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Study of Effect of Nutritional Factors for Optimization of Lipase Production by Lipolytic Fungi

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Authors' contributions

This work was carried out in collaboration between all authors. Author VR designed the experiment and study, author PG performed the experimental work. Authors PP and NG carried out the statistical analysis and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To isolate lipase producing fungi from soil samples and study the effect of nutritional factors on lipase production by the isolated fungi.
Study Design: Lipase producing fungi were isolated from oil contaminated soil samples. Different carbon, nitrogen and lipid sources and their different concentrations were tested for optimum lipase production. T-test and ANOVA were performed to validate the results.
Place and Duration of the Study: Department of Biotechnology, National Institute of Technology, Raipur, From February to August, 2012.
Methodology: Isolation of lipase producing fungi from oil contaminated soils was done using Saboraud's agar medium. Identification of isolated fungi was done by Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi. Different sources and concentrations of carbon, nitrogen and lipids (inducers) were tested for optimization of extracellular lipase production. Lipase assay was performed by photometric method. Tukey's test and one way ANOVA tests were performed to validate the results obtained.

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Results: Three fungi isolated in the study were *Aspergillus ochraceous, Aspergillus fumigatus* and *Penicillium purpurogenum*. Culture amendments study indicated that maximum lipase activity for *A. ochraceous* was reported with 0.5% maltose as carbon source and 0.5% peptone as nitrogen source. For *A. fumigatus* sucrose at 1.0% was the best carbon source, while soybean meal at 1.5% as nitrogen source reported maximum lipase activity. For *P. purpurogenum* maximum lipase activity was reported with 1.5% sucrose as carbon source and 1.5% peptone as nitrogen source. Tween 80 at 1.0% was the best inducer for all the three isolates.
Conclusion: Lipase producing fungi were isolated from oil contaminated soils and culture amendments study was done to optimize extracellular lipase production.

Keywords: Lipase; nutritional modification; ANOVA; optimization.

1. INTRODUCTION

Lipases (triacylglycerol acylhydrolases EC 3.1.1.3) are enzymes that catalyze the hydrolysis of triacylglycerol and release glycerol and free fatty acids at the oil water interface [1]. This hydrolysis reaction is reversible and the enzyme can catalyse ester synthesis and transesterifcation in the reactions containing low water concentrations [2]. Lipases offer several advantages over chemical catalysts such as substrate specificity, regio- and enantio-selectivity, and lower temperature and pressure requirements [3]. They can utilize wide spectrum of substrates, are highly stable towards extreme temperatures, pH and organic solvents [4]. The fact that lipases can be produced by microorganism by utilizing a variety of substrates adds to their advantage in industrial applications. Materials such as waste vegetable oils [5], derivatives of tallow [6], wastewater from slaughterhouse [7] are some of the cheapest sources which have been reported to be used for lipase production.

Microbial lipases belong to a major group of biocatalysts in applied biotechnology. These enzymes are known for catalyzing highly specific biotransformation reactions and have been used in production of biopolymers; biodiesel, agrochemicals and many other compounds with pharmaceutical values [8]. Biodiesel production by lipase catalysis is a major area of current research. Chemical catalysts such as NaOH or KOH although result in approximately 99% transesterifcation yield, however, problems related to glycerol recovery and formation of byproducts limit the utility of chemical catalysis for biodiesel production. Accordingly, much attention has been given to enzymatic catalysis using lipase for biodiesel production. Although the cost of enzymatically catalyzed biodiesel production is higher, considering the environmental issues with chemical catalysts, the costs are comparable [9]. Thus different culture strategies have been developed in order to optimize industrial relevant production [10]. Microbial commercial lipases are mainly produced from *Pseudomonas*, *Mucor*, *Geotrichum*, *Rhizopus* and *Candida* sp. concretely; *Candida rugosa* is a well-known industrial lipase producer [11]. Other fungal species known to be extremely good producers of lipases are *Aspergillus* and *Penicillium*. Li and Zong [12] have presented an excellent review on the production, purification and application of commercial lipases from *Penicillium sp.*

Mostly microbial lipases are extracellular, which is either produced by solid state or submerged fermentation. Purification is one of the most important and complex step in extracellular lipase production [13]. However, the cost of many novel techniques of purification exceeds the cost of production [14]. Considering the high cost of purification of extracellular lipase, an alternative method is to use whole cells as biocatalysts. This is referred to as intracellular lipase [15] and it significantly reduces the cost of purification which is otherwise necessary in extracellular lipase [16].

Being inducible, the yield of these enzymes is determined by a number of factors. Major factors controlling lipase production are culture practices (liquid or solid fermentation), bioreactor designing and media composition [17]. Sources of carbon, nitrogen and lipids mainly govern the media requirements for optimum lipase production. A number of studies on optimizing lipase production by media optimization have been designed along these factors.

Considering the huge economic paybacks from lipases, it is imperative to optimize lipase production. Many attempts have been made to improve the production of whole-cell lipase and study the culture conditions systematically.

Keeping in view the role of nutritional factors, the present study was conducted with an aim of investigating the effect of nutritional sources and production optimization of extracellular lipases.

2. MATERIALS AND METHODS

2.1 Isolation and Identification of Fungi

Lipolytic fungi were isolated from the soil samples collected from oil contaminated area in Saboraud's Agar media using standard methods of isolation. The media was composed of (per liter) 10.0g Peptone; 40.0g Dextrose; 15.0g agar-agar; adjusted to pH 5.6. All the chemicals, reagents and nutritional sources used in the whole study were purchased from Himedia Labs, Mumbai, India unless otherwise stated. The fungal isolates under study were identified by Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi as *Aspergillus ochraceous, Aspergillus fumigatus*, and *Penicillium purpurogenum*.

2.2 Plate Assay Method for Lipase Production

The lipolytic activities of fungi were qualitatively detected by plate assay method of Sierra (1957). Polyoxyethylene sorbitan monolaurate (Tween 20) was used as a lipid substrate in a media that contained (per litre), 10.0g Peptone, 5.0g NaCl, 0.1g $CaCl_2.2H_2O$, 20.0g Agar-agar, and the pH was adjusted to 6.0. Tween 20 was sterilized separately by autoclaving (15 minutes, 121℃) and 1ml of it was added to 100 ml of sterile and cooled basal medium. The lipolytic activity as observed by the development of a zone of precipitate formed around a fungal colony, which were point inoculated on media plates and incubated at 28℃ for 3-5 days.

2.3 Lipase Production and Preparation of Enzyme Extract

The fungi were grown on Czapek's Dox medium with composition (per litre), 30.0g Sucrose; 2.0g $NaNO_3$; 1.0g KH_2PO_4; 0.5g KCL; 0.5g $MgSO_4$; 0.001g $FeSO_4$ and pH was adjusted to 6.0. Twenty ml of culture medium was taken in a 100 ml conical flask, inoculated with fungal mat disc cut from a fungal plate using borer of diameter 0.4 cm, and incubated at 28°C for culturing. The cultures were harvested after 5 days of incubation. The culture broth was filtered through Whatman No. 1 filter paper and wet mycelial mat was separated. The mycelial mats were washed with cold normal saline. One gram of fresh mycelium was weighed and thoroughly homogenized in cold normal saline with sterilized sand with pestle and mortar at 4℃. The mycelia extract was centrifuged at 1900 g for 15 minutes at 4℃ to collect intracellular crude enzyme for assay of activity. After harvesting the fungal mat, the culture filtrate was centrifuged at 700 g for 15 minutes at room temperature to obtain cell free supernatant, which served as extracellular enzyme.

2.4 Enzyme Assay

The fungal isolates were analysed for their potential as lipase producers by assessment of their intracellular and extracellular lipase activity by photometric method. The enzyme assay was done by estimating the amount of free fatty acids released in the reaction according to the method of Itaya and Ui [18]. The site of action of lipase is the interface between the oil drops and the aqueous phase so that the degree of emulsification plays an important role in establishing the active substrate concentration. A stabilized olive oil suspension was used as a substrate for enzyme assay. The free fatty acids (FFA), released upon enzyme action were extracted using chloroform and were allowed to react with copper nitrate. The resulting complex was then allowed to react with diethyl dithiocarbamate. The yellow colour thus developed was measured at 440 nm. One unit of specific enzyme activity was defined as 1 µmoles free fatty acids released/mg protein/min. Protein content of the supernatant (enzyme sample) was estimated by the method of Lowry et al. [19] using crystalline bovine serum albumin as standard protein.

2.5 Enzyme Production under Different Nutritional Conditions

To study the effect of various nutritional factors on the production of lipase, a basal medium as described by Okeke and Okolo (20) was used which contained, (in liters), 20.0g Sucrose; 10.0g Peptone; 10g Tween 80; 6.0g KH_2PO_4; 1.0g KCl; 5g $MgSO_4$, and the pH was adjusted to 6.0. Effects of different carbon sources, nitrogen sources, carbon and nitrogen concentration and inducers were carried out in media based on the report of Okeke and Okolo [20] to optimize the production of extracellular lipases of the three fungi.

2.6 Effect of Carbon Sources and their Concentrations on the Production of Extracellular Lipase

To study the effect of carbon sources on the production of extracellular lipase glucose, galactose, xylose, maltose, mannitol and starch were selected as carbon sources. Sucrose was replaced in the basal medium by equal amounts of other sugars to be tested, while all other ingredients remaining the same. The fungal isolates were grown in duplicate flasks for 5 days with each carbon source and lipase activity was assayed in culture filtrate. The carbon source found to be the best for enzyme production for respective fungi was selected and their different concentrations i.e. 0.5%, 1%, 1.5%, 2%,3%, 4%, 5%and 6% were used in the culture medium and extracellular enzyme activity was determined. During these trials nitrogen and inducer source were kept constant as peptone (10g/l) and Tween 80 (10g/l) respectively.

2.7 Effect of Nitrogen Sources and Their Concentration on the Production of Extracellular Lipase

Different nitrogen sources selected for the present study were tryptone, soybean meal, yeast extract, sodium nitrate and ammonium sulphate. The peptone was substituted by other nitrogen sources in the culture medium for all the three isolates. The culture filtrate was taken and lipase activity was determined. The nitrogen sources giving the maximum lipase activity for each fungi was taken in different concentrations i.e. 0.25%, 0.5%, 1%, 1.5%, 2%, 3%, 5% and 7% in culture medium and the extracellular lipase activity was assayed. During these trials carbon and inducer source were kept constant as sucrose (20g/l) and Tween 80 (10g/l) respectively.

2.8 Effect of Inducers on Production of Extracellular Lipase

Effect of different lipids as inducers on enzyme production was observed. Tween 80 and four different oils i.e. olive oil, soybean oil, groundnut oil and mustard oil at a concentration of 1% were selected for the present study. The concentration of Tween 80 was also varied and different concentrations i.e. 0.5%, 1%, 1.5% and 2% of Tween 80 were added to the culture medium to select the suitable concentration for production. Table 1 presents the fatty acid composition of the various oils used as inducer in this study. During these trials carbon and nitrogen source were kept constant as sucrose (20g/l) and peptone (10g/l) respectively.

2.9 Statistical Analysis

The results are the mean of three replicates with value of standard deviation. Significance of the differences among the replicates were analysed by using probability values (*p*). One way ANOVA test was also performed to determine whether the variation in enzyme activity is attributable to change in concentration of carbon, nitrogen or lipid source.

Table 1. The fatty acid composition of various oils used as inducer for extracellular lipase production

Oil used as inducer	Palmitic acid (hexadecanoic) C16	stearic acid (n-octadecanoic) C18	oleic acid (C18:1)	Linolenic acid (C18:3)	Lenoleic acid (C18:3)	Arachidic acid (Eicosanoic) C20
Groundnut oil	6-9%	3-6%	52-60%	----	13-27%	2-4%
Olive oil	7-16%	1-3%	65-80%	----	4-10%	0.1-0.3%
Soybean oil	7-1 %	2-6%	22-34	5-11 %	43-56%	----
Mustard oil	1.5 %	0.4%	22%	6.8 %	14.2%	----

(Source: http://www.chempro.in/fattyacid.htm)

3. RESULTS

3.1 Enzyme Activity

A total of 38 fungi were isolated from the mentioned soil samples. Initial qualitative screening was done by plate assay method and of the 38 isolates, 3 highest lipase producing fungi were taken up for quantification studies. Figs. 1 and 2 shows the lipase activity of *Aspergillus ochraceous* and *Penicillium purpurogenum* in plate assay respectively. In the second level of screening, lipase production from the 3 isolates was quantified spectrophotometrically by measuring intra and extracellular lipase production. The Intracellular lipase activity recorded in the culture filtrates of *Aspergillus ochraceous*, *Aspergillus fumigatus* and *Penicillium purpurogenum* were 0.899, 0.492 and 0.851 µmoles FFA released/mg protein/min., respectively, while extracellular lipase activity was 6.221, 3.947 and 10.263 µmoles FFA released/mg protein/min., respectively. As extracellular activity is higher than of intracellular enzyme activity, and thus, the extracellular enzyme was selected for further study to optimize its production.

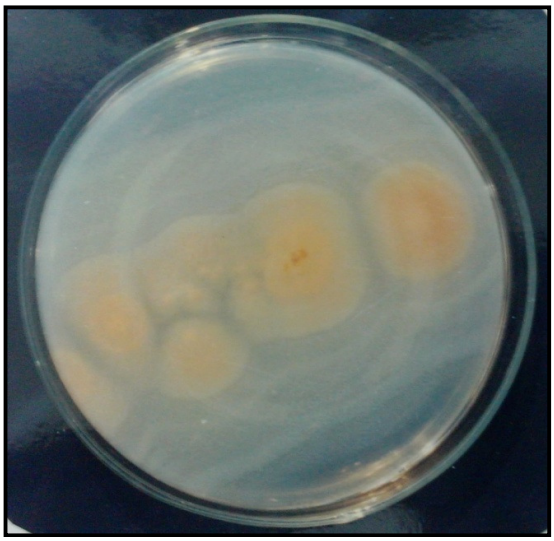

Fig. 1. Lipase activity of *Aspergillus ochraceous* by plate assay method

3.2 Culture Amendment Study for Optimum Production of Lipase

In order to optimize the production of extracellular lipase in the three fungi, culture amendment studies were carried out, wherein different carbon, nitrogen and lipid sources as well as different concentrations of the same were tested for optimal lipase production.

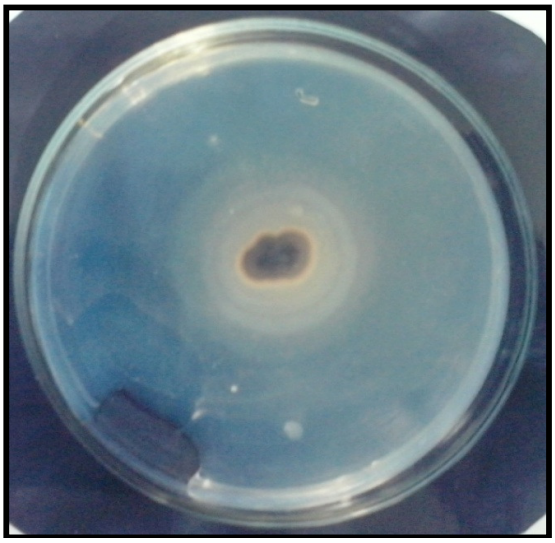

Fig. 2. Lipase activity of *Penicillium purpurogenum* by plate assay method

3.2.1 Effect of carbon sources on the production of extracellular lipase

The lipase production obtained by using different carbon sources has been tabulated in Table 2. Of the 6 different carbon sources used, maltose and sucrose gave the highest enzyme activity. It was found that the lipase activity of *Aspergillus ochraceous* reached the maximum level when maltose was used as carbon source, the value being 1.145 ± 0.073 µmoles FFA released/mg protein/min. Similarly the significant highest level of lipase activity recorded in *Aspergillus fumigatus*, and *Penicillium purpurogenum* was in presence of sucrose, the values were 1.606 ± 0.187 and 1.126 ± 0.107 µmoles FFA released/mg protein/min., respectively.

The concentrations of the best carbon source for all the three cultures were also varied and the lipase production observed has been graphically represented in Fig. 3. Maltose at a concentration of 0.5% gave the highest yield of lipase in case of *Aspergillus ochraceous*. A concentration of 1.5% and 2.0% were the most appropriate with *Aspergillus fumigatus* and *Penicillium purpurogenum* respectively. ANOVA results imply significant variation in enzyme activity with change in concentration.

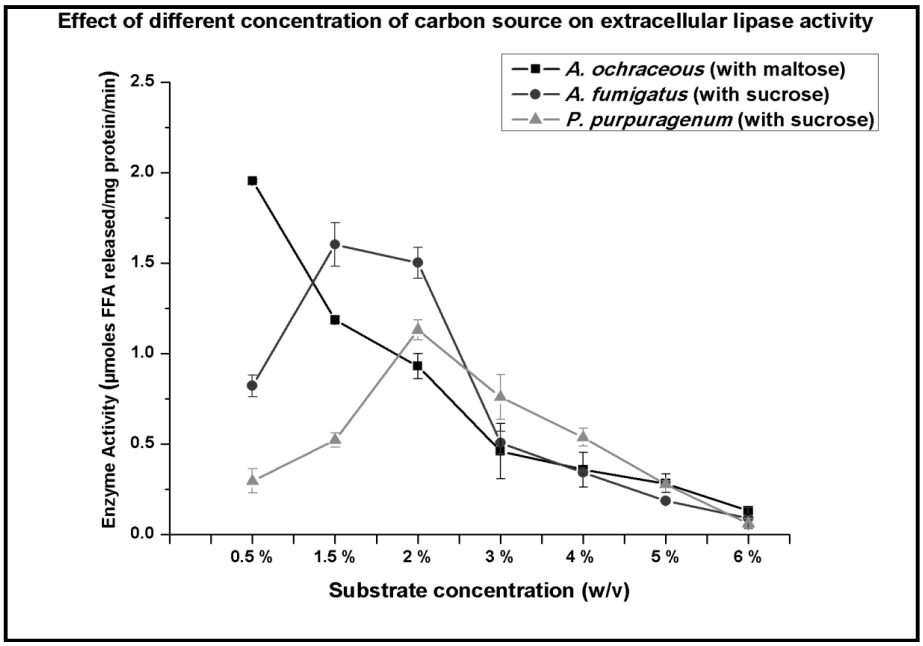

Fig. 3. Effect of different concentration of carbon sources on extracellular lipase production
For A. ochraceous the carbon source was maltose, while for A. fumigatus and P. purpurogenum it was sucrose

3.2.2 Effect of nitrogen sources on the production of extracellular lipase

The yield of lipase obtained by varying the nitrogen sources in the media are formulated in Table 3. The highest level of extracellular lipase activity was found in the culture filtrate of *Aspergillus ochraceous* in the presence of peptone, the value being 1.329±0.162 µmoles FFA released/mg protein/min. The presence of soybean meal was recorded to be the best nitrogen source for the production of extracellular lipase by *Aspergillus fumigatus*, and *Penicillium purpurogenum* the values being 1.337±0.171 and 0.991±0.372 µmoles FFA released/mg protein/min. respectively. Also the concentration of the best nitrogen source for all the three cultures was also carried over a range to identify the best concentration of the inducer. Peptone at a concentration of 0.5% gave the highest yields of lipase with *Aspergillus ochraceous* , while soybean meal at concentrations of 1.5% was the best inducer for lipase production for both *Aspergillus fumigatus* and *Penicillium purpurogenum* as can be seen from Fig. 4. ANOVA results indicate that change in concentration of nitrogen source significantly affects the extracellular lipase activity.

3.2.3 Effect of inducer

Five different lipid sources were tested to investigate the effect of different inducers on the lipase production. All the three cultures gave the highest yield with Tween 80 as shown in Table 4. *Aspergillus fumigatus* gave the highest yield of Lipase with a value of 1.558±0.373 µmoles FFA released/mg protein/min, closely followed by *Penicillium purpurogenum* (1.548±0.522 µmoles FFA released/mg protein/min) and then *Aspergillus ochraceous* (1.225±0.010 µmoles FFA released/mg protein/min). The optimum concentration of Tween 80 for the highest production of lipase was obtained by varying its concentration and measuring the yield. It was observed, and as evident from Fig. 5 that Tween 80 at 1% concentration yielded the highest amount of lipase as shown by the data for *Aspergillus ochraceous* (1.213 µmoles FFA released/mg protein/min.), *Aspergillus fumigatus* (1.128 µmoles FFA released/mg protein/min.), and *Penicillium purpurogenum* (1.026 µmoles FFA released/mg protein/min.) However, with increasing the concentration of Tween 80, a decrease in the lipase activity was recorded at 1.5 and 2% Tween 80 in all the three fungi. ANOVA results suggest that changing TWEEN 80 concentration has significant effect on lipase production only in *Penicillium purpurogenum* and not in other two isolates, i.e. *Aspergillus fumigatus* and *Aspergillus ochraceous*.

Table 2. Effect of different carbon sources on the production of extracellular lipase (µmoles FFA released/mg protein/min), at 20 g/l concentration

S. no	Fungal Isolates	Sucrose	Maltose	Xylose	Glucose	Starch	Galactose	Mannitol
1	*Aspergillus ochraceous*	1.071±0.298	1.145±0.073	1.024±0.328	0.729±0.015	0.868±0.088	0.859±0.156	0.634±0.143
2	*Aspergillus fumigatus*	1.606±0.187[a]	1.241±0.184[a]	0.753±0.128	0.971±0.146	0.653±0.490	0.764±0.494	0.664±0.292
3	*Penicillium purpurogenum*	1.126±0.107[a]	0.933±0.047	0.942±0.046	0.532±0.189	0.732±0.153	0.940±0.012[a]	0.485±0.200

**Compared with the lowest mean, irrespective of groups, level of significance p=.05 (a), p=.01 (b), p<.001 (c).*

Table 3. Effect of different nitrogen sources on the production of extracellular lipase (µmoles FFA released/mg protein/min), at 10g/l concentration

S. no.	Fungal isolates	Soybean meal	Peptone	Yeast extract	Tryptone	Sodium nitrate	Ammonium sulphate
1	*Aspergillus ochraceous*	1.017±0.016[b]	1.329±0.162[a]	1.098±0.065	0.679±0.044	1.242±0.353	1.192±0.187[a]
2	*Aspergillus fumigatus*	1.337±0.171[b]	0.992±0.132[a]	1.121±0.439	0.429±0.276	0.672±0.276	0.845±0.209
5	*Penicillium purpurogenum*	0.991±0.372	0.725±0.150	0.693±0.132	0.455±0.143	0.584±0.023	0.785±0.139

** Compared with the lowest mean, irrespective of groups, level of p=.05 (a), p=.01 (b)*

Table 4. Effect of different lipid sources on the production of extracellular lipase (µmoles FFA released/mg protein/min)

S. no	Fungal isolates	Control	Olive oil	Soybean oil	Groundnut oil	Mustard oil	Tween 80
1	*Aspergillus ochraceous*	0.311±0.072	1.192±0.114[b]	1.023±0.253[a]	0.808±0.035[b]	0.324±0.101	1.225±0.010[c]
2	*Aspergillus fumigatus*	0.426±0.110	0.878±0.070[a]	0.587±0.049	0.900±0.014[b]	0.187±0.079	1.558±0.373[a]
3	*Penicillium purpurogenum*	0.443±0.176	0.696±0.056	0.583±0.000	0.661±0.042	0.361±0.019	1.548±0.522

** Compared with the mean value of control group, level of significance p=.05 (a), p=.01 (b), p<.001 (c)*

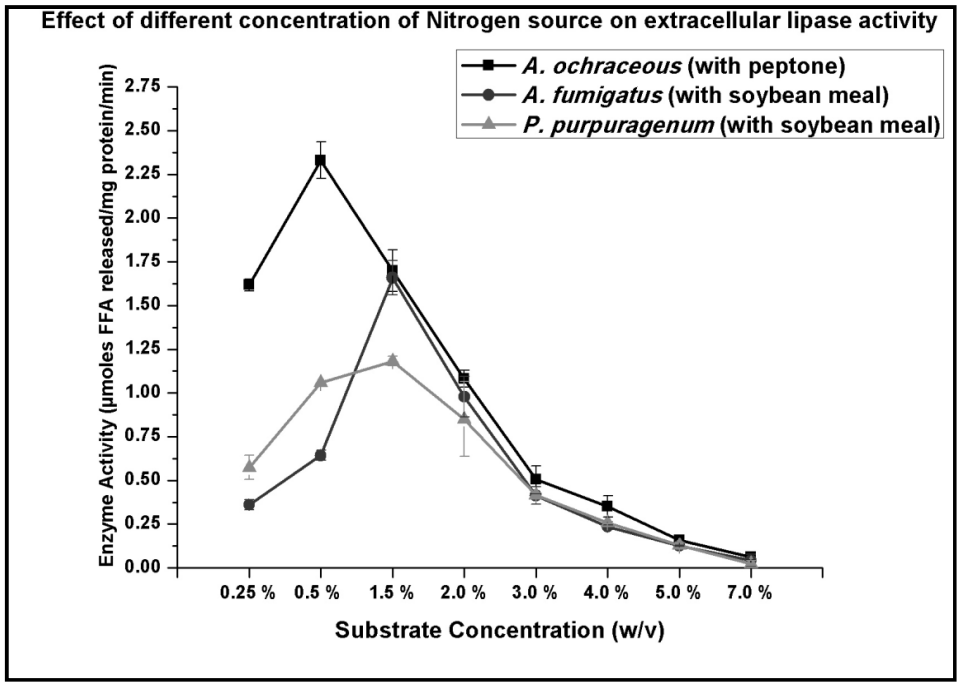

Fig. 4. Effect of different concentration of nitrogen sources on extracellular lipase production
For A. ochraceous the nitrogen source was peptone, while for A. fumigatus and P. purpurogenum it was soybean meal

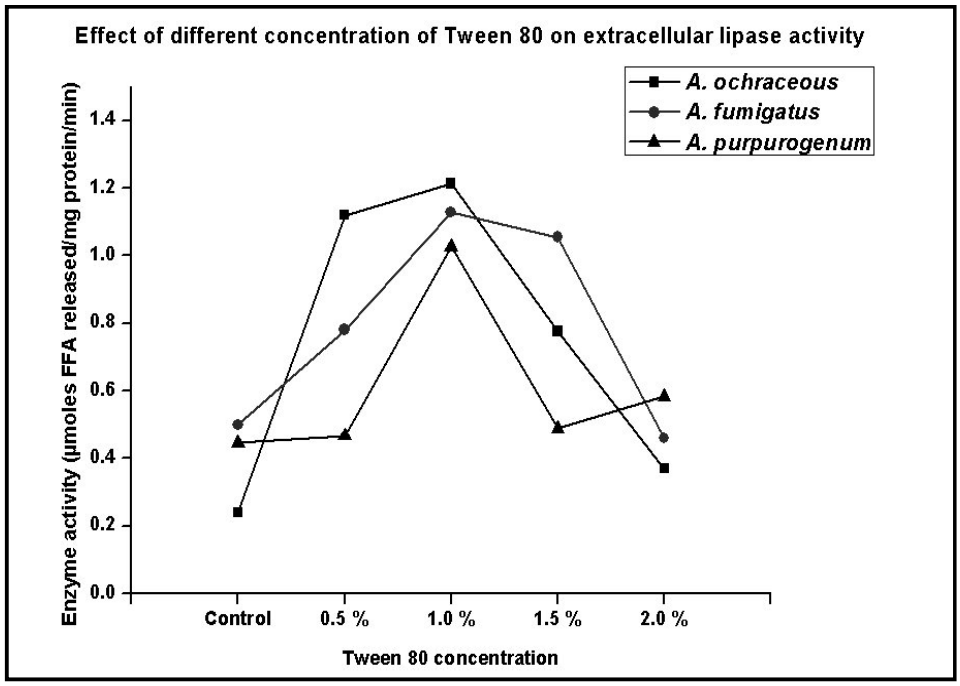

Fig. 5. Effect of different concentration of Tween 80 on extracellular lipase production

4. DISCUSSION

The present study consists of evaluation of different culture conditions in different fungal isolates for their capacity to produce lipase. Three fungi, out of total thirty eight isolates were screened out for their lipolytic activity by plate assay method. Plate assay method of qualitative screening has been reported by several other workers [21,22,23]. The three fungi were identified as *Aspergillus ochraceous*, *Aspergillus fumigatus*, and *Penicillium purpurogenum*. *Aspergillus fumigatus* has been known to be an efficient lipase producer, though not much literature is available about *Aspergillus ochraceous* or *Penicillium purpurogenum* [24,25,26,27,28].

These fungal isolates were then tested for their capacity for production of extracellular lipase under varied nutritional conditions. Culture amendments were done such as different carbon, nitrogen and lipid sources were used to test the lipase production. This was followed by assessing for lipase production in different concentrations of the best carbon, nitrogen and lipid source.

Type and concentration of carbon source is important regulating factor in lipase production. Other than carbohydrate source, vegetable oils, lipids, fatty acids and triglycerols have also been used for lipase production [29]. Generally a concentration of 0.5-1.0% of carbon source has been reported to be optimum for lipase induction [30,31]. A mixture of carbohydrate source has also been suggested for enhanced lipase production [32]. Our results indicate that maltose and sucrose to be the best carbohydrate based carbon source among the others tested in the study. The probable reason for higher lipase activity of *A. ochraceous* at lower sugar concentration may be because the carbon requirements of the strain could be low, or else peptone, which is used as nitrogen source can suffice for the carbon requirements of the strain.

Nitrogen is another important nutritional factor responsible for higher lipase production. Nitrogen source can either be organic or inorganic. Complex organic nitrogen sources such as yeast extract, peptone, and soybean extract have been traditionally used for lipase production [33,34,35,36]. Ammonium sulphate is the most common inorganic nitrogen source reported to give higher yields of lipase [32,37]. Other than that, KNO_3 has also been reported to yield high yields of lipase [38]. Inorganic nitrogen sources such as metal salts are consumed fast, while organic nitrogen sources also supply essential nutrients, amino acids and other growth factors required for metabolism and enzyme synthesis [1]. A complete synthetic medium without organic components is thus not suitable for growth.

Since lipases are inducible enzymes, inducers, such as oils and lipids play an important role in their production, although the mechanism is poorly understood [39]. The length of carbon chain moiety as well as the degree of unsaturation play important role in lipase induction. Natural oils such as soybean oil, olive oil, groundnut oil etc. have been extensively used as inducers for lipase production. Our results show Tween 80 to be the best inducer of lipase production. This is because Tween 80 acts as a substrate as well as a surfactant thereby strongly inducing the lipase activity.

Other surfactants such as polyethylene glycol (PEG), SDS and Triton have also been reported to induce higher titers of lipase. Addition of surfactant changes the permeability of cell membrane which aids in increased released of lipase outside the cell. It can also desorb mycelia bound lipases or destabilize the membrane and enhance the lipase secretion. However, the effect of surfactant varies depending upon its type, concentration and microorganism.

There are many reports in literature about lipase production and optimization from fungal sources. Falony et al. [40] tested extracellular lipase production from *A. niger* using submerged and solid state fermentation. They reported a maximum lipase activity of 1.46 IU/ml in submerged fermentation with a mixture of glucose (2%) and olive oil (2%) as carbon source and 4.8 IU/ml in solid state fermentation with glucose (1.5 %) and olive oil (1.5%) as carbon source and 0.75% ammonium sulphate and 0.34% urea as nitrogen source. Abdel-Fattah and Ahmed [41] used lipids as carbon source and reported 0.4% corn oil as optimum concentration for maximum lipase production from *A. niger* (32.7 µg ml^{-1}) and *A. terreus* (26.65 µg ml^{-1}). They reported KNO_3 to be best inorganic and L-glutamic acid to be the best organic nitrogen source. This is contrary to our report where peptone and soybean meal were the best nitrogen source. This may be due to different suitability of nitrogen sources for lipase production by different fungi. Similar work on isolation and study of effect of carbon and

nitrogen sources on fungal lipase production was done by Rajeshkumar and Illyas [42].They reported Sucrose to be the best carbon source and Sodium nitrate to be the best nitrogen source for lipase production. Rani and Panneerselvam [43] also studied effects of nutritional factors on lipase production. They reported sucrose (2%) and peptone (3%) to be the best carbon and nitrogen sources which partially concurs with our results.

5. CONCLUSION

Lipases are one of the most important biocatalysts used in a number of biotechnological applications. Optimization of lipase production, thus, is an imperative commercial necessity for various industries. The present work deals with optimization of extracellular lipase production from isolated fungi by changing the nutritional composition of production medium. The change in nutritional composition such as carbon, nitrogen and lipid sources along with their concentration affects the production of extracellular lipase by all the fungi as confirmed by t-test and ANOVA. Hence, their optimization is obligatory to achieve maximum yield of enzyme.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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