academicJournals

Vol. 15(41), pp. 2292-2300, 12 October, 2016
DOI: 10.5897/AJB2016.15600
Article Number: E3EEA1E61057
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Lipase production from a wild (LPF-5) and a mutant (HN1) strain of *Aspergillus niger*

Arun Kumar Sharma[1], Vinay Sharma[1]*, Jyoti Saxena[2] and Arindam Kuila[1]

[1]Department of Bioscience and Biotechnology, Banasthali University, Rajasthan, India.
[2]Department of Biochemical Engineering, Bipin Tripathi Kumaon Institute of Technology, Dwarahat, Uttrakhand, India.

Received 5 August, 2016; Accepted 26 September, 2016

In this study, a wild (LPF-5) and a mutant (HN1) strain of *A. niger* were compared for lipase production. Several physical parameters (carbon source, nitrogen source, pH, temperature and incubation period) were optimized for maximization of lipase production. Lipase activity between wild type and mutant strain were compared. Among all carbon sources, mixture of glucose (1%, w/v) and olive oil (1%, v/v) exhibited maximum increase in the production of lipases by both the wild (94.91 ± 0.60 U mL^{-1} min^{-1}) and mutant (118.23 ± 0.73 U mL^{-1} min^{-1}) strain. Addition of glucose into the production medium (containing olive oil) increased the production of lipase up to 20% in case of both the strains. The production of lipase by both the strains was higher in the medium of pH 7.0 containing peptone (1%, w/v) as nitrogen source after 3 days of incubation at 28°C. The activity of lipase from HN1 strain in optimized medium was 40% higher (147.65 ± 1.14 U mL^{-1} min^{-1}) than in un-optimized medium (105.19 ± 0.91 U mL^{-1} min^{-1}), while it was 38% higher for LPF-5 strain in optimized medium. Therefore the mutant strain (*A. niger* HN1) is prospective for the development of industrial biotechnology for production of extracellular lipase. Lipase enzyme was partially purified by ammonium sulfate precipitation and 70% precipitate showed highest specific activity of 66.12 U mg^{-1} for mutant strain as compared to specific activity of 29.88 U mg^{-1} in crude lysate.

Key words: Wild strain, mutant strain, *Aspergillus niger,* lipase activity, specific activity, ammonium sulfate.

INTRODUCTION

Lipases (triacyl glycerol acylhydrolases, EC 3.1.1.3) belonging to the class hydrolases catalyze hydrolysis of insoluble triacylglycerols to generate free fatty acids, mono and diacylglycerols and glycerol (Das et al., 2016). Lipases are capable of catalyzing hydrolysis, esterification and transesterification (alcoholysis, acidolysis, aminolysis and interesterification) reactions (Sharma and Kanwar, 2014).

Lipases can be categorized into three groups: Animal, plant and microbial according to their origin. The most broadly applied lipases are microbial due to their stability and chemical properties (Hasan et al., 2009). Microbial lipases are secreted by several bacteria, yeasts, actinomycetes and moulds (Toscano et al., 2011). Filamentous fungi are the preferred sources of lipases among lipase-producing microorganisms. The

*Corresponding author. E-mail: vinaysharma30@yahoo.co.uk.

advantages of using filamentous fungi as industrial producers of extracellular lipases in comparison with the rest of lipase producing microorganisms are as follows: (i) Capability to utilize broad range of agricultural and other waste products as source of nutrients; (ii) Ability to produce lipases extracellularly in the fermentation broth; (iii) Separation of fungal mycelium is very simple from fermentation broth by vacuum filtration in comparison with yeast and bacterial biomasses, and (iv) Capability to produce lipases both in solid state fermentation (SSF) and submerged fermentations (SmF) (Toscano et al*.,* 2011). The main producers of commercial lipases are *Rhizopus* sp., *Pencillium* sp., *Aspergillus* sp., *Mucor* sp., *Candida rugosa*, *Acremonium alcalophilum*, *Lipomyces starkeyi*, *Humicola lanuginosa*, *Cunninghamella verticillata* and *Geotrichum candidum* (Thakur, 2012).

Lipolytic microorganisms have been isolated from different sources such as edible oil extraction factories, diesel and edible oil contaminated soil, industrial wastes, dairies, etc. (Veerapagu et al., 2013). Presently, the demand of the industries for new sources of lipases with diverse catalytic features promotes the isolation and screening of new lipolytic microbial strains (Thakur, 2012).

The amount of enzyme produced by wild strains is generally low. Therefore, overproduction of the enzymes requires strain improvement and optimization of culture conditions (Pathak et al., 2015). In order to increase production of enzyme from fungal organisms, optimization studies are of great importance. A number of reports describe the effect of various culture media and environmental factors including; pH, temperature, nitrogen, carbon, and lipid sources, dissolved oxygen concentration and agitation, on lipase production (Sundar and Kumaresapillai, 2013).

Two fermentation processes, including solid state fermentation (SSF) and submerged fermentation (SmF) have been used for lipase production. Submerged fermentation is an attractive technique because all process parameters can be easily controlled to optimize the enzyme production, biomass can be easily determined by centrifugation or filtration, space requirement is less, higher amounts of enzyme can be extracted, and recovery of enzyme is easier using this fermentation method. The majority (more than 75%) of industrial enzymes are produced by SmF (Subramaniyam and Vimala, 2012). The genetic engineering approach can increase the productivity of enzyme. The overall mutability and mutation rate of specific genes can be increased by the recent technique of site specific mutagenesis (Pathak et al., 2015).

Purification of the enzyme is important in various industries such as cosmetics, fine chemicals and pharmaceuticals in efforts to understand three dimensional structure (Saxena et al., 2003). In other industries, crude enzyme is preferred over purified enzyme for specific applications as the cost of purification can be avoided.

Following carbohydrases and proteases, lipases are believed to be the third largest group based on total sales volume. Lipases found promising application in various industries such as detergent, agrochemical, paper, chemical processing, dairy, pharmaceuticals, oleo-chemical, cosmetics, polymer synthesis, synthesis of surfactants and personal care products (Ray, 2012). Due to increased demand of lipases in various industries we have made attempt to enhance extracellular lipase production by optimizing culture conditions and physiological parameters for mutagenic culture of the fungus *Aspergillus niger* LPF-5. Lipase enzyme was further partially purified by ammonium sulfate precipitation.

MATERIALS AND METHODS

Microorganism

The lipolytic fungal culture (*A. niger* LPF-5) was obtained from the Department of Bioscience and Biotechnology, Banasthali University and maintained on potato dextrose agar (PDA) slants.

Strain improvement

A culture of *A. niger* LPF-5 was mutagenized by incubation of spores with a 0.01 M sodium nitrite solution for various time intervals and hyperproducer mutant cultures were selected by quantitative screening as described by Karanam and Medicherla (2008) (data not shown). The composition of the production medium (g L^{-1}) was as follows: Bacteriological peptone, 10; olive oil, 1%; $MgSO_4.7H_2O$, 0.6; KH_2PO_4, 1.0; NH_4NO_3, 1.0 and pH was adjusted to 7.0. The wild and hyperproducer nitrous acid mutant culture were assigned the name of *A. niger* LPF-5 and *A. niger* HN1, respectively.

Optimization of culture conditions for extracellular lipase production in shake flasks

A. niger hyperproducer nitrous acid mutant (HN1) and wild (LPF-5) strain were utilized for optimization studies in order to increase the extracellular lipase production in SmF. Culture conditions (carbon source and nitrogen source) and physiological parameters such as temperature, pH and incubation time were optimized for enhanced production of lipase.

Effect of various carbon sources on lipase production

Various carbon sources including; olive oil, soybean oil, mustard oil, Tween-20, Tween-80, Tributyrin, Triton-X-100 and glucose were used to investigate their effect on extracellular lipase production by both LPF-5 and HN1 strain. These carbon sources were added individually to the production medium at a constant concentration (1%, w/v) by replacing the original carbon source of the broth. Spore suspensions (10^8 spores mL^{-1}) were prepared and inoculated in fermentation broth containing alternative carbon sources followed by incubation at 28°C for three days. Lipase assays were conducted in the cell free supernatant as described by Winkler and Stuckmann (1979).

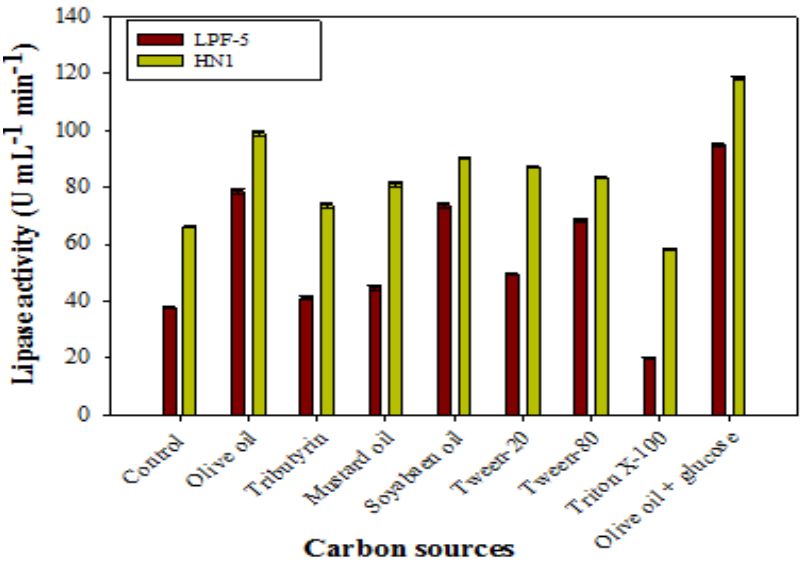

Figure 1. Impact of different carbon sources (1%, w/v) on lipase activity of wild (LPF-5) and mutant (HN1) strain of *A. niger* (constant parameters were: peptone (1%), pH 7, 28 ^{o}C and incubation time 3 days).

Effect of various nitrogen sources (organic and inorganic) on lipase production

Organic nitrogen sources (peptone, urea, yeast extract and beef extract) and inorganic nitrogen sources ($(NH_4)_2SO_4$, $NaNO_3$ and KNO_3) were evaluated for their effect on extracellular lipase production from LPF-5 and HN1 strains. These nitrogen sources were added individually in the fermentation broth at a constant concentration (1%, w/v) using the previously optimized carbon source.

Effect of pH on lipase production

Fermentation broth was prepared using previously optimized carbon and nitrogen sources. The pH of the fermentation broth was adjusted to 4, 5, 6, 7, 8, 9, 10 or 11 using 1 N HCl or 1 N NaOH.

Effect of incubation temperatures on lipase production

The effect of various incubation temperatures on extracellular lipase production was investigated by placing the inoculated flasks for three days in a shaker incubator at 18, 28, 37 or 50°C.

Effect of incubation period on lipase production

To study the effect of incubation period on lipase production, inoculated flasks were placed in a shaker incubator for 24, 48, 72, 96, 120 and 144 h at 28°C at 120 rpm. Samples were withdrawn at the end of each incubation period and extracellular lipase activities in the cell free supernatants were determined.

Partial purification of extracellular lipase enzyme

Precipitation of proteins was performed according to the chart as described by Gomori et al. (1955). Lipase produced in SmF by both the wild and mutant strains was partially purified by ammonium sulfate precipitation. Crude enzyme extracts were obtained from culture broth of both LPF-5 and HN1 strains. The crude extracts were then subjected to protein fractionation by differential ammonium sulfate precipitation to obtain three fractions (0-30, 30-70 and 70-80%) (Rifaat et al., 2010). The total protein content in the crude lysate and different fractions of ammonium sulfate was determined by spectrophotometric method (Lowry et al., 1951). All the above experiments were carried out in triplicates and mean values presented in this report.

RESULTS AND DISCUSSION

Impact of different carbon sources on lipase production

The influence of various carbon sources was investigated on lipase production by LPF-5 and HN1 strains. Figure 1 reveals that among the all carbon sources, a mixture of glucose and olive oil resulted in the highest production of lipase by LPF-5 (94.91 ± 0.60 U mL^{-1} min^{-1}) and HN1 (118.23 ± 0.73 U mL^{-1} min^{-1}). The activity of lipase was 78.59 ± 1.10 U mL^{-1} min^{-1} for LPF-5 and 99.03 ± 0.92 U mL^{-1} min^{-1} for HN1 with olive oil (1%, v/v) but increased to 94.91 ± 0.60 U mL^{-1} min^{-1} for LPF-5 and 118.23 ± 0.73 U mL^{-1} min^{-1} for HN1 in the presence of glucose (1%, w/v) and olive oil (1%, v/v). It indicates that glucose favours the growth of the fungus, while olive oil stimulates production of lipase. Triton X-100 inhibited lipase production and decreased lipase activity to 20.13 ± 0.17 U mL^{-1} min^{-1} from 38.02 ± 0.30 U mL^{-1} min^{-1} for LPF-5 and 57.98 ± 0.36 U mL^{-1} min^{-1} from 66.20 ± 0.60 U mL^{-1} min^{-1} for HN1. For the HN1, a combination of olive oil and glucose was superior, followed by olive oil, soybean oil,

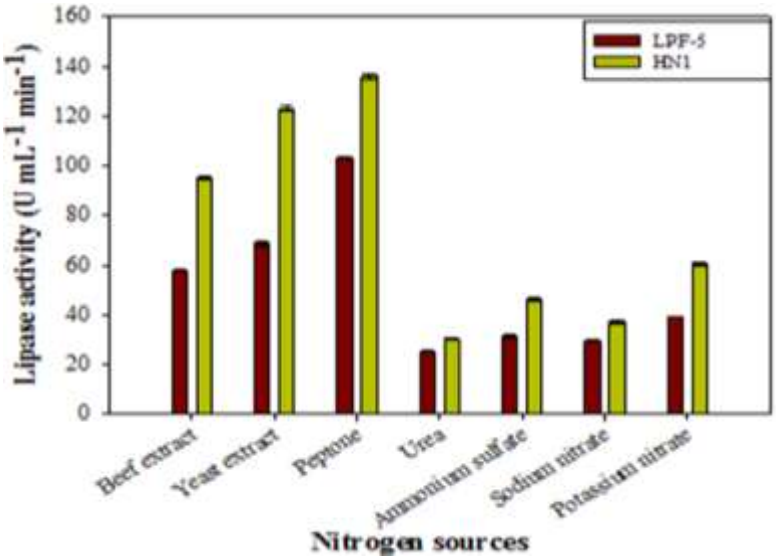

Figure 2. Impact of different nitrogen sources (1%, w/v) on lipase activity of wild (LPF-5) and mutant (HN1) strain of *A. niger* (constant parameters were: Olive oil (1%), glucose (1%), pH 7, 28°C and incubation time 3 days).

Tween-20, Tween-80, mustard oil, tributyrin and Triton X-100 in decreasing order. Hence, the mixture of glucose and olive oil was selected as the optimum carbon source for the production of lipase in all subsequent experiments.

These findings suggest that different oils are efficiently used by the fungus and the lipidic carbon sources at a concentration of 1%, v/v act as inducer for the production of lipase. It indicates that the lipidic carbon sources have the capability to enhance the lipase production at significant level due to inducible nature of lipase.

Previously published articles have shown that the production of lipase was significantly increased when lipidic carbon sources were added to the medium (Maia et al*.,* 2001; Silva et al., 2005; Nwuche and Ogbonna, 2011). Olive oil (1%, v/v) was also reported as a superior carbon source for production of lipase by *Aspergillus awamori* (Xia et al., 2011), *Aspergillus sydowii* (55 U mL^{-1}) (Bindiya and Ramana, 2012) and *Aspergillus brasiliensis* (565 U mL^{-1}) (Reshma and Shanmugam, 2013). Falony et al. (2006) reported 0.53 U mL^{-1} lipase activity by *A. niger* in the presence of olive oil (2%, v/v). This was enhanced to 0.99 U mL^{-1} when the medium was further supplemented with glucose (2%, w/v). Similar results were also obtained by Sarkar and Laha (2013) and Adham and Ahmed (2009) showing that the mixture of glucose and olive oil is effective at increasing lipase production by *A. niger*.

Impact of different nitrogen sources on lipase production

The impact of different inorganic and organic nitrogen sources on the activity of extracellular lipases by LPF-5 and HN1 was evaluated. The highest levels of activity of extracellular lipase was 103.31 ± 0.54 U mL^{-1} min^{-1} for LPF-5 and 136.19 ± 1.07 U mL^{-1} min^{-1} for HN1 when peptone was the source of nitrogen in the production medium (Figure 2). Peptone was followed by yeast extract, beef extract, KNO_3, $(NH_4)_2SO_4$, $NaNO_3$ and urea in decreasing activity. Hence, peptone was chosen as the optimum nitrogen source for the production of lipase in all the subsequent experiments. All the tested organic nitrogen sources increased the production of lipase except urea. Urea was likely toxic to the cells at the tested concentration. Urea probably denatured the cellular proteins, therefore the growth of the fungus and productivity of lipase was decreased.

Cihangir and Sarikaya (2004), whose findings are in favour of present results, reported that the optimum lipase activity (14.83 U mL^{-1}) by a novel isolate of *Aspergillus* sp. was achieved when the medium was supplemented with peptone (1%, w/v). Peptone was the optimum source of nitrogen followed by yeast extract, $(NH_4)_2SO_4$, NH_4NO_3, soyabean meal and urea. Peptone was also reported as the most suitable nitrogen source for production of lipase by *Aspergillus* sp. (21.80U mL^{-1}) (Aulakh and Prakash, 2010), *A. niger* (Mukhtar et al., 2015) and *A. carbonarius* (Ire and Ike, 2014). Besides organic sources, production of lipase was increased by *A. niger* when the medium was fed with $(NH_4)_2SO_4$ (0.35%, w/v) (Salihu et al., 2015) and NH_4NO_3 (Pokorny et al., 1994).

Impact of different initial pH on the lipase production

The impact of the initial pH of the culture medium on lipase production by LPF-5 and HN1 was studied. The

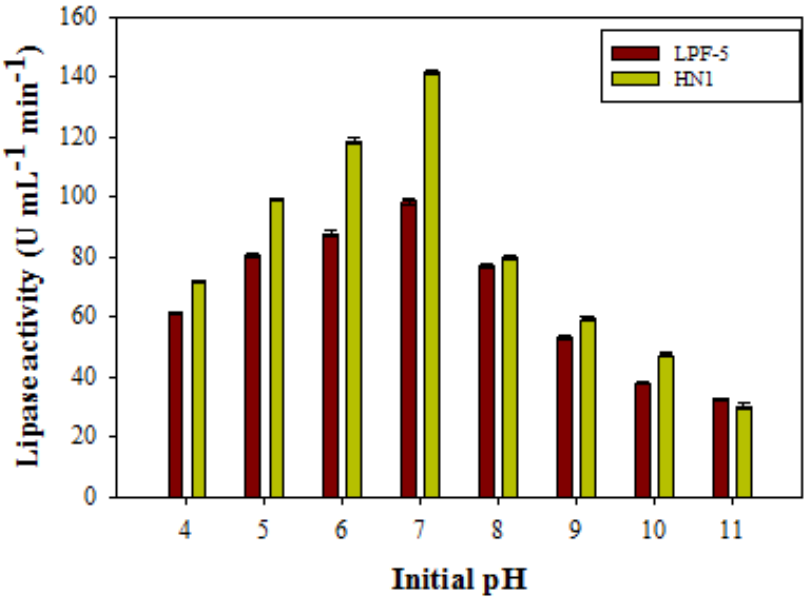

Figure 3. Impact of pH on lipase activity of wild (LPF-5) and mutant (HN1) strain of *A. niger* (constant parameters were: olive oil (1%), glucose (1%), peptone (1%), 28 ^{o}C and incubation time 3 days).

production of lipase by both LPF-5 (98.42 ± 0.84 U mL^{-1} min^{-1}) and HN1 (141.48 ± 0.91 U mL^{-1} min^{-1}) strain of *A. niger* were found to be maximum at pH 7.0. Therefore, pH 7.0 was chosen for lipase production in subsequent studies of optimization. At pH 7.0, the production of lipase by HN1 was 43% higher than LPF-5. As the pH of the fermentation medium was changed from the optimum value, activity of lipase was decreased. It might be due to that the alterations in the external pH optima reduce the availability of nutrients to the organism by altering the ionization of the nutrient molecules. It indicates that production of lipase by fungal culture is very specific to pH. Lipase activity was highest at pH 7.0, followed by pH 6.0, 5.0, 8.0, 4.0, 9.0, 10.0 and 11.0 (Figure 3). The activity of lipase by HN1 in the acidic pH range (4.0-6.0) was always higher than in LPF-5, indicating that HN1 is more tolerant of low pH conditions than LPF-5. A pH of 7.0 was also found to be the best for maximum lipolytic activity by *A. awamori* HB-03 (Xia et al., 2011) and *A. brasiliensis* (Reshma and Shanmugam, 2013). However, Abdel-Fattah and Hammad (2002) reported optimum lipase activity by *A. niger* and *A. terreus* at pH 6.0 while Mahmoud et al. (2015) reported optimum pH in the alkaline range of 7.0-9.0 for lipase production by *A. terreus*.

Impact of incubation temperatures on lipase production

Figure 4 illustrates the impact of various incubation temperatures on the production of extracellular lipases by LPF-5 and HN1. Among the various temperatures tested, the maximum production of lipase was achieved at 28°C by both LPF-5 (103.37 ± 0.92 U mL^{-1} min^{-1}) and HN1 (126.98 ± 1.25 U mL^{-1} min^{-1}). Therefore, 28ºC was chosen as the incubation temperature for subsequent studies. Lipase production was increased with the increase of incubation temperature from 18 to 28ºC but at temperatures exceeding 37ºC, it declined and reached a minimum (38.86 ± 0.28 U mL^{-1} min^{-1} for LPF-5 and 32.04 ± 0.31 U mL^{-1} min^{-1} for HN1) at 50ºC (Figure 4). This might be due to the the effect of the elevated temperature on the growth of the *A. niger*. An optimal incunbation temperature of 30ºC was also reported for lipase production by Cihangir and Sarikaya (2004) for *Aspergillus* sp., Rajan and Nair (2011) for *A. fumigatus* MTCC 9657 and Xia et al. (2011) for *A. awamori* HB-03. However, for lipase production by *A. niger*, optimum temperatures of 35ºC (Ghasemi et al., 2014) and 40ºC (Falony et al., 2006) have been reported. Iftikhar et al. (2010) have reported that the highest levels of lipase production by both wild (3.25 ± 0.01 U mL^{-1} min^{-1}) and mutant (11.28 ± 0.01 U mL^{-1} min^{-1}) strains of *R. oligosporus* IIB-63 was obtained at 30ºC.

Impact of incubation time on lipase production

Lipase activity increased with incubation period from 24 h (37.47 ± 0.48 U mL^{-1} min^{-1} for LPF-5 and 71.09 ± 0.65 U mL^{-1} min^{-1} for HN1) to 72 h of incubation (112.39 ± 0.99 U mL^{-1} min^{-1} for LPF-5 and 147.65 ± 1.14 U mL^{-1} min^{-1} for HN1). At incubation periods of longer than 72 h, this trend

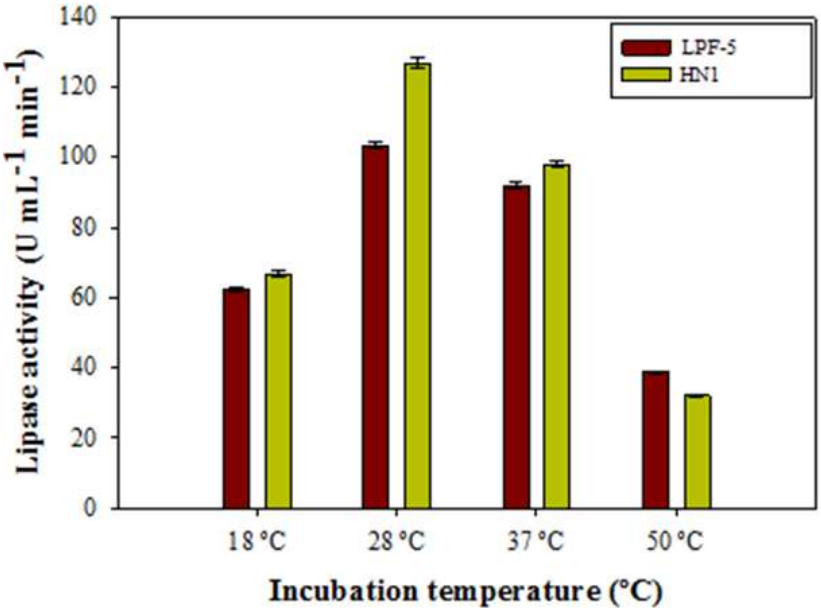

Figure 4. Impact of incubation temperatures on lipase activity of wild (LPF-5) and mutant (HN1) strain of *A. niger* (constant parameters were: olive oil (1%), glucose (1%), peptone (1%), pH 7 and incubation time 3 days).

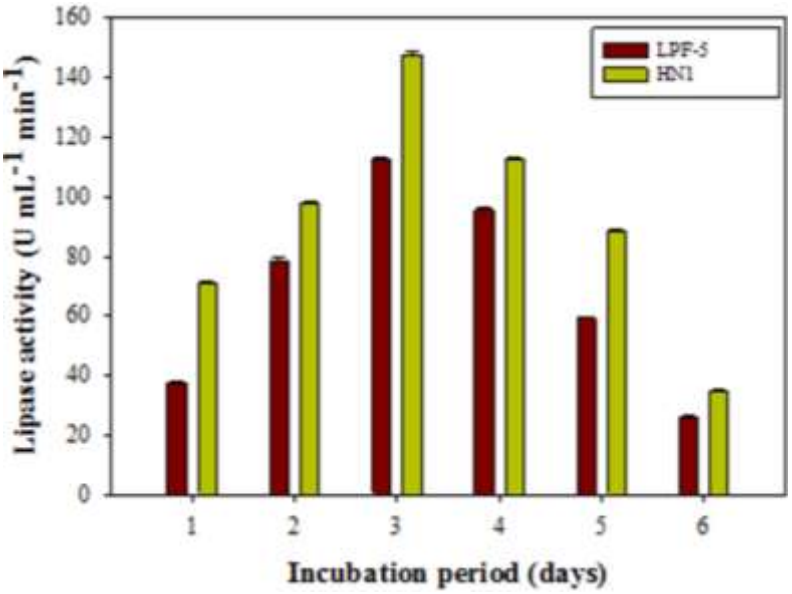

Figure 5. Impact of incubation time on lipase activity of wild (LPF-5) and mutant (HN1) strain of *A. niger* (constant parameters were: Olive oil (1%), glucose (1%), peptone (1%), pH 7 and 28°C).

reversed. Lipase production gradually decreased at incubation periods of from 96 to 144 h by both LPF-5 and HN1 (Figure 5). This declines might be due to the depletion of the culture media nutrients and the synthesis of metabolic byproducts (inhibitors) in the production medium.

The present results support the similar pattern of lipase activity at various incubation periods as reported by Mukhtar et al. (2015) where activity was highest (4.98 ± 0.05 U mL^{-1}) at 72 h of incubation, followed by 96, 48, 24 and 120 h. Niaz et al. (2013) reported maximum productivity of lipase by *A. niger* when cultured in SmF for

Table 1. Summary of the partial purification of extracellular lipase by wild (LPF-5) and mutant (HN1) strain of *A. niger*.

Purification stage	Total Vol. (mL)		Lipase activity (U mL^{-1} min^{-1})		Protein content (mg mL^{-1})		Total lipase activity		Total protein content		Specific activity of lipase (U mg^{-1})		Fold purification		% Recovery	
	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	
Crude protein extract																
	10	106.40	146.29	3.25	4.89	1064	1463	32.5	48.9	32.73	29.88	1	1		100	100
Ammonium sulfate fractionate (%)																
30%	3	117.28	154.15	2.96	4.10	351.84	462.47	8.88	12.3	39.62	37.62	1.23	1.25		33.06	31.61
70%	3	**142.06**	**204.33**	2.41	3.09	426.18	612.99	7.23	9.27	**58.94**	**66.12**	**1.81**	**2.21**		**43.43**	**41.89**
80%	3	126.95	174.71	3.09	4.36	380.85	524.14	9.27	13.08	41.08	40.07	1.26	1.34		36.02	35.82

W, Wild strain (*A. niger* LPF-5); M, Mutant strain (*A. niger* HN1); %, Percentage.

72 h. The highest levels of lipase production after 72 h of incubation were also reported by Pagori et al. (2008) for *R. chinensis* and Dheeman et al. (2011) for *Penicillium* sp DS-39.

Partial purification of extracellular lipase using ammonium sulfate

The lipases recovered after the optimization study of LPF-5 and HN1 were subjected to partial purification by $(NH_4)_2SO_4$ precipitation. Table 1 shows that among the three precipitates (30, 70 and 80%), the enzyme precipitated at 70% saturation exhibited maximum specific lipase activity of 58.95 U mg^{-1} for LPF-5 and 66.12 U mg^{-1} for HN1 when compared to specific activity of the initial crude protein lysate (32.73 U mg^{-1} for LPF-5 and 29.83 U mg^{-1} for HN1). The $(NH_4)_2SO_4$ precipitation resulted in a 1.81 fold purification for LPF-5 and a 2.21 fold purification for HN1 strain at 70% saturation at recovery rates of 43.43 and 41.89% by LPF-5 and HN1, respectively (Table 1). The partially purified lipase had lipase activity of (142.06 U mL^{-1} min^{-1} for LPF-5 and 204.33 U mL^{-1} min^{-1} for HN1) at 70% saturation. It may be due to that our lipase contains more number of hydrophilic amino acids, which strongly interact with surrounding water molecules, forming solvated layer and keep the enzyme in soluble state. Therefore, at low ionic strength (30% $(NH_4)_2SO_4$ concentration) is not sufficient to interfere this interaction but when further ionic strength is increased (70% $(NH_4)_2SO_4$) concentration), which is sufficient to disrupt the solvated layer. Under these conditions, hydrophobic interaction of enzyme molecules is dominated, which further results in aggregation and precipitation of the lipase.

The present results support the study of Jayaprakash and Ebenezer (2012) who reported that 70% $(NH_4)_2SO_4$ precipitation saturation resulted in the highest lipase activity by *Aspergillus* sp. In *Antrodia cinnamomea,* the highest levels of lipase activity were present in the 70% saturation fraction of the cell free supernatant broth and specific lipase activity also increased to 12.70 U mg^{-1} as compared to the crude lysate (10.9 U mg^{-1}) (Shu et al., 2006). Ammonium sulfate concentrations higher and lower than 70% saturation have also been cited in the literature as optimal for concentrating lipase activity. Adham and Ahmed (2009) reported maximum partial purification of lipase of *A. niger* NRRL3 at 60% saturation. Chahinian et al. (2000) reported reveals extracellular lipase activity of 25.4 U mL^{-1} by *P. cyclopium* at 80% $(NH_4)_2SO_4$ fractionation.

The study reveals that the physico-chemical environment greatly influences the enzyme production and that its optimization needs a careful manipulation of the cultural environment.

Conclusion

The optimization studies were carried to identify culture conditions that would enhance lipase production by both the LPF-5 (wild) and hyper-producer mutant strain (HN1) of *A. niger*.

The carbon and nitrogen sources and physiological parameters such as pH, temperature and incubation time were optimized and the comparison in lipase activity between mutant and wild type strains was made. During the optimization study, lipase activity of HN1 was always

found to be higher than LPF-5. The highest activity of lipase by A. niger HN1 occurred at pH 7.0 after 3 days of incubation at 28ºC using combination of glucose (1%, w/v) and olive oil (1%, w/v) as carbon source and peptone (1%, v/v) as nitrogen source. In most of the industrial applications, the use of crude enzyme is preferred over purified preparation. Hence, the crude protein extracts obtained from both strains were partially purified using $(NH_4)_2SO_4$. Among the three precipitates, the highest lipase activity by HN1 and LPF-5 strains was obtained at 70% saturation of the crude lysate. These findings suggest that partially purified lipase from *A. niger* HN1 could be potential source of enzyme for industrial use.

Conflict of interest

Authors confirms that they do not have conflict of interest in the publication.

REFERENCES

Abdel-Fattah GM, Hammad I (2002). Production of Lipase by Certain Soil Fungi, Optimization of Cultural Conditions and Genetic Characterization of Lipolytic Strains of *Aspergilli* Using Protein Patterns and Random Amplified Polymorphic DNA (RAPD). Online J. Biol. Sci. 2(10):639-644.

Adham NZ, Ahmed EM (2009). Extracellular lipase of *Aspergillus niger* NRRL3; production, partial purification and properties. Indian J. Microbiol. 49(1):77-83.

Aulakh SS, Prakash R (2010). Optimization of medium and process parameters for the production of lipase from an oil-tolerant *Aspergillus* sp. (RBD-01). J. Basic Microbiol. 50(1):37-42.

Bindiya P, Ramana T (2012). Optimization of lipase production from an indigenously isolated marine *Aspergillus sydowii* of Bay of Bengal. J. Biochem. Technol. 3(5):503-211.

Chahinian H, Vanot G, Ibrik A, Rugani N, Sarda L, Comeau LC, (2000). Production of extracellular lipases by *Penicillium cyclopium* purification and characterization of partial acylglycerol lipase. Biosci. Biotechnol. Biochem. 64(2):215-222.

Cihangir N, Sarikaya E (2004). Investigation of lipase production by a new isolated of *Aspergillus* sp. World J. Microbiol. Biotechnol. 20(2):193-197.

Das A, Shivakumar S, Bhattacharya S, Shakya S, Swathi SS (2016). Purification and characterization of a surfactant-compatible lipase from *Aspergillus tamarii* JGIF06 exhibiting energy-efficient removal of oil stains from polycotton fabric. 3 Biotech. 6(2):1-8.

Dheeman DS, Frias JM, Henehan GTM (2011). Purification and characterization of an extracellular lipase from a novel strain *Penicillium* sp DS-39 (DSM23773). J. Mol. Catal. B Enzym. 72(3-4):256-262.

Falony G, Armas JC, Mendoza JCD, Hernandez JLM (2006). Production of Extracellular Lipase from *Aspergillus niger* by Solid-State Fermentation. Food Technol. Biotechnol. 44(2):235-240.

Ghasemi M, Farahbakhsh A, Farahbakhsh A, Safari AA (2014). Using Submerge Fermentation Method to Production of Extracellular Lipase by *Aspergillus* niger. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng. 8(9):1020-1023.

Gomori G, Colwick SP, Kaplan NO (1955). Preparation of buffers for use in enzyme active studies. In. Methods in Enzymology. Academic Press Inc. Pub., New York, 1: 138-146.

Hasan F, Shah A, Hameed A(2009). Methods for detection and characterization of lipases: A comprehensive review. Biotechnol. Adv. 27(6):782-798.

Iftikhar T, Niaz M, Haq IU (2010). Comparative studies on the lipolytic potential of wild and mutant strains of *Rhizopus oligosporous* var. *microsporous* IIB-63 isolated from lipid rich habitats. Pak. J. Bot. 42(6):4285-4298.

Ire FS, Ike VC (2014). Screening and Optimization of Process Parameters for the Production of Lipase in Submerged Fermentation by *Aspergillus carbonarius* (Bainer) IMI 366159. Annu. Res. Rev. Biol. 4(16):2587-2602.

Jayaprakash A, Ebenezer P (2012). Purification and characterization of *Aspergillus japonicus* lipase from a pig fat production medium. J. Acad. Ind. Res. 1(1):1-7.

Karanam SK, Medicherla NR (2008). Enhanced lipase production by mutation induced *Aspergillus japonicus.* Afr. J. Biotechnol. 7(12):2064-2067.

Lowry OH, Rosenbrough NJ, Farr AL, Randall A (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Mahmoud GA, Koutb MMM, Morsy FM, Bagy MMK (2015). Characterization of lipase enzyme produced by hydrocarbons utilizing fungus *Aspergillus terreus*. Eur. J. Biol. Res. 5(3):70-77.

Maia MMD, Heasley A, Morais MMCD, Melo EHM, Morais MA, Ledingham WM, Lima JL (2001). Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation. Bioresour. Technol. 76(1):23-27.

Mukhtar H, Hanif M, Rehman AU, Nawaz A, Haq IU (2015). Studies on the Lipase Production by *Aspergillus niger* through Solid State Fermentation. Pak. J. Bot. 47:351-354.

Niaz M, Iftikhar T, Zia MA (2013). Effect of Nutritional Factors on Lipase Biosynthesis by *Aspergillus niger* in Solid State Fermentation. Ext. J. App. Sci. 1(1):9-16.

Nwuche CO, Ogbonna JC (2011). Isolation of Lipase Producing Fungi from Palm Oil Mill Effluent (POME) Dump Sites at Nsukka. Braz. Arch. Biol. Technol. 54(1):113-116.

Pagori N, Cheikhyoussef A, Xu Y, Wang D (2008). Production and biochemical characterization of extracellular lipase from *Rhizopus chinensis* CCTCC M201021. Biotechnol. J. 7(4):710-717.

Pathak SS, Sandhu SS, Rajak RC (2015). Mutation Studies on Fungal Glucoamylase: A Review. Int. J. Pharma Bio Sci. 5(2):297-308.

Pokorny D, Friedrich J, Cimerman A (1994). Effect of nutritional factors on lipase biosynthesis by *Aspergillus niger*. Biotechnol. Lett. 16(4):363-366.

Rajan A, Nair AJ (2011). A comparative study on alkaline lipase production by a newly isolated *Aspergillus fumigatus* MTCC 9657 in submerged and solid-state fermentation using economically and industrially feasible substrate. Turk. J. Biol. 35:569-574.

Ray A (2012). Application of Lipase in Industry. Asian. J. Pharm. Tech. 2(2):33-37.

Reshma CH, Shanmugam P (2013). Isolation and Characterization of the Lipase from *Aspergillus Brasiliensis*. Int. J. Biotechnol. Bioeng. Res. 4(5):481-486.

Rifaat HM, El-Mahalawy AA, El-Menofy HA, Donia SA (2010). Production optimization and partial purification of lipase from *Fusarium oxysporum*. J. Appl. Sci. Environ. Sanit. 5(1):39-53.

Salihu A, Bala M, Alam MZ (2015). Lipase production by *Aspergillus niger* using sheanut cake: An optimization study. J. Taibah Univ. Sci. In Press.

Sarkar D, Laha S (2013). Optimization of extracellular lipase enzyme production from *Aspergillus niger* by submerged and solid state fermentation process. Int. J. Pharma Bio Sci. 4(4):978-985.

Saxena RK, Davidson WS, Sheoran A, Bhoopander G (2003). Purification and characterization of an alkaline thermostable lipase from *Aspergillus carneus*. Proc. Biochem. 39(2):239-247.

Sharma S, Kanwar SS (2014). Organic solvent tolerant lipases and applications. Sci. World J. 2014:1-15.

Shu CH, Xu CJ, Lin GC (2006). Purification and partial characterization of a lipase from *Antrodia cinnamomea*. Proc. Biochem. 41(3):734-738.

Silva WOB, Mitidieri S, Schrank A, Vainstein MH (2005). Production and extraction of an extracellular lipase from the entomopathogenic fungus *Metarhizium anisopliae*. Process Biochem. 40(1):321-326.

Subramaniyam R, Vimala R (2012). Solid State and Submerged Fermentation for the Production of Bioactive Substances: A

Comparative Study. Int. J. Sci. Nat. 3(3):480-486.
Sundar WA, Pillai NK (2013). Isolation, Purification and Medium Optimization of Lipase Enzyme Producing Strains of *Aspergillus niger* Isolated from Natural Sources. Int. J. Pharm. Pharm. Sci. 5(2):321-324.
Thakur S (2012). Lipases, its sources, Properties and Applications: A Review. Int. J. Sci. Eng. Res. 3(7):1-29.
Toscano L, Gochev V, Montero G, Stoytcheva M (2011). Enhanced Production of Extracellular Lipase by Novel Mutant Strain of *Aspergillus niger.* Biotechnol. Biotechnol. Equip. 25(1):2243-2247.
Veerapagu M, Narayanan AS, Ponmurugan K, Jeya KR (2013). Screening, selection, identification, production and optimization of bacterial lipase from oil spilled soil. Asian J. Pharm. Clin. Res. 6(3):62-67.
Winkler UK, Stuckmann M (1979). Glycogen hyaluronate and some other pohysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J. Bacteriol. 138(3):663-670.
Xia JJ, Huang B, Nie ZY, Wang W (2011). Production and characterization of alkaline extracellular lipase from newly isolated strain *Aspergillus awamori* HB-03. J. Cent. South Univ. Technol. 18(5):1425-1433.