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MUTAGENIC STRAIN IMPROVEMENT OF *ASPERGILLUS NIGER* (MBL-1511) AND OPTIMIZATION OF CULTURAL CONDITIONS FOR BOOSTED LIPOLYTIC POTENTIAL THROUGH SUBMERGED FERMENTATION

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Abstract

In present study an isolated hyper producer of *Aspergillus niger* (MBL-1511) was treated for sodium azide mutagenesis. Results showed 147.27 % enhanced extracellular lipase activity after 150 minutes of sodium azide treatment. Wild and mutant hyper lipase producer strains were exploited to submerged fermentation (SmF). Brassica meal as an additive agro waste product to the basal medium was optimized. Experimental conditions optima were 10% inoculum size, 30°C temperature, 96 h rate of fermentation and pH 6 for maximum lipases production. Molasses and Ammonium nitrate were optimized as the best carbon and nitrogen sources (0.6% and 0.4%) w/v respectively and sunflower oil 1% (v/v) as better inducer. Finally, an effective mutant [MBL-1511^{SA-4}(150 min)] having of 176.10% enhanced extracellular lipases production over wild (MBL-1511) strain was acquired.

Key words: Fermentation, *Aspergillus*, Lipases, SmF.

Introduction

Lipases play vital role to facilitate hydrolysis of lipids into glycerol, and fatty acids, also play role as enantioselective (Salihu *et al*., 2012). Microorganisms not only give high enzyme yields, can be manipulated genetically, cultivate rapidly on economical fundamentally available medium and can give consistent results in terms of product synthesis (Saxena *et al*., 2003). Microorganism's derived lipases are captivating biocatalysts due to their easy handling and their multiple properties (Snellman *et al*., 2002). A number of fungi including *Aspergillus, Mucor, Geotrichum, Penicillium, Candida* and *Rhizopus* are industrially exploited because of the fate to produce lipases (Elibol & Ozer, 2000). Industrially valuable enzymes are being obtained from genus *Aspergillus* so, it is regarded as a model microorganism for synthesis of fungal enzymes (Holker *et al*., 2004).

Mutagenesis is now widely employed for strains improvement in connection to better results in terms of industrial products both qualitatively and quantitatively. It is considered that strains can be improved for better lipases yields (Iftikhar *et al*., 2010). So, various techniques are used and submerged fermentation is considered convenient because extraction and purification of enzyme by this process is simple as well easy to handle and control (Ito *et al*., 2001; Subramaniyam & Vimala, 2012). Submerged fermentation technique (SmF) is preferred over Solid state fermentation (SSF) due to multiple facts such as to control, mediate, recovery of elements at any stage (Sandhya *et al*., 2005).

Lipases being third major group of biocatalysts on account of their industrial employment are widely produced (Saxena *et al*., 2003). Currently to meet this industrial demand, different enzymes are imported from other countries. The objective of the present work is to improve the strain through mutagenesis. Moreover, the wild strain of *Aspergillus niger* (MBL-1511) was exploited for improving its lipolytic potential using SmF. Further, from results it was also intended that it would be accommodating in designing the medium for commercial production of lipases. The results will also be helpful for establishment of industrial unit for lipases production.

Materials and Methods

Microorganism: A purified culture of *Aspergillus niger* (MBL-1511) was obtained from the laboratory of Mycology and Biotechnology, Department of Botany, Government College University, Faisalabad. The culture was regularly revived using 4% potato dextrose agar (PDA) medium (Iftikhar *et al*., 2010).

Inoculum preparation: In the present study concentration 10 % inoculum was used (Parveen & Manikandaselvi, 2011). Vogel medium was prepared in conical flask, cotton wool plugged and was sterilized at 15 lb/inch^2 pressure (121°C) for 15 min. One milliliter of spore suspension was aseptically transferred to the flask. The flask was incubated at 30°C in an incubator shaker at 200 rpm for 24 h (Iftikhar *et al*., 2010).

Media preparation: Various fermentation medium were tested in order to check their effect on lipases potential using submerged fermentation technique.

M_1: Peptone 2, yeast extract 0.5, NaCl 0.5, Na_2CO_3 0.025, and olive oil 1g/100 mL (Rajan & Nair, 2011).

M_2: (KH2PO4 2, MgSO4·7H2O 0.5, KCl 0.5, and yeast extract 0.5) g/L (Hosseinpour *et al*., 2012).

M_3: (g L^{-1} KH_2PO_4 0.5, NH_4NO_3 0.2, $(NH_4)_2SO_4$ 0.4, $MgSO_4.7H_2O$ 0.02, Peptone 0.1, Trisodium citrate 0.5, Yeast Extract 0.2, Glucose 50% (w/v) and pH 5.5 (Iftikhar *et al*., 2010).

M_4: (Glucose 10, Peptone 20, NaCl 5, Yeast extract 5) g/L and pH 6.0 (Adinarayan *et al*., 2004).
M_5: (Ammonium Sulphate 0.1, potassium chloride 0.7, olive oil 100, Urea 0.1, peptone 2) g/L and pH 8.0 (Kashmiri *et al*., 2006).
M_6: peptone 5g, yeast extract 3g and sodium chloride 3 g per liter of distilled water (Imandi, 2008).
M_7: (peptone 2, yeast extract 0.5, Glucose 1, NaCl 0.5) g/100mL (Niaz *et al*., 2014).

Strain improvement by Sodium azide mutagenesis: After mutagenesis the strain obtained while using sodium azide gave better results. Spore suspension of *A. niger* (MBL-1511) was prepared by using phosphate buffer pH 7.0 (Elliaiah *et al.,* 2002). Then it was subjected to mutagenic treatment by adding 1 mL of sterile solution of sodium azide (250µg mL^{-1} in phosphate buffer) to 9 mL spore suspension. The reaction was allowed to proceed. Control tubes were also kept without any chemical mutagen. Time interval of mutagenic treatment oscillated from 30 to 150 min and then incubated at 30ºC. Hyper producer was then washed thrice at 5000 rpm for 10 min with sterilized distilled water and again re-suspended in 10mL sterilized buffer. The samples were serially diluted with the same buffer and plated on Sabouraud's Dextrose agar medium as it inhibits bacterial growth (Bapiraju *et al*., 2004). Further screening and selection of mutants was performed as reported by (Iftikhar *et al*., 2010).

Screening and selection of mutants: For the selection of mutants, first of all concentration of Oxgall at 1% as colony restrictor was optimized. Selection of best mutant among various variants was done by formation of kill curve (Petruccioli *et al*., 1999). Strain was subjected to catabolite repression, 2-deoxy-D-glucose was used at 1 mg/ml (Fiedurek *et al*., 1987; Gromada & Fiedurek, 1997). The colonies that appeared as background growth were picked and pursued to the lipases identification. PDA plates [g/L Tryptone 10.0, NaCl 5.0, yeast extract 5.0, Tributyrin (Sigma) 0.5, Agar 20.0 and pH 7.0] were used for the detection of potent lipolytic producer (Lee & Rhee, 1993). Then large zone producing strains were scratched, dissolved into buffer, filtered and let the reaction for extracellular lipases activity was determined titrimetrically (Kundu & Pal, 1970).

Production of extracellular lipases

Shake flask fermentation: The selected mutant strain of *Aspergillus niger* MBL-$1511^{SA\text{-}4}$(150 min) along with wild (MBL-1511) strain were screened for their lipolytic potential through submerged fermentation using the best production medium. Fifty mL of fermentation medium was transferred to each cotton wool plugged Erlenmeyer flask. The flasks were sterilized in autoclave at 15 lb / $inch^2$ pressure at 121°C for 15 min and cooled at room temperature. 10% inoculum was transferred aseptically to each flask. Flasks were then placed in the orbital shaking incubator at 30°C with shaking speed of 200 rpm. After specific incubation time the content of the flasks was used for the estimation of lipases. All experiments were carried out in triplicate (Iftikhar *et al*., 2010).

Extracellular lipase assay

Titrimetric analysis of lipases: After specific time interval lipase activity was assayed titrimetrically using olive oil hydrolysis according to the method reported by (Iftikhar *et al.*, 2008).

Protein estimation: Protein micro-assay was performed following Bradford (1976).

Dry cell mass determination: The mycelium was filtered through Whatman filter # 1. Ten mL of the sample was poured through filter paper and placed in the oven at 80°C for 24 h for constant mass (Colen *et al.*, 2006).

Statistical analysis: Experiments were performed in triplicate and the results were statistically analyzed using computer software Costat.

Results

Screening of sodium azide treated mutants: (MBL-1511)**:** In present studies, a selected hyper producer of *Aspergillus niger* (MBL-1511) was subjected to mutagenic treatment by adding 1 mL of sterile solution of sodium azide (250µg mL^{-1} in phosphate buffer) to 9 mL spore suspension. Colony formation on the petriplates, kill curve was formulated and view of different zones as shown (Fig. 1a, b & c). Results indicate 147.27% increased extracellular lipases production after 150 min of the mutagenic treatment with sodium azide (Table 1). Mutation showed significant impact on lipases production. The selected mutant was therefore assigned the code MBL-$1511^{SA\text{-}4}$(150 min) and used for further studies in parallel with wild strain.

Screening of fermentation medium: Fermentation medium play key role for enhanced lipases production by fungi. Various culture media were trialed in order to optimize the lipolytic potential of wild (MBL-1511) and mutant MBL-$1511^{SA\text{-}4}$(150 min) strains of *A. niger* (Fig. 2a & b). For this purpose, seven different synthetic media *i.e.*, M_1, M_2, M_3, M_4, M_5, M_6, and M_7 were evaluated. Of all the media evaluated, M_3 gave the highest units of lipases by both wild (MBL-1511) and mutant MBL-$1511^{SA\text{-}4}$(150 min) strains of *A. niger* (Fig. 2a & b). Therefore, M_3 medium was selected for further studies.

Volume of fermentation medium: Different volume concentrations also have great impact on the lipases production. In the present study different volume concentrations were used by *A. niger* by wild (MBL-1511) and mutant MBL-$1511^{SA\text{-}4}$(150 min) strains of *A. niger i.e.,* (25mL, 50mL, 75mL, 100mL,125mL and 150mL) for the production of lipases through SmF (Fig. 3a & b). Maximum lipases production was achieved when volume of fermentation was 50mL Fig. 3(a & b). Therefore 50 mL volume of the fermentation medium was used for further studies.

Table 1. Activity of extracellular lipases produced by wild (MBL-1511) and various potent mutants of *Aspergillus niger* MBL-1511^{SA-4}(150 min) using solid state fermentation technique.

Strain improvement by sodium azide treatment.			
Wild/mutant	**Zone size (mm)**	**Lipase activity (IU/mL)**	**% Increase or decrease in activity**
MBL$^{SA-1}_{(150\ min)}$	12	6.70 ± 0.37	121.81
MBL$^{SA-2}_{(150\ min)}$	10	6.60 ± 0.31	120
MBL$^{SA-3}_{(150\ min)}$	9	7.00 ± 1.00	127.27
MBL$^{SA-4}_{(150\ min)}$	14	8.10 ± 1.05	147.27
MBL$^{SA-5}_{(150\ min)}$	11	6.51 ± 0.1	118.36
MBL$^{SA-6}_{(150\ min)}$	13	6.95 ± 0.05	126.36
MBL$^{SA-7}_{(150\ min)}$	8	5.94 ± 0.02	108
MBL$^{SA-8}_{(150\ min)}$	9	5.75 ± 0.2	104.54
MBL$^{SA-9}_{(150\ min)}$	13	5.0 ± 0.5	90.90
MBL$^{SA-10}_{(150min)}$	10	4.80 ± 0.42	87.27

Each value is mean of triplicate and ± denote the standard error value
*Incubation temperature 30ºC, Incubation period 48 h and pH 7.0

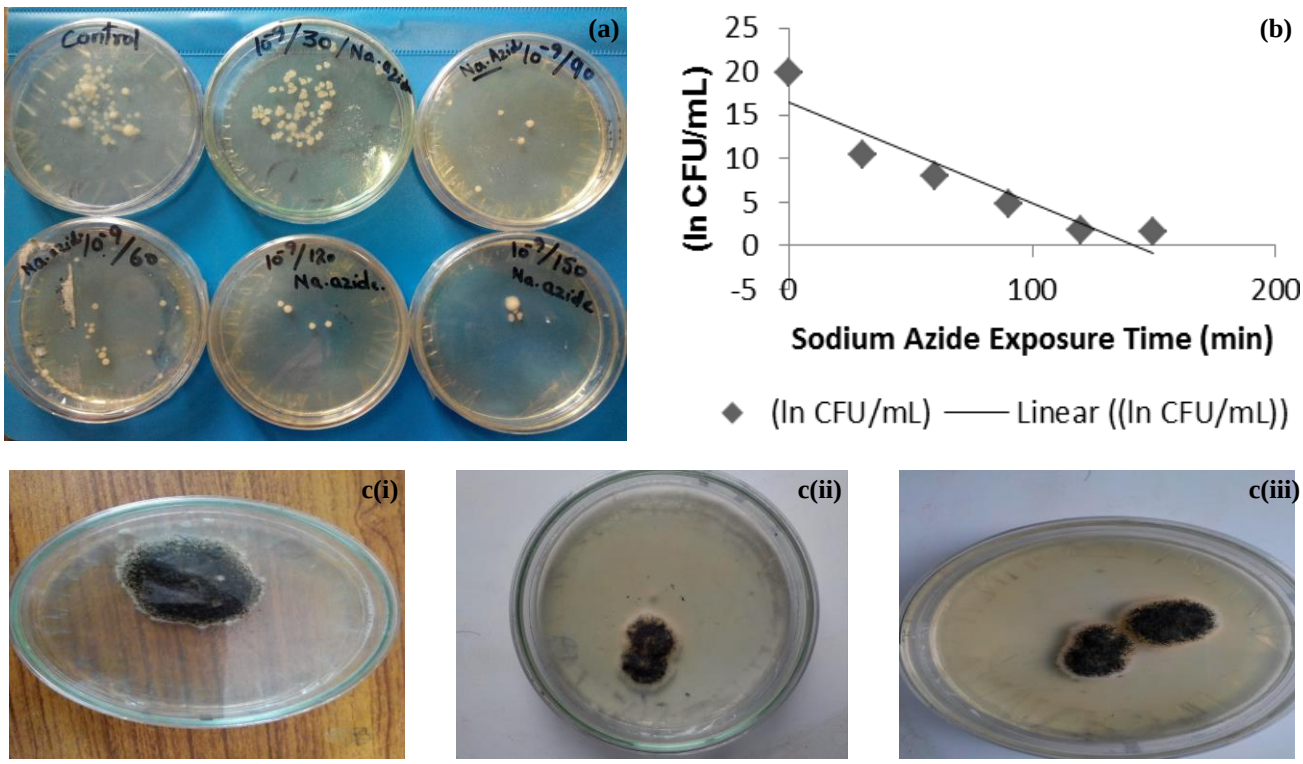

Fig. 1. a) Colony formation on the petriplates; b) Dose plot to formulate the kill curve (% of survival = 1.5/19.9 *100 = 7.53%, % of killing=100-7.53=92.47%; c) i-iii Different zones sizes of *A. niger* (MBL-1511) on the PDA Petriplates.

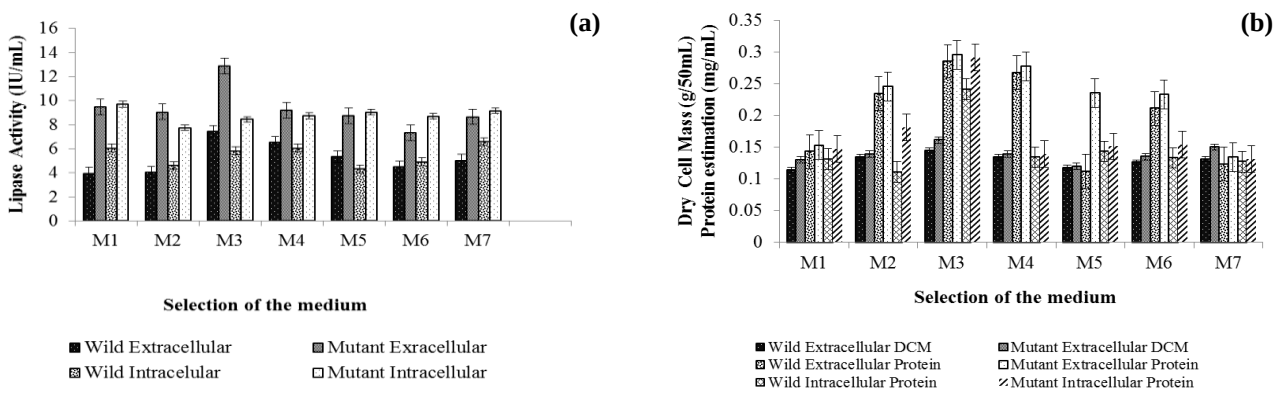

Fig. 2. Effect of media on the (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

Effect of the temperature: Temperature also effect lipases production considerably. In the present work, different incubation temperature ranges includes 20, 25, 30, 35, 40, 45, 50 and 55°C were tested for the lipases production (Fig. 4a & b). Results indicate lipases production and mycelial growth were gradually increased with the increase in temperature up to 30°C and significantly decreased thereafter. Therefore, 30°C temperature was optimized for further studies.

Effect of the pH: Initial pH also has great effect on the lipases production; a slight change in pH has great impact on the production of lipases. In present study eight different pH including 3, 4, 5, 6, 7, 8, 9 and10 pH were tested for the production of lipases by *A. niger* of wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains through SmF. Maximum units were obtained at pH 6.0 by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger* (Fig. 5a & b). Therefore, pH of 6.0 was optimized for further studies.

Selection of agro-industrial by-product as additive to the fermentation medium: Different agro industrial byproducts like 1% (w/v) sesame, rice husk, coconut, soybean, sunflower, brassica, almond and wheat bran were used as additive under submerged fermentation medium by using wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger* and the results were recorded, (Fig. 6a & b). Brassica meal was proved a better hyper-producer among all trialed additive substrates.

Rate of the fermentation: Incubation period plays crucial role in the enzyme production. In the present work different incubation time *i.e.*, 12-144 hours were experimented for obtaining maximum lipases productivity (Fig. 7a & b). Lipases production were recorded maximum at the fermentation time period of 96 h by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger*. It might also be possible that in the beginning, micro-organism was adapting to the environmental conditions. Therefore, incubation time of 96 h was optimized for further studies.

Effect of additional carbon sources: Several additional carbon sources *i.e.,* glucose, sucrose, maltose, lactose, dextrose, tween 80, fructose, starch and molasses were tested (1% w/v) with regard to their effect on lipases yield by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger* (Fig. 8a, b, c & d). Among additional organic carbon sources the molasses showed best lipase activity in both wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger*. Further, concentration of molasses *i.e.,* 0.2, 0.4, 0.6, 0.8 and 1 % (w/v) were experimented by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger* through SmF. Molasses at 0.6 % (w/v) gave the maximum units both by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger*. Therefore, Molasses at 0.6% (w/v) was used for further studies.

Effect of additional organic and inorganic nitrogen sources: In the present work, different organic and inorganic nitrogen sources including peptone, urea, yeast extract, casein, nutrient broth, malt extract, ammonium chloride, ammonium nitrate, sodium nitrate and ammonium acetate were tested (1% w/v) for hyper production of lipases and results showed in (Fig. 9a, b, c & d). Ammonium nitrate gave the maximum production both by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min). Further concentration of ammonium nitrate at 0.4% w/v gave maximum units of lipases (Fig. 9c & d). Therefore 0.4% (w/v) Ammonium nitrate was optimized for further use.

Effect of additional oils as organic carbon sources: Organic carbon sources (various oils) were used for hyper lipases productions. In the present work different organic carbon sources (1% v/v) *i.e.*, brassica oil, almond oil, coconut oil, sunflower oil, olive oil, cotton seed oil, sesame oil, castor oil, soybean oil and canola oil were tested for enhanced production of lipases (Fig. 10a & b). Results endorsed sunflower oil at 1% (v/v) for the maximum lipases production. Therefore, sunflower oil at 1% (v/v) was optimized for further studies.

Discussion

Screening of sodium azide treated mutants: (MBL-1511)**:** Mutation has great impact on the lipases production. Similar findings are reported by (Bapiraju *et al*., 2004) who stated that the UV and chemically treated fungal strains of *Rhizopus* sp. showed 133% to 232% higher production of lipases than the wild strain. The mutant strains of *Aspergillus niger* and *Aspergillus fumigatus* by sodium azide mutagenesis reported two folds increased enzyme production over wild strain, *Penicillium* sp. by UV radiation and *Penicillium* sp. by sodium azide showed 1.5 folds increased enzyme production over wild strain (Rajeshkumar & Ilyas, 2011). Mala *et al*. (2001) reported that mutant strains of *Aspergillus niger* showed 5 to 7 fold enhanced productivity of lipases over the wild strain. The selected mutant was therefore assigned the code MBL-1511^{SA-4}(150 min) and used for further studies in parallel with wild strain.

Screening of fermentation medium: Fermentation medium play key role for enhanced lipases production by fungi. Different media components have different effect on product production. It might be due to the reason that glucose and peptone are easily metabolizable carbon and nitrogen sources respectively, while KH_2PO_4 proved readily available source of potassium and phosphorous. In the present studies M_3 medium gave the maximum production, therefore M_3 medium was selected for further studies.

Volume of fermentation medium: Volume concentrations also have great impact on the lipases production. In the present studies 50 mL of the medium gave the maximum production. The improper agitation and inadequate aeration which consequently decreased enzyme production (Martinez *et al.*, 1993). Maximum production was also reported to be achieved at 45 mL as mentioned (Niaz *et al.*, 2014). Our finding is in line with previous research (Ebrahimpour *et al*., 2008). So, 50 mL of the medium was selected for further studies.

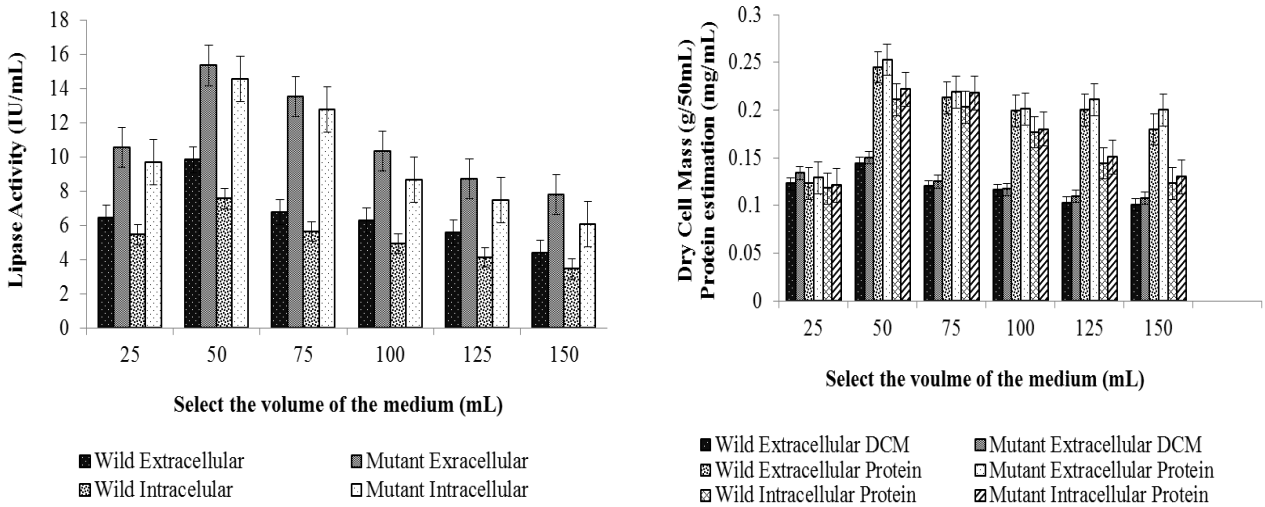

Fig. 3. Effect of volume of the medium on the (a) Extracellular and intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

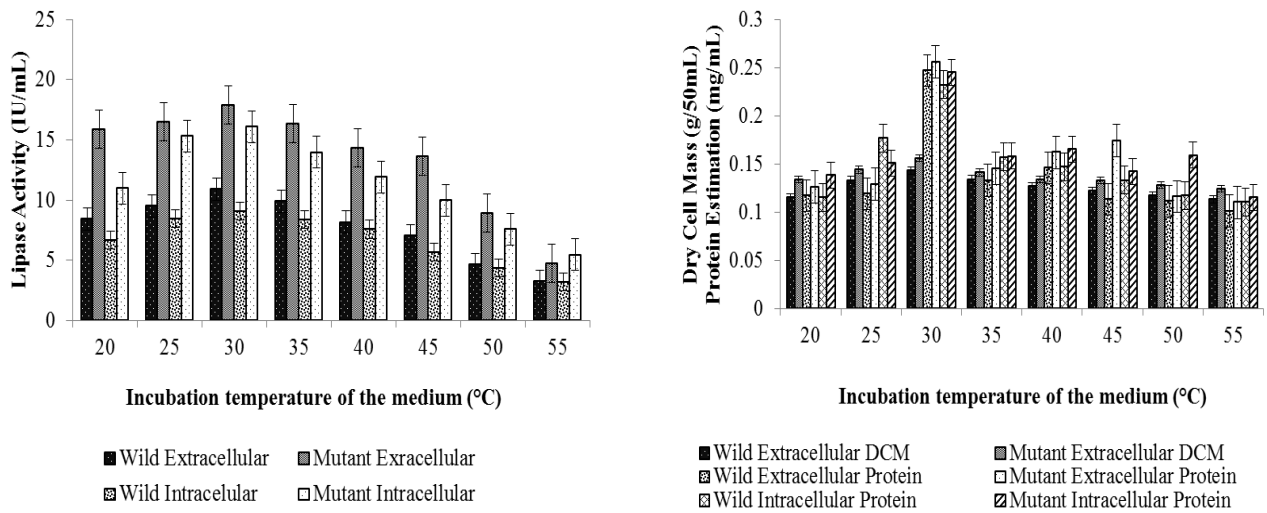

Fig. 4. Effect of incubation temperature of the medium on the (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

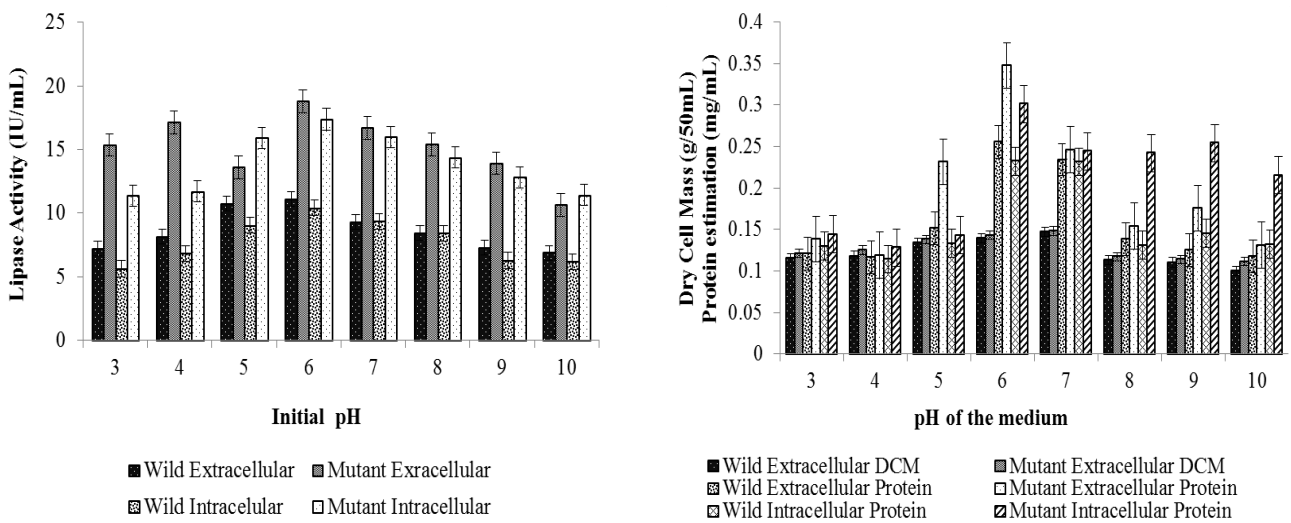

Fig. 5. Effect of initial pH of the medium on the (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

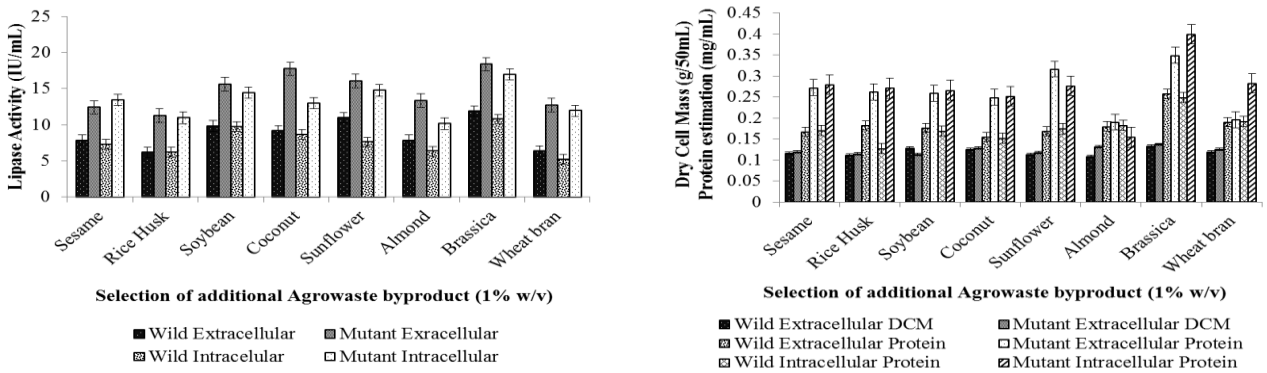

Fig. 6. Effect of agro-industrial by-products on the (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

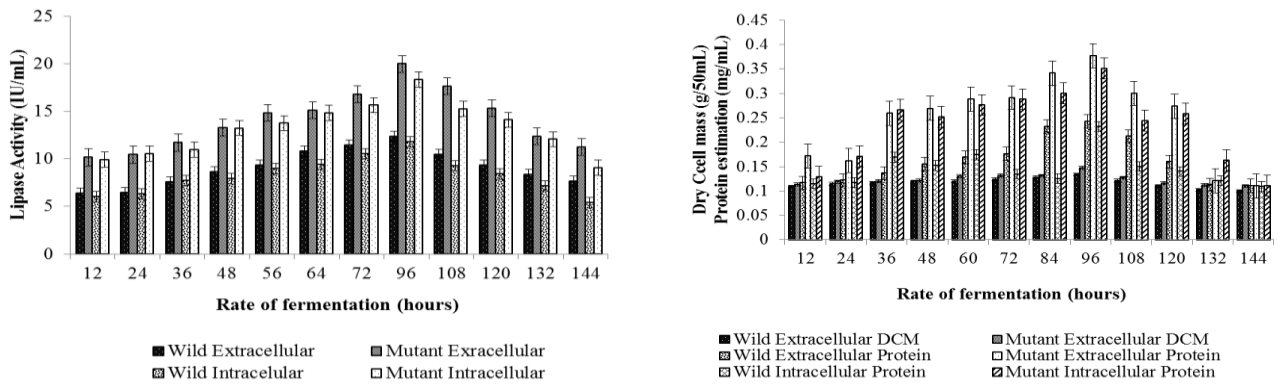

Fig. 7. Effect of Rate of fermentation of the medium on the (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

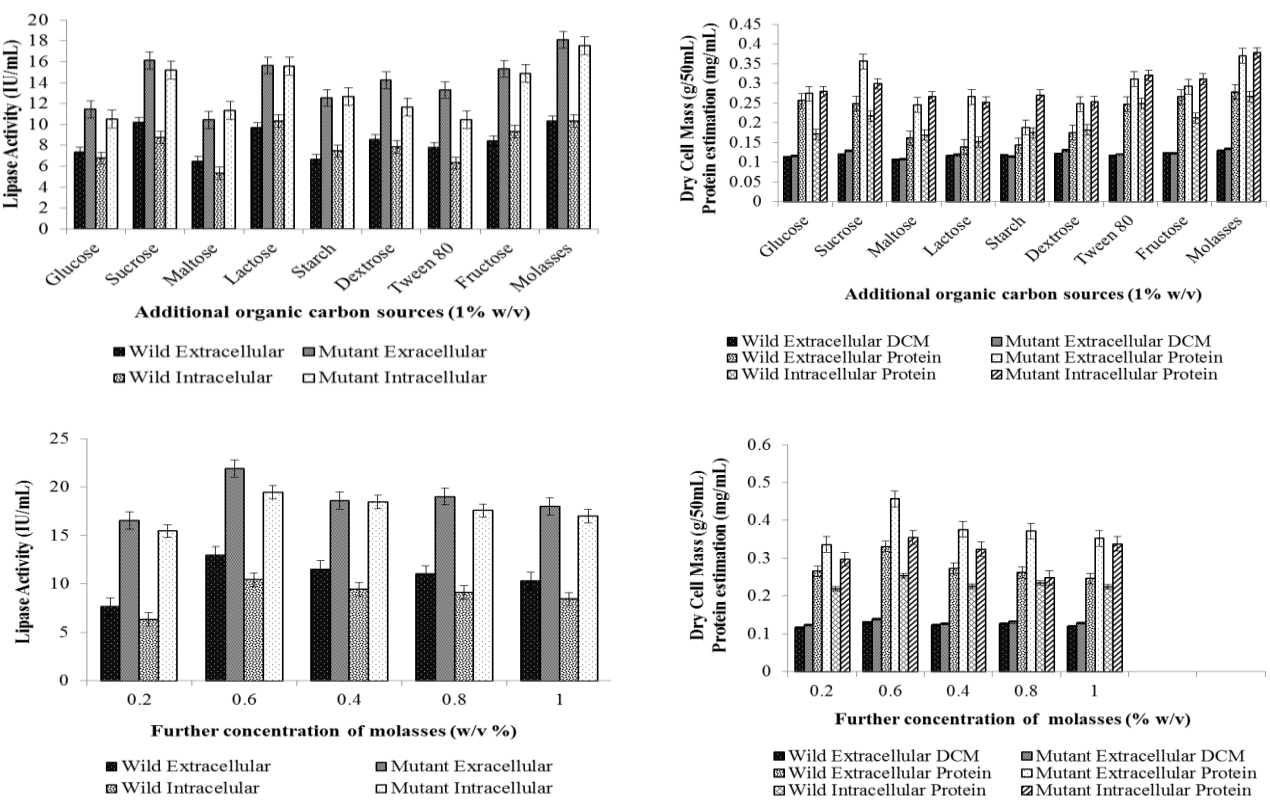

Fig. 8. Effect of additional organic carbon sources (a & c) Extracellular and Intracellular; (b & d) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

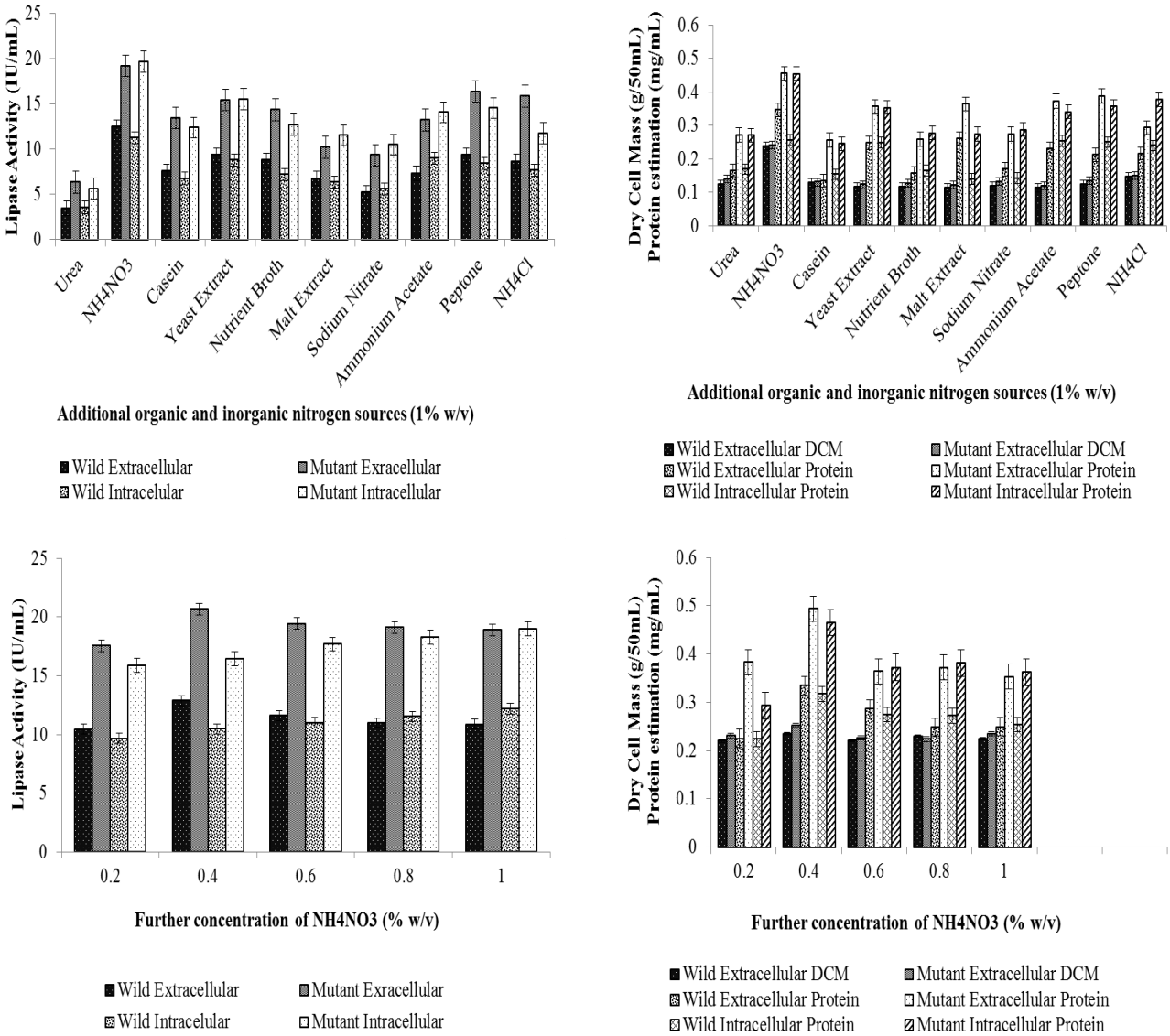

Fig. 9. Effect of additional organic and inorganic nitrogen sources (a & c) Extracellular and Intracellular; (b & d) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

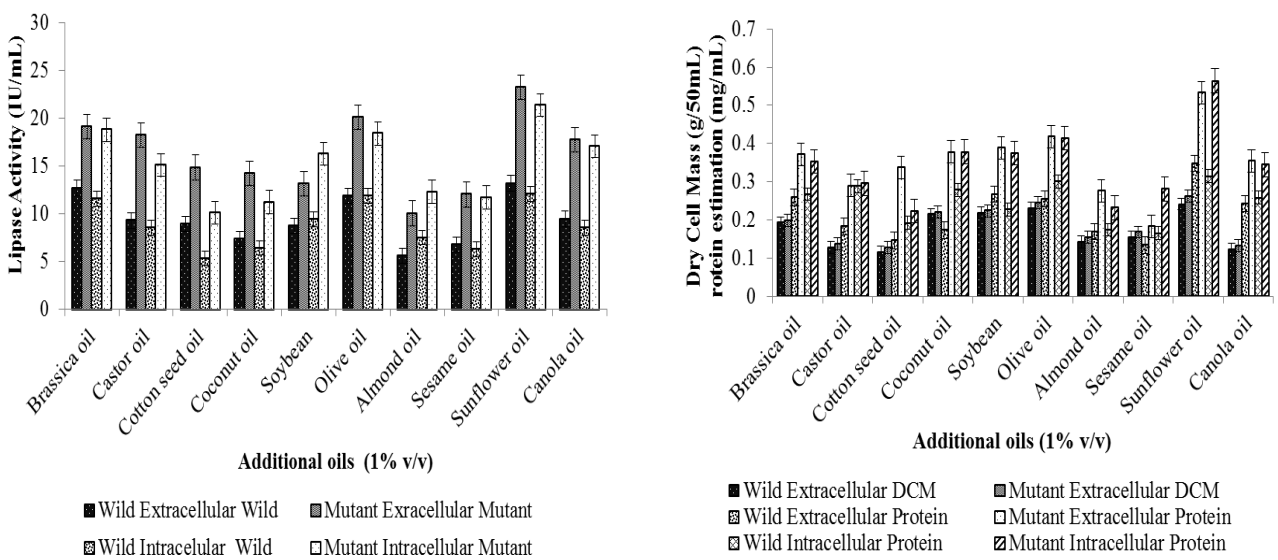

Fig. 10. Effect of additional oils as carobon sources (a) Extracellular and Intracellular; (b) Dry cell mass and protein estimation by wild (MBL-1511) and mutant (MBL-1511^{SA-4}(150 min) strains of *A. niger* on lipases production by SmF.

Effect of the temperature: Temperature also effect lipases production considerably. In the present work, results indicate lipases production and mycelial growth are gradually increased with the increase in temperature up to 30°C and significantly decreased thereafter. It has been suggested that higher temperatures may cause enzyme deactivation leading to lower lipase activity. It might be due to the reason that higher temperature alters the cell membrane structure and stimulates protein catabolism, thus causes cell death (Amin *et al*., 2014). Our results correlate with the findings of (Anbu *et al*., 2011; Hosseinpour *et al.,* 2012; Amin *et al*., 2014) who reported optimal activity at 30°C. Therefore, 30°C temperature was optimized for further studies.

Effect of the pH: Initial pH also has great effect on the lipase production; a slight change in pH has great impact on the production of lipases. In the current finding maximum units were obtained at pH 6.0. Maximum activity was achieved at pH of 6.8 (Hosseinpour *et al.,* 2012). It might be due to the necessity of acidic pH for lipase production and as a result the increase in pH resulted in decrease of lipases activity. Literature indicated maximum lipase production was achieved at the acidic pH ranges from (4.0- 5.0) Peter, (1995). Iftikhar *et al*., (2015) obtained the maximum lipase production at pH of 5. Again maximum units were obtained at pH 7.0 (Niaz *et al.,* 2014). Our results are in line with the findings of Mohanasrinivasan *et al.,* (2009), Anbu *et al*., (2011), Iftikhar *et al*., (2014). Therefore, pH of 6.0 was used for further studies.

Selection of agro-industrial by-product as additive to the fermentation medium: Different agro industrial byproducts also play a vital role in lipases production. Brassica meal was proved a better hyper-producer among all trialed additive substrates. Oil cakes contain significant amount of oil along with the seed proteins that become available to the lipolytic organisms as carbon and nitrogen source for their growth. Apart from this some essential nutrients and minerals are also found in meals. Our results are in line with findings of other workers (Dharmendra & Parihar, 2012). Therefore, brassica meal was optimized as the best substrate and further studies were conducted with this substrate.

Rate of the fermentation: Incubation period plays crucial role in the enzyme production. In the present work maximum production was recorded at the fermentation time period of 96 h by wild and mutant strains. It might also be possible that in the beginning, micro-organism was adapting to the environmental conditions. It might also be possible that in the beginning, micro-organism was adapting to the environmental conditions. It might be due to the reason that exhaustion of nutrients in substrate, which resulted in the inactivation of enzyme after specific incubation period. Decline in exponential curve might be due to the exhaustion of nutrients or loss of moisture after specific incubation period. Our results are in line with other researchers (Sethi *et al*., 2013; Amin *et al*., 2014). It is also noted that further incubation after 96 h did not enhance lipases production, but a steep decrease in lipase activity. Therefore, incubation time of 96 h was optimized for further studies.

Effect of additional carbon sources: Several additional carbon sources have great influence on the lipases production. Among additional organic carbon sources the molasses showed best lipases production in both wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger*. High enzymatic production was obtained when the microorganism was cultured in the medium supplemented with 1% sucrose as organic carbon source (Anbu *et al.,* 2011). Molasses gave the higher productivity (Potumarthi *et al.,* 2008). In the present study molasses fulfill the nutritional requirement of the medium as well as it is economical source that contains sucrose. Molasses at 0.6 % (w/v) gave the maximum units both by wild (MBL-1511) and mutant MBL-1511^{SA-4}(150 min) strains of *A. niger*. Therefore, Molasses at 0.6% (w/v) was used for further studies.

Effect of additional organic and inorganic nitrogen sources: Nitrogen sources greatly affect the lipase production. Microorganisms generally produce higher lipase levels on organic N sources (Sharma *et al*., 2001). Inorganic N sources, such as $(NH_4)_2SO_4$ and NH_4NO_3 were reported for maximum production (Dekker *et al*., 2007). It might be due to the reason it fulfilled the nutritional requirement of the medium and best for the growth of fungus as compared to other nitrogen components. Further increase the concentration didn't increase the enzyme production. Our results are in line with results of (Sujatha & Dhandayuthapani, 2013). Further concentration of ammonium nitrate at 0.4 % gave maximum units of lipases. Therefore 0.4% (w/v) Ammonium nitrate was optimized for further use.

Effect of additional oils as organic carbon sources: Organic carbon sources (various oils) produce the lipases at higher level. In the present work sunflower oil at 1% (v/v) gave the maximum lipase production. This might be due to the fact that sunflower did not affect the nutrient uptake from fungus (Martinez *et al*., 1993). It might be due to the conversion of the oil to fatty acids and then to alkyl esters with acids catalysis. Literature indicates maximum lipases production by olive oil 1% (v/v) in the medium (Wang *et al*., 2008). The results of the present findings are in accordance with studies of researchers (dos Santos *et al*., 2014).

Conclusion

In the present study, *Aspergillus niger* (MBL-1511) strain yields lipases efficiently in Submerged fermentation (SmF). SmF is an efficient, less costly, simple and directly applicable technique for the optimization of different parameters and resulted in considerably increased lipases production. Lipases activity is significantly influenced by 10% inoculum, pH 6.00, and incubation temperature 30°C, at 96 h, molasses, ammonium nitrate and sunflower oil respectively. There was 176.10% increased lipase production by mutant strain of *Aspergillus niger* over the wild strain when 1% (v/v) sunflower oil was used. These

observations suggested that every fungus requires different nutrients and physicochemical parameter for growth and yield of enzyme and each fungal strain needs optimization. Therefore, the recurrent strain of wild (MBL 1511) and mutant MBL-1511^{SA-4}(150 min) strain of *Aspergillus niger* were optimized. It will help in designing industrial scale medium that can interchangeably be used for lipases production.

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