, 2012.

Research Note

$MgSO_4 \cdot 7H_2O$ 1.5, $CaCl_2 \cdot 2H_2O$ 1.0, $FeCl_2 \cdot 4H_2O$ 0.08, $ZnSO_4 \cdot 7H_2O$ 0.001, diammonium tartarate 1.5, olive oil (1%)] were used in the study for production of lipase by the test fungus.

3. Lipase production

The fungus was screened for its lipase-producing capacity by observing the zone of hydrolysis around the growth on tributyrin agar plates (malt extract agar medium containing 1% tributyrin) following the method of Adinarayana *et al*. (2004). Initially lipase production was carried out in medium A (Malt extract liquid medium supplemented with 1% olive oil as substrate) at 30°C for 4 days.

4. Lipase assay

Lipase activity was estimated by a titrimeteric method (Ray *et al.* 1999) by measuring the amount of fatty acid released from the substrate by the enzyme. The substrate olive oil for lipase was emulsified by mixing 25 ml olive oil and 75 ml 2.0% polyvinyl alcohol solution. For the lipase assay, a reaction mixture containing 5 ml olive oil emulsion, 4 ml 0.2M phosphate buffer (pH 7.0), 1 ml 110 mM $CaCl_2$ and 1 ml enzyme solution was incubated at 45°C for 20 min. Control containing inactivated enzyme was treated similarly. Immediately after 20 min, the emulsion was destroyed by the addition of 20 ml of acetone-ethanol (1: 1, v/v) mixture and the liberated free fatty acid was titrated with 0.02N NaOH using phenolphthalein as indicator. One unit of lipase activity was defined as the amount of enzyme that liberated 1 µmol of fatty acid/min. 1.0 ml of 0.02N NaOH is equivalent to 100 µmol of free fatty acid.

Biomass determination

To determine the fungal biomass 50 ml culture was centrifuged at 8,000 rpm for 10 min (at 4°C) and the pellet was washed with distilled water. The washed mycelium was dried at 100°C for 24 h in a pre-weighed dry Petri dish to constant weight and expressed as g dry weight/50 ml medium.

Optimization of culture conditions for maximum lipase production

1. Effect of incubation period

To determine the optimum incubation time for maximum enzyme production, the medium was inoculated with a disc of freshly grown (48 h) culture of *G. indicus* and incubated at 30°C for 5 days. Biomass and lipase activity were estimated up to 5 days at regular intervals of 24 h.

2. Effect of pH and temperature on enzyme production

The effect of initial pH of the medium was studied by inoculating an inoculum of *G. indicus* in medium A of pH range 6.0 to 8.5 and incubated at 30°C for 3 days. The effect of incubation temperature on enzyme production by the fungus was studied by growing the culture at 30, 35, 40 and 45°C.

3. Effect of carbon and nitrogen sources

Lipases, being inducible enzymes, require a lipid carbon source in the form of oils for higher enzyme yield. The production medium was therefore supplemented with different substrates (1%) as the sole carbon source, viz. olive oil (control), mustard oil, coconut oil, linseed oil, amla oil, til oil, sunflower oil, ricinus oil and soyabean oil and inoculated with a 48-h-old seed culture. Inoculated flasks were incubated at 30°C for 3 days and lipase activity was determined. Different organic and inorganic nitrogen supplements (1%) such as malt extract, peptone, beef extract, yeast extract, tryptone, urea, $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl, $NaNO_3$ were also tested to achieve enhanced lipase production.

Biochemical characterization of the lipase

1. Substrate specificity of lipase

To determine substrate specificity, 1 ml of crude lipase was incubated with 5 ml of emulsion of different substrates (olive oil, Tween-20, Tween-80, glycerol and linseed oil) and the lipase assay was performed as mentioned earlier.

2. Temperature optima and thermal stability

The optimum temperature for the crude enzyme was determined by subjecting the reaction mixture at different temperatures ranging from 30-55°C and lipase activity was calculated. To determine thermal stability, the crude enzyme was incubated at 45°C for 2 h and lipase activity was determined at regular 30-min intervals.

3. Effect of organic solvents

The effect of organic solvents on the stability of crude enzyme was determined by incubating the reaction mixture with different organic solvents for 20 min and lipase activity was calculated.

4. Effect of additives (metal ions and detergents)

The effect of various metal ions (NaCl, KCl, $CaCl_2$, $MgCl_2$, $MnCl_2$, $HgCl_2$ and $FeCl_3$), detergents (SDS, Tween-20, Tween-80 and Triton X-100), β-mercaptoethanol and EDTA on lipase activity were studied. The reaction mixture containing additive was incubated at 45°C for 20 min and lipase activity was calculated.

All fermentations and assays were carried out in triplicate and the mean value was presented.

RESULTS AND DISCUSSION

Lipase production from *Gliomastix indicus*

The lipolytic potential of *G. indicus* was tested by observing the zone of hydrolysis on tributyrin agar plates. The appearance of a clear zone around the fungal growth indicated the lipolytic activity of this new culture producing 10.6 U/ml of lipase activity in malt extract medium (medium A) at 30°C after 4 days. Further, different nutritional and cultural conditions were studied to enhance lipase production from *G. indicus*.

1. Screening media for maximum production of lipase

Four different media viz. medium A, B, C and D were tested in an attempt to improve lipase production from *G. indicus.* Among the different media tested, medium A (pH 7.0) used initially, supported maximum growth and lipase production than all other media (**Table 1**). Hence, medium A (malt extract medium supplemented with 1% olive oil) was used in further studies on lipase production.

2. Growth profile and effect of incubation period

Growth and lipase production from *G. indicus* were determined at 24-h intervals up to 96 h by growing the fungus in medium A (pH 7.0). Maximum growth (expressed in terms of biomass) as well as lipase activity (10.6 U/ml) was detected after 72 h incubation (**Fig. 1**) indicating that enzyme production was associated with the growth phase/ stage. Ray *et al.* (2004) also observed maximum lipase pro-

Table 1 Screening of medium for maximum lipase production.

Medium	Lipase activity (U/ml)
Medium A	10.2
Medium B	8.3
Medium C	4.2
Medium D	5.6

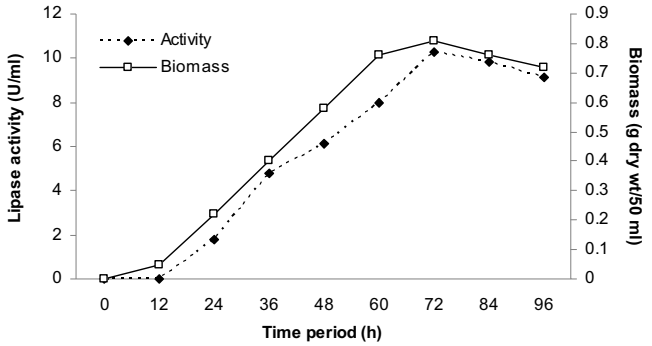

Fig. 1 Effect of incubation period on enzyme production.

Table 2 Effect of pH on lipase production

pH	Lipase activity (U/ml)
6.0	6.2
6.5	9.8
7.0	10.8
7.5	11.6
8.0	4.1
8.5	2.0

duction (26.0 U/ml) from *Corynebacterium* sp. after 72 h of growth. Cho *et al.* (2007) studied the effect of incubation time on lipase production by *Penicillium chrysogenum* and found maximum lipase activity (68.0 U/ml) and fungal biomass on the 5th day of incubation. Rajesh *et al.* (2010) obtained maximum lipase production (3.26 U/ml) from *Trichoderma reesei* after 96 h of incubation.

3. Effect of culture medium pH

Growth and lipase production were maximum at pH 7.5 indicating the alkalophilic nature of the isolate (**Table 2**). Lipase production under alkaline condition is desirable since this is the most common condition in industrial applications. Hence, most researchers have tested alkalophilic cultures for lipase production. Abada *et al.* (2008) reported maximum lipase production from *Bacillus stearothermophilus* AB-1 in a pH 7.5 medium. Rani and Panneerselvam (2009) tested different fungal cultures for lipase production and observed maximum activity of 23.7 U/ml from *Aspergillus terreus* in the medium of pH 8.0. Rajan *et al.* (2011) reported lipase production from alkaline lipase-producing fungus *Aspergillus fumigatus* MTCC 9657 at a pH range of 8.5-10.5 and observed maximum lipase activity of 5.8 U/ml at pH 8.5.

4. Effect of incubation temperature

In order to determine the suitable incubation temperature, the culture was grown at 30-45°C under stationary condition. After recording enzyme activity and growth at different temperatures, the culture yielded maximum lipase at 30°C (**Table 3**). A decline in growth and enzyme production were observed above 30°C. Maximum lipase production at 30°C was also reported by other groups. Rani and Panneerselvam (2009) reported maximum lipase activity of 19.2 U/ml by *Aspergillus terreus* at 30°C. Rajesh *et al.* (2010) observed maximum lipase production (3.5 U/ml) at 30°C from *Trichoderma reesei*. The maximum lipase production of 17.0 U/ml was obtained from *Fusarium oxysporum* at 30°C (Rifaat *et al.* 2010). Sneha *et al.* (2012) found 30°C as optimal incubation temperature for lipase production (23.0 U/ml) from *Aspergillus heteromorphus*.

5. Effect of carbon source

Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition, besides

Table 3 Effect of incubation temperature.

Temperature (°C)	Lipase activity (U/ml)
30	10.8
35	4.6
40	4.8
45	N.D.

N.D. = Not detected

Table 4 Effect of carbon source on lipase production.

Carbon source (1%)	Lipase activity (U/ml)
Olive oil (control)	11.2 (0.66)
Mustard oil	9.6 (0.60)
Coconut oil	11.8 (0.80)
Linseed oil	17.3 (0.78)
Amla oil	10.1 (0.58)
Til oil	4.2 (0.12)
Sunflower oil	6.8 (0.33)
Ricinus oil	3.8 (0.22)
Soyabean oil	10.1 (0.58)

Values in parenthesis represent **b**iomass (g dry weight/50 ml)

physicochemical culture conditions. The major factor for expression of lipase activity has always been reported as being the carbon source since lipases are inducible enzymes. A number of oils (olive, amla, coconut, linseed, mustard, ricinus, til, sunflower, soyabean) were therefore tested as carbon source as well as inducers for lipase production by *G. indicus*. Among the various oils, linseed oil (*Linum usitatissmum*) at 1% (v/v) resulted in a marked increase in lipase production (17.3 U/ml) compared to olive oil (11.2 U/ml) used initially (**Table 4**). Coconut (11.8 U/ml), amla (10.1 U/ml) and soyabean (10.1 U/ml) oil also supported a desirable level of lipase production. Many researchers have reported the production of lipase using different oils as the carbon source. Savitha *et al.* (2007) observed highest lipase production (12.1 U/ml) when coconut oil (1% v/v) was used as the substrate. Shukla and Gupta (2007) found optimum lipase production of 48.3 U/ml from *Rhizopus oryzae* KG-5 using Tween-20 as a carbon source. Lau *et al.* (2010) reported optimal lipase production (179 U/ml) using a combination of sunflower oil and Tween-80 (1% v/v each) as the carbon source. Brassica oil (1%) as a carbon source produced highest lipase activity (3.78 U/ml) from *Fusarium solani* (Iftikar *et al.* 2012). However, in most of the reports olive oil has been used as the substrate for lipase production. Rajesh *et al.* (2010) found maximum lipase activity of 3.43 U/ml in medium supplemented with olive oil (2%) as compared to different oils (coconut oil, groundnut oil, sesame oil, sunflower oil) tested. Olive oil (2%) was also found to be the best carbon source for optimum lipase production from *Fusarium oxysporum* (17.0 U/ml) (Rifaat *et al.* 2010) and *Aspergillus heteromorphus* (23.0 U/ml) (Sneha *et al.* 2012).

6. Effect of nitrogen source

The effect of different nitrogen sources on lipase production by *G. indicus* was studied by supplementing the medium with various organic (1%) and inorganic (0.1%) sources. Among the organic nitrogen sources, malt extract at 1% ensured better growth of the organism with enhanced lipase activity (24.8 U/ml) compared to 2% (control) followed with peptone, yeast extract and beef extract (**Table 5**). Ammonium sulphate and ammonium nitrate also supported appreciable enzyme production. However, the organic nitrogen sources were better for lipase production than inorganic nitrogenous salts. Similar observations have been reported by many others. Peptone (3%) was the best nitrogen source for lipase production from *Aspergillus terreus* (Rani and Panneerselvam 2009). Rifaat *et al.* (2010) also observed maximum lipase production (17.0 U/ml) from *Fusarium oxysporum* with peptone as well as malt extract at 4%. Among inorganic nitrogen sources, only sodium nitrate was

Table 5 Effect of nitrogen source on lipase production.

Nitrogen source (1%)	Lipase activity (U/ml)
Malt extract (2%) (control)	16.3 (0.85)
Malt extract	24.8 (0.78)
Peptone	18.3 (0.70)
Tryptone	12.6 (0.40)
Yeast extract	17.8 (0.50)
Beef extract	16.8 (0.52)
Urea	10.3 (0.44)
$(NH_4)_2SO_4$	15.3 (0.48)
NH_4NO_3	14.6 (0.40)
$NaNO_3$	10.2 (0.22)
NH_4Cl	6.8 (0.10)

Values in parenthesis represent biomass (g dry weight/50 ml)

Table 6 Substrate specificity of lipase.

Substrates (1%)	Lipase activity (U/ml)
Olive oil	24.3
Linseed oil	26.8
Tween-20	20.3
Tween-80	22.1
Glycerol	12.1

found to be effective, resulting in 14.0 U/ml lipase activity. Sodium nitrate (1%) also supported maximum lipase activity (5.17 U/ml) from *Fusarium solani* (Iftikar *et al.* 2012). Highest lipase activity (16 U/ml) was determined when 1% ammonium sulfate was the nitrogen source (Kebabci and Cihangir 2012).

The optimization of different nutritional and environmental parameters (such as linseed oil (1% v/v) as carbon source, 1% malt extract, incubation period 72 h and pH of the medium 7.5) resulted in a 2.62-fold increase in lipase production compared to initial culture conditions (malt extract medium of pH 7.0 supplemented with 1% olive oil, malt extract 2% incubated for 96 h).

Biochemical characterization of lipase

Some properties of lipase which are desirable for its industrial applications were characterized.

1. Substrate specificity

To study the substrate specificity of lipase from *G. indicus*, the enzyme was incubated with different substrates [olive oil (control), Tween-20, Tween-80, glycerol and linseed oil]. Olive oil and linseed oil were the best substrates for the enzyme (**Table 6**). Substrate specificity of lipases is often crucial to their application for analytical and industrial purposes (Saxena *et al.* 1999). Cho *et al.* (2007) also studied the effect of different substrates on lipase activity and found highest activity with tributyrin (1%) among the various synthetic and natural oil substrates tested.

2. Temperature optima and thermal stability

The optimum temperature of crude lipase was 45°C (**Fig. 2**). Many others reported thermophilic lipases with temperature optima ranging from 40-60°C. Lipase from *Mucor* sp. exhibited maximum activity (13.2 U/ml) at 40°C (Savitha *et al.* 2007). The optimum temperature for lipase from *Trichoderma reesei* (4.23 U/ml) and *Aeromonas hydrophila* (10.0 U/ml) was determined to be 50°C by Rajesh *et al.* (2010) and Neelambri *et al.* (2011), respectively. Kukreja and Bera (2005) reported temperature optimum for lipase from *Pseudomonas aeruginosa* to be 60°C.

The enzyme was stable at the optimum temperature for 90 min retaining 80% of its original activity (**Fig. 3**). Thereafter, activity decreased sharply. Hence, the lipase from *G. indicus* can be considered as a thermostable enzyme.

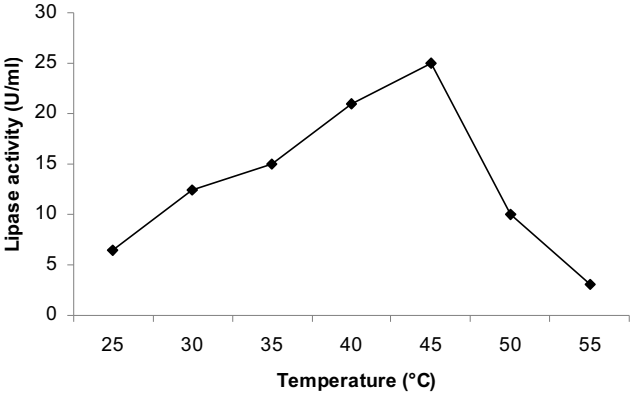

Fig. 2 Temperature optima of crude lipase.

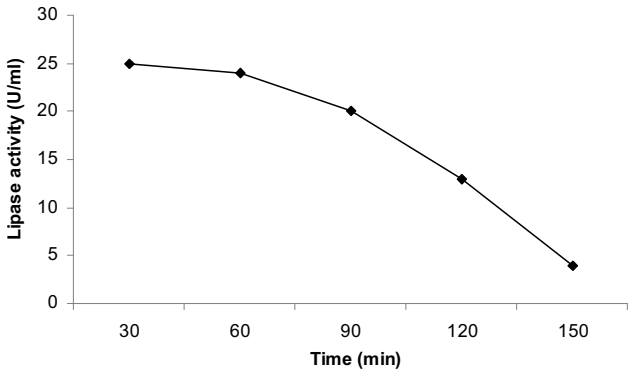

Fig. 3 Thermal stability of lipase at 45°C.

3. Effect of organic solvents

The effect of organic solvents on lipase activity was studied keeping in mind that lipases often catalyze reactions in organic solvents, since the substrates of lipases are fats which are insoluble in aqueous solution. Hence, lipases stable in the presence of organic solvents could be industrially useful, as they do not require a stabilizing agent. In this respect, lipase from *G. indicus* exhibited appreciable stability when organic solvents (30%) were added to the reaction mixture (**Table 7**). Lipase activity was not affected by any solvent except for *n*-butanol. Lipase from *Penicillium aeruginosa* was also found to be stable in the presence of organic solvents (30%) specifically acetone, methanol and ethanol retaining 100, 92.6 and 90.4%, respectively of original activity up to 2 h (Kukreja and Bera 2005). Neelambri *et al.* (2011) reported stability of lipase from *Aeromonas hydrophila* in the presence of organic solvents (50%) such as acetone (12.0 U/ml), ethanol (11.5 U/ml) and methanol (10.0 U/ml).

4. Effect of metal ions and detergents

The lipase from *G. indicus* exhibited an increase in enzyme activity in the presence of Ca^{2+}, but was inhibited by EDTA, suggesting it to be a Ca^{2+}-dependent metalloprotein. The metal ions Na^{+}, K^{+}, Mg^{2+} and Fe^{3+} and β-mercaptoethanol did not affect lipase activity. However, Mn^{2+} and Hg^{2+} and detergents (SDS and Triton X-100) strongly inhibited lipase activity (**Table 8**). The stimulation of lipase activity by Ca^{2+} ions (5 and 10 mM) has been previously reported by Ray *et al.* (2004) and Kukreja and Bera (2005) respectively. Detergents like Tween-20, -40, -60 and -80 had no effect on lipase from *Corynebacterium* sp. (Ray *et al.* 1999) and *Pseudomonas aeroginosa* MTCC 2488 (Kukreja and Bera 2005), while SDS resulted in a complete loss of enzyme activity.

Table 7 Effect of organic solvents on lipase activity.

Organic solvent (30%)	Lipase activity (U/ml)
Control	23.2 (100.0)
n-Propanol	23.6 (100.6)
iso-Propanol	24.0 (103.4)
Hexane	22.6 (97.4)
Ethanol	23.0 (99.1)
n-Butanol	16.1 (69.3)
Methanol	24.0 (103.4)
Acetone	24.3 (104.0)

Values in parenthesis represent relative activity (%)

Table 8 Effect of additives on lipase activity.

Additives (1 mM)	Relative activity (%)
Control	100.0
NaCl	92.7
KCl	89.5
$CaCl_2$	123.3
$MgCl_2$	93.5
$MnCl_2$	43.5
$HgCl_2$	16.9
$FeCl_3$	16.1
EDTA	24.2
SDS	19.3
β-Mercaptoethanol	84.6
Triton-X-100	39.5
Tween-20	95.2
Tween-80	87.9

CONCLUSION

The present work has been taken up with a view of exploring the possibilities of using *G. indicus* as a source for production of industrially important enzymes and metabolites. The results of the investigation demonstrated that under optimized culture conditions *G. indicus* can be used as a new potent microbial source of lipase. The lipase from *G. indicus* was found to be extracellular, inducible, thermostable and also exhibited stability in different organic solvents. These biochemical properties of *G. indicus* lipase make it suitable for applications in oil and detergent industries.

ACKNOWLEDGEMENTS

The authors express sincere gratitude to Prof A. Subrahmanyam, Head, Department of Biotechnology, Meerut Institute of Engineering & Technology, Meerut for providing the culture of *Gliomastix indicus* for the present work. The authors thank Dr. Jaime A. Teixeira da Silva for extensive improvements to language, grammar and figure quality.

REFERENCES

Abada EAE (2008) Production and characterization of a mesophilic lipase isolated from *Bacillus stearothermophilus* AB-1. *Pakistan Journal of Biological Sciences* **11 (8)**, 1100-1106

Adinarayana K, Bapi Raju KVVSN, Zargar MN, Bhavani Devi R, Jhansi Lakshmi P, Ellaiah P (2004) Optimization of process parameters for production of lipase in solid-state fermentation by newly isolated *Aspergillus* species. *Indian Journal of Biotechnology* **3**, 65-69

Cho HY, Bancerz R, Ginalska G, Leonowicz A, Cho NS, Ohga S (2007) Culture conditions of psychrotrophic fungus, *Penicillium chrysogenum* and its lipase characteristic. *Journal of the Faculty of Agriculture, Kyushu University* **52 (2)**, 281-286

Gulati R, ISAR J, Kumar V, Prasad AK, Parmar V, Saxena RK (2005) Production of a novel alkaline lipase by *Fusarium globulosum* using neem oil and its applications. *Pure and Applied Chemistry* **77 (1)**, 251-262

Gupta P, Upadhyay LSB, Shrivastva R (2011) Lipase catalysed-transesterification of vegetable oils by lipolytic bacteria. *Research Journal of Microbiology* **6 (3)**, 281-288

Iftikhar T, Niaz1 M, Zia MA, Sadaf M, Jabeen R (2012) Production potential of locally isolated strain of *Fusarium solani* (Mbl 24) for extracellular lipases. *Pakistan Journal of Botany* **44 (1)**, 393-397

Immaneul G, Esakkiraj P, Jeladhas A, Lyapparaj P, Arunachalam P (2008) Investigation of lipase production by milk isolate *Serratia rubidaea. Food Technology and Biotechnology* **46 (1)**, 60-65

Kebabci O, Cihangir N (2012) Comparison of three *Yarrowia lipolytica* strains for lipase production: NBRC 1658, IFO 1195, and a local strain. *Turkish Journal of Biology* **36**, 15-24

Kukreja V, Bera MB (2005) Lipase from *Pseudomonas aeruginosa* MTCC 2488: Partial purification, characterization and calcium dependent thermostability. *Indian Journal of Biotechnology* **4**, 222-226

Lau HL, Ariff A, Woo KK, Ling TC, Hii SL (2011) Production and optimization of alkalostable lipase by alkalophilic *Burkholderia cenocepacia* ST8. *African Journal of Biotechnology* **10 (36)**, 7002-7009

Nagalakshmi S, Vijayalakshmi M, Subrahmanyam A (2009) *Gliomastix indicus sp. nov. Current Trends in Biotechnology and Pharmacy* **3 (1)**, 111-112

Neelambri V, Vasathabharthi V, Balasubramanian R, Jayalakshmi S (2011) Lipase from marine *Aeromonas hydrophila. Research Journal of Microbiology* **6 (8)**, 658-668

Rajan A, Kumar DRS, Nair AJ (2011) Isolation of a novel alkaline lipase producing fungus *Aspergillus fumigatus* MTCC 9657 from aged and crude rice bran oil and quantification by HPTLC. *International Journal of Biological Chemistry* **5 (2)**, 116-126

Rajesh EM, Arthe R, Rajendran R, Balakumar C, Pradeepa N, Anitha S (2010) Investigation of lipase production by *Trichoderma reesei* and optimization of production parameters. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **9 (7)**, 1177-1189

Rani C, Panneerselvam A (2009) Influence of environmental and nutritional parameters on lipase production. *ARPN Journal of Agricultural and Biological Sciences* **4 (5)**, 39-43

Ray N, Ray L, Chattopadhyay P (2004) Production of lipase in a fermentor using a mutant strain of *Corynebacterium* sp.: Its partial purification and immobilization. *Indian Journal of Experimental Biology* **42**, 202-207

Ray N, Ray L, Srimani BN, Chattopadhyay P (1999) Isolation and identification of alkaline thermostable lipase producing microorganism, culture conditions, nutritional requirements and some properties of crude enzyme. *Indian Journal of Experimental Biology* **37**, 818-824

Rifaat HM, Ahmed El-Mahalawy A, Amin El-Manofy H, Donia SA (2010) Production, optimization and partial purification of lipase from *Fusarium oxysporum*. *Journal of Applied Sciences and Environmental Sanitation* **5 (1)**, 39-53

Savitha J, Srividya S, Jagat R, Payal P, Priyanki S, Rashmi GW, Roshini KT, Shantala YM (2007) Identification of potential fungal strains for the production of inducible, extracellular and alkalophilic lipase. *African Journal of Biotechnology* **6 (5)**, 564-568

Saxena RK, Ghosh PK, Gupta R, Davidson WS, Bradoo S, Gulati R (1999) Microbial lipases: Potential biocatalysts for the future industry. *Current Science* **77 (1)**, 101-115

Shukla P, Gupta K (2007) Edological screening for lipolytic molds and process optimization for lipase production from *Rhizopus oryzae* KG-5. *Journal of Applied Science and Environment Sanitation* **2 (2)**, 35-42

Sneha PM, Parimala T, Rao CSVR, Satish Babu R (2012) Effect of various sources and parameters on the production of an extracellular lipase from *Aspergillus heteromorphus* and optimization using response surface methodology. *Journal of Chemical, Biological and Physical Sciences* **2 (1)**, 212-222