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EXPLOITATION OF WILD AND MUTANT STRAINS OF *ASPERGILLUS NIGER* (MBL-33) FOR ENHANCED LIPOLYTIC POTENTIAL

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Abstract

The present investigation describes the production of lipases (Triglycerol acyl-hydrolases; E.C. 3.1.1.3) by using nitrous acid, a mutagen affecting non-replicating DNA, to enhance the enzyme potential of the strain using submerged fermentation technique. A native fungal strain of *Aspergillus niger* (MBL-33) was subjected to mutagenic treatment @ the dose rate of 0.1M nitrous acid. Time interval of mutagenic treatment ranged from 30 to 180 minutes. A potent mutant of *A. niger* (MBL-33) with an increased activity of 423 % over the wild strain was obtained after screening out of fifty mutants. Various basal medium was tested in order achieve ideal combination of carbon and nitrogen sources for enhanced enzyme production. Medium M-7 supported highest output of enzyme by both wild and mutant strains. The optimum conditions for the best lipases activity were attained by using 1.0 mL inoculum level at 30^{o}C after 32 h at pH of 9. Sucrose & peptone were the best additional carbon & nitrogen source @ 1% concentration respectively. Calcium (Ca^{2+}) proved the best metal ion as it supports the maximum lipases production by wild (24.52±0.37^{a} U/mL) and mutant (98.21±0.24^{a} U/mL) strain at the concentration of 1mM.

Key Words: Fermentation, *Aspergillus,* Lipases, SmF

Introduction

Lipases, triglycerol acyl-hydrolases (E.C. 3.1.1.3), split the triacylglycerol into glycerol and fatty acids. Ester synthesis in aqueous medium is another role of lipase. Lipase is the industrial enzyme that is used in various industries like detergents, leather, food, bakery and other chemical industries which enhances their commercial demand and value (Hasan *et al.,* 2010). Different sources can be exploited for the production of lipases such as animals, plants and microorganisms. In all these sources, the cheapest source is microorganism like bacteria and fungi. Fungal sources are considered more preferable than bacterial sources due to its cheap and easy availability as well as high growing capability (Houde *et al.,* 2004; Salihu *et al.,* 2012). Different fungi are used to get the maximum production of lipases like *Aspergillus nidulans, Alternaria alternata, Fusarium* sp, *Aspergillus niger, Penecilium* sp, *Rhizopus* sp *etc*., (Sztajar *et al.,* 1988; Herrgard *et al.,* 2000).

Different techniques are used for the production of lipases like solid substrate fermentation (SSF) and submerged fermentation (SmF) (Socole and Vandenbergh, 2003). About 90% of the total production of all industrial enzymes and most of the commercial biotechnological developments was achieved through submerged fermentation conditions (Holker *et al*., 2004). It is need of the time to improve the lipases qualitatively and quantitatively before its exploitation at industrial scale. Strain improvement is most widely used strategy for enhancing the production of lipases (Bapiraju *et al.,* 2004). Gromada & Fiedurek (1997) have also developed efficient screening procedures for the selection of mutants. Triton X-100 (0.01-0.1%) is also reported as colony restrictor besides Oxgall (Khattab & Bazaraa, 2005).

Lipase is considered as the third major enzyme group on account of its production potential for industrial usage (Saxena *et al.,* 2003b). Pakistan is in dire need of local enzyme production units in order to fulfill the industrial demand. Lipases are considered as one of the enzyme which is gaining importance in connection with detergent and pharmaceutical industry. Currently to meet this industrial demand, different enzymes are imported from other countries. This is the additional load on economy of Pakistan. So, it is necessary for us to produce lipases by using cheap fungal sources. From the last few years various studies have been presented on *A. niger*, presumably the most important fungus for production and emission of protein. This piece of work was designed in order to provide the basis for comprehensive studies which leads to the exploitation of local fungal flora for commercial scale enzyme production. The results will be helpful for establishment of industrial unit of lipases in order to reduce the economic burden of country.

Materials and Methods

Microorganism: An axenic culture of *Aspergillus niger* (MBL-33) was obtained from Department of Botany, GC University, Faisalabad. The culture was revived after regular intervals using 4% potato dextrose agar medium (Rhodes & Stanbury, 1997).

Mutagenesis using nitrous acid: Cell culture was subjected to 0.1M nitrous acid at an interval of 30, 60, 90, 120, 150 and 180 min. In order to remove the traces of mutagen, the cell culture was washed thrice with saline and was plated on PDA plates having 1% Oxgall as colony restrictor. Initially the mutants were picked on the basis of larger zones and then further tested quantitatively (Carlton & Brown, 1981).

Production of extracellular lipases

Shake flask fermentation: The selected mutant hyper-producer of *Aspergillus niger* (MBL-33) obtained after 150min of mutagenic treatment was subjected to submerged fermentation technique in comparison to the wild strain after following Rajeshkumar & Ilyas (2011). Various fermentation medium tested in order to check their effect on lipases potential using submerged fermentation technique (Table 1).

Table 1. Composition of various culture media tested for the enhanced production of lipases using submerged fermentation technique by wild and mutant strains of *Aspergillus niger* (MBL-33).

M_1: (Iftikhar *et al*., 2015)		M_2: (Iftikhar *et al*., 2015).		M3: (Licia *et al*., 2006).		M4: Elwan *et al*., (1986).		M5: (Hosseinpour *et al*., 2011).		M6: (Kashmiri *et al*., 2006).		M7: (Iftikhar *et al*., 2015).	
Ingredients	**g/L**	**Ingredients**	**g/L**	**Ingredients**	**g/L**	**Ingredients**	**g/L**	**Ingredients**	**g/L**	**Ingredients**	**g/L**	**Ingredients**	**g/L**
Soybean meal	10	KH_2PO_4	0.5	Peptone	30	Sucrose	2	Yeast extract	0.25	Ammonium Sulphate	0.1	Peptone	20
K_2HPO_4	2	NH_4NO_3	0.2	NaH_2PO_4	12	$MgSO_4.7H_2O$	0.05	Glucose	12.5	Potassium chloride	0.7	Olive oil	8
$MgSO_4.7H_2O$	0.5	$(NH_2)_2SO4$	0.4	KH_2PO_4	2	KCL	0.05	KCl	0.5	Olive oil,	100	$MgSO_4$	0.6
$NaNO_3$	0.5	$MgSO4.7H_2O$	0.02	$MgSO_4.7H_2O$	0.3	KH_2PO_4	0.1	Olive oil	12	Urea	0.1	KH_2PO_4	1.0
Glucose	10	Peptone	0.1	$CaCl_2$	0.25	$(NH_4)_2SO_4$	0.23	KH_2PO_4	2	Peptone	2	NH_4NO_3	1.0
Olive oil	20	Glucose	50	Olive oil	10	pH	5.4	$MgSO_4.7H2O$	0.5	pH	8.0	Glucose	10
pH	7.0	pH	5.5	pH	6.5			Peptone	0.25			pH	7.0
								pH	7.0				

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 Kempka *et al*., (2008). One unit of enzyme activity is defined as the amount of enzyme that released 1µmole fatty acid per minute under specified assay conditions.

Dry Cell Mass Determination: The mycelium was filtered through Whatman filter paper # 1. The washed mycelium was dried at 105±1°C to constant mass (Kashmiri *et al*., 2006).

Statistical analysis: The experiments were statistically analyzed by the method of Snedecor & Cochran (1980) using a computer based software (CoStat 3.03). Duncan multiple range tests was applied under one way ANOVA. Significance was presented in the form of probability ($p>0.05$) values.

Results

Microbial lipases are ubiquitous in nature and this is very importance industrial enzyme. This fact brings the lipases in lime light. Recently, much interest has been developed for the process optimization for quantitative enhancement of lipases by fungi as reported by Sharma *et al.,* (2001). Lipase production is dependent upon various cultural parameters as far as the organism's growth and enzyme production is concerned. Various process parameters which play pivotal role in enhanced enzyme production includes initial pH, incubation temperature, carbon sources, nitrogen sources, substrate concentration, inoculum size, inducer sources and concentration. The present study was conducted to investigate the production of lipases by a wild strain of *Aspergillus niger* (MBL-33) in comparison to the mutant strain by optimization of cultural conditions through submerged fermentation technique.

Screening of Nitrous acid treated mutants: In the present studies, a purified native culture of *Aspergillus niger* (MBL-33) was subjected to nitrous acid mutagenesis at the dose of 0.1M for different time intervals ranging from 30-180 min. Mutants were selected after formulation of kill curves (Data not given). Among all mutants tested, mutant-9 (M-9) derived from wild was found to be the best producer of extracellular lipases with an enzyme production of 72.16 ±0.52[a] U/mL showing 423% increase in enzyme activity over the wild strain (Fig. 1). Therefore, mutant M-9 was selected for further studies.

Screening of fermentation medium: Fermentation medium plays key role for enhanced lipases production from fungi. Various culture media were tested in order to optimize the lipolytic potential of wild & mutant strains of *A. niger* (MBL-33) (Fig. 2). For this purpose, seven different synthetic media *i.e.,* M1, M2, M3, M4, M5, M6, and M7 were evaluated. Of all the media tested, M7 gave the highest units of extracellular lipases by both wild and mutant strains of *A. niger* (MBL-33). As far as the intracellular lipases was concerned, wild (24.96±0.05[a]

U/mL) was supported by M-3 while mutant gave maximum production (33.37±0.107[a] U/mL) with M6. It was also revealed from the results although M-7 gave maximum extracellular lipases production but there is no significant difference observed in the intracellular lipases production as far as the wild and mutant strains were concerned (Fig. 2). Total protein & specific activity was also calculated (data not given). Therefore, M7 medium was selected for further studies.

Rate of fermentation: Rate of fermentation is a key factor for the enzyme production through submerged fermentation. Figs. 3a & b showed the effect of rate of fermentation on Dry cell mass (DCM), extracellular and intracellular lipases production. The wild strain showed the highest extracellular units (34.1±0.53[a] U/mL) after 72h of incubation period while mutant strain exhibits maximum activity (89.10±0.95[a] U/mL) after 32h of fermentation. On the other hand, maximum intracellular lipases activity (39.77±1.95[a] U/mL) was obtained after 72h in case of wild while mutant showed maximum intracellular activity (89.44 ± 0.58[a] U/mL) after 96h of incubation. Further, kinetic parameters were studied in order to check the rate of the production of enzyme (Table 2). Therefore, 32h was optimized for further studies.

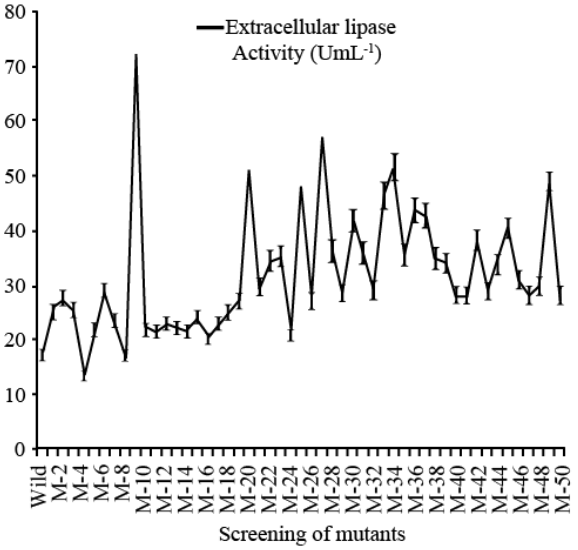

Fig. 1. Screening of mutants for the production of extracellular lipases by *Aspergillus niger* (MBL-33)

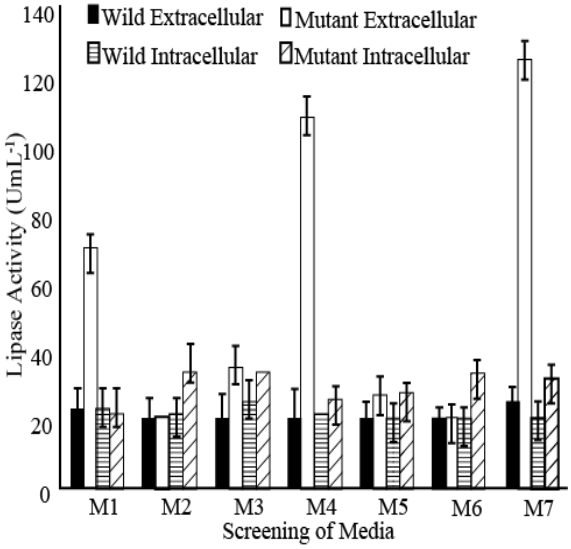

Fig. 2. Effect of fermentation medium on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

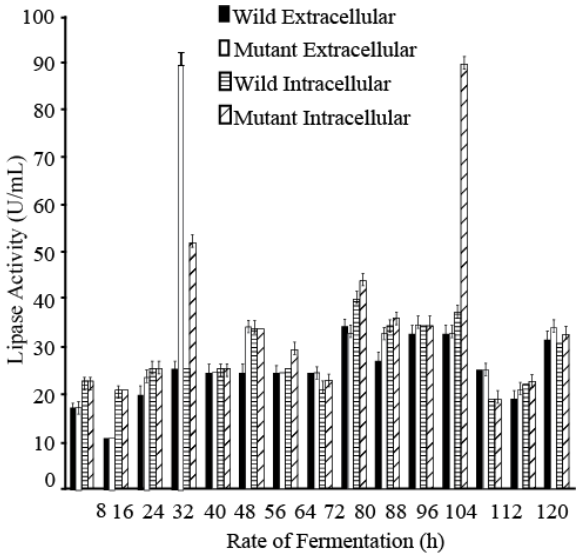

Fig. 3a. Effect of incubation period on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

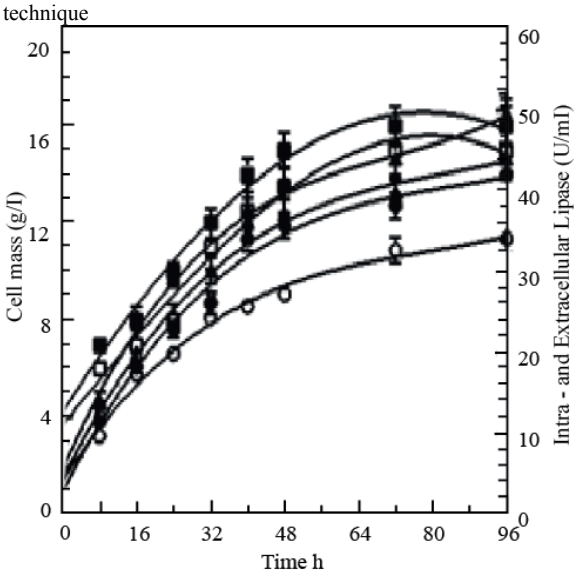

Fig. 3b. Effect of incubation period on the production of Dry cell mass (DCM) in comparison with extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

Table 2. Kinetic evaluation of selected parameters for the rate of fermentation for the production of extracellular & intracellaular lipases by *Aspergillus niger* (MBL-33) and its mutant derivative in through submerged fermentation technique.

Kinetic Parameters	Wild		Mutant	
μ (h^{-1})	0.22		0.24	
	Wild Extracellular	**Mutant Extracellular**	**Wild Intracellular**	**Mutant Intracellular**
q_p (U/g. h)	501.01	630	550	735
$Y_{p/x}$ (U/g. cells)	2277.33	2625	2500	3062.5

Kinetic parameters: $\mu(h^{-1})_{max}$ Specific growth rate, q_p Unit product produced/g cells/h, $Y_{p/x}$ Unit product produced/g cells formed

Effect of additional Carbon sources: Several additional carbon sources *i.e.,* glucose, sucrose, galactose, glycine and starch were tested with regard to their effect on lipases yield by *Aspergillus niger* (MBL-33) (Fig. 4). The highest extracellular wild lipase activity (38.5±2.27[a] U/m L) was obtained when glycine was added as the additional carbon sources in case of wild strain while sucrose supported maximum extracellular lipase production (85.0±1.10[a]U/mL) by mutant strain. Therefore, sucrose @ 1% was selected as additional carbon source for further studies.

Effect of additional organic and inorganic nitrogen sources: Additional nitrogen sources (organic and inorganic) are very effective in terms of lipases production from fungi (Fig. 5). Fermentation medium was supplemented with 1% (w/v) of nitrogen source (NH_4CI, peptone, $NaNO_3$, yeast extract and urea). Lipase activity was significantly increased when peptone was used as additional nitrogen source. The highest extracellular wild lipase production was achieved by wild (25.24±0.105[a] U/mL) and mutant (75.13±0.105[a] U/mL) strains when peptone was used as an additive. Therefore, peptone at the rate of 1% was optimized for further studies.

Effect of initial pH: The influence of initial pH of fermentation medium on lipase production by wild and mutant strains of *Aspergillus niger* (MBL-33) was investigated (Fig. 6). The best lipase yield of extracellular enzyme by both wild (58.11±0.19[a] U/mL) and mutant (90.94±1.004[a] U/mL) strains and intracellular enzyme activity by both wild (24.38±0.537[a] U/mL) and mutant (46.44±0.48[a] U/mL) strains of *Aspergillus niger* (MBL-33) was obtained. Therefore, pH 9 was selected for further studies.

Effect of incubation temperature: Incubation temperature also affected lipases production. In the present work, different incubation temperature ranges including 15, 30, 45 and 60°C were tested for the production of lipases from wild & mutants strains of through submerged fermentation technique (Fig. 7). Therefore, 30°C is optimized for further studies.

Effect of size of inoculum: Various inoculum levels have great influence on the lipases production. The size of inoculum was ranged from 0.5-2.5 mL with an interval of 0.5mL (Fig. 8). The highest extracellular output of enzyme was obtained by wild (41.5±3.04[a] UmL^{-1}) @ 2.5mL and mutant (87.22±2.54[a] UmL^{-1}) @ 1mL while intracellular yield by both wild (21.33±0.76[a] UmL^{-1}) and mutant (46.61±1.20[a] UmL^{-1}) strains of *Aspergillus niger* (MBL-33) was achieved when 0.5 mL level of inoculum was used. As the size of inoculum changes, the highest extracellular yield of mutant lipases was decreased. As the maximum production of enzyme was achieved in the presence of 1mL of inoculum, that's why it was selected for further studies.

Effect of metal ions: Different metal ions were also tested with regard to their effect on lipase production (Fig. 9). Ca^{+2} exhibited the best lipases activity at 1mM concentration. Same metal ion supported the maximum specific activity of the enzyme (data not given). Therefore, Ca^{+2} at 1mM concentration was optimized for enhanced lipases potential of microorganism.

Discussion

In the present studies, a purified native culture of *A. niger* (MBL-33) was subjected to nitrous acid mutagenesis at the dose of 0.1M for different time intervals ranging from 30-180 min. It is evident from the literature that nitrous acid mutants exhibited better efficiency as compared to the UV generated mutants in the submerged culture (Mala *et al.,* 2001). Therefore, mutant M-9 was selected for further studies.

Different medium components have different effect on product production. In the present study M7 gave the maximum lipase 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 as the source of potassium & phosphorous. Olive oil also supported the maximum production of lipases as a part of the medium. The present findings are in line with the earlier reported results (Chahinian *et al.,* 2000; Jianghua *et al*., 2000; Elibol & Ozer, 2001).

Incubation period plays crucial role in the enzyme production. In the present work different incubation time were tested to check the liplytic potential. Lipase production was achieved maximum after the fermentation time period of 32 h. This might to be due to this reason that growth of the organism is linked with the incubation time (Valeria *et al.,* 2003; Kashmiri *et al.,* 2006; Vargas *et al*., 2008; Sztajar & Maliszewska, 1989). Extracellular lipase production was higher than intracellular lipase production as reported by Peter, (1995).

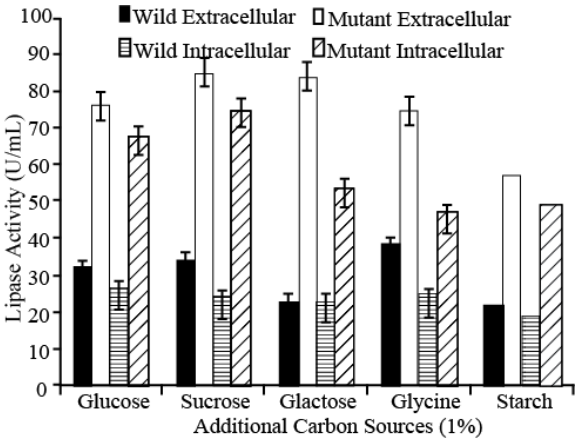

Fig. 4. Effect of additional carbon sources on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

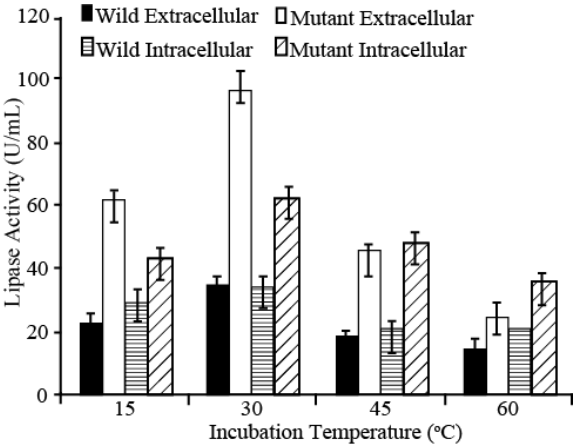

Fig. 7. Effect of incubation temperature on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

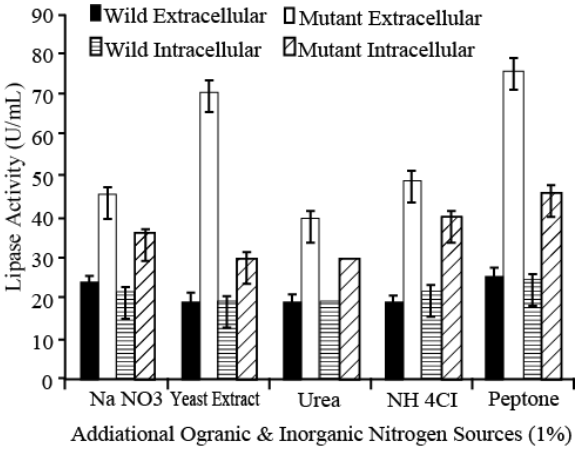

Fig. 5. Effect of additional nitrogen sources on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

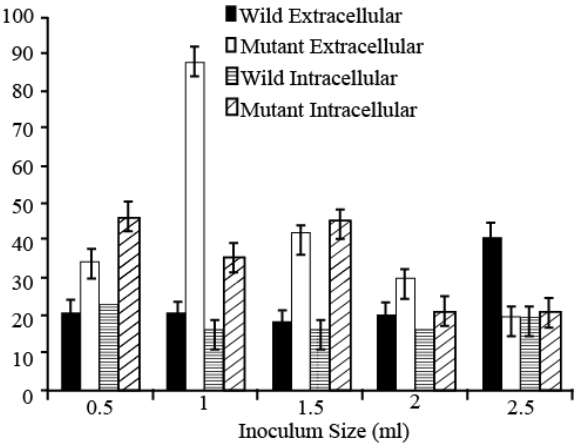

Fig. 8. Effect of inoculum size on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

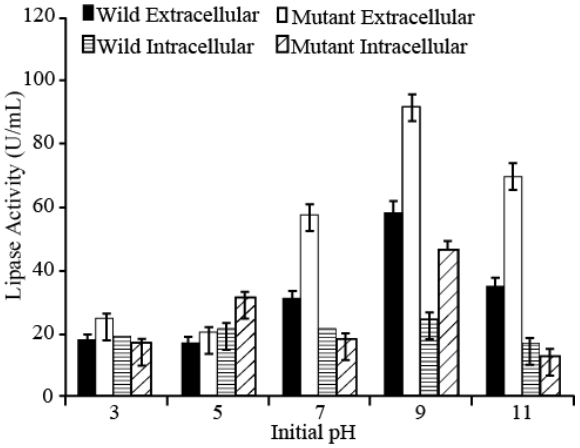

Fig. 6. Effect of initial pH on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

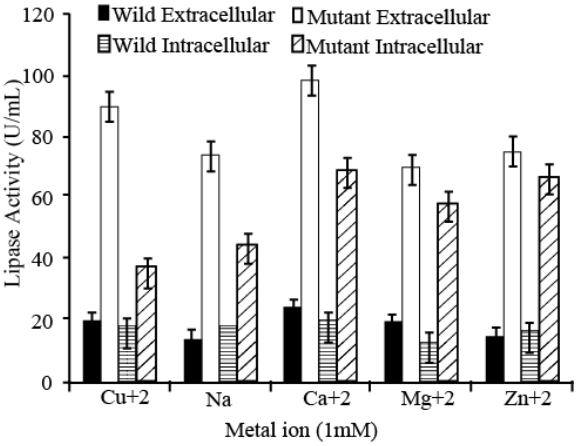

Fig. 9. Effect of metal ions on the production of extracellular & intracellular lipases by wild & mutant strains of *Aspergillus niger* (MBL-33) through submerged fermentation technique

Additional carbon sources also play a vital role in the production of lipase. In the present study several different carbon sources ***i.e.,*** glucose, sucrose, galactose, glycine and starch were tested with regard to their effect on lipases yield by *Aspergillus niger* (MBL-33). Among additional carbon sources, sucrose showed best lipase activity. It might be due to the reason that it is reported as best inducer for lipases using various microorganisms (Benjamin & Pandey, 1997; Elibol & Ozer, 2001; Chunhua *et al*., 2002). Our results were not in accordance with several researchers as they have reported galactose as the best inducer (Banerjee *et al*., 1985; Corzo & Revah, 1999). Our results are in line with the findings of Kaushik *et al*., (2006). Therefore, sucrose @ 1% was selected as additional carbon source for further studies.

Organic and inorganic nitrogen source play a vital role in the synthesis of lipase Shah *et al*., (2007). Fermentation medium was supplemented with 1% (w/v) of nitrogen source (NH_4CI, peptone, $NaNO_3$, yeast extract and urea). In the present study peptone gave the maximum production. It might be due to the fact that lipase production is greatly affected by type and concentration of nitrogen sources (Iwai & Tsujisaka, 1984; Hatzinikolaou *et al*., 1996; Sharma *et al*., 2001). Peptone was also reported as best lipases inducer in the case of *Aspergillus* sp., which is in accordance with the findings of Cihangir & Sarikaya (2004). Our findings are not in line accordance with Sztajar & Maliszewska (1989). . Therefore, peptone at the rate of 1% was optimized for further studies.

A slight change in pH has great impact on the production of lipase. Maximum activity was obtained when initial pH of the medium was adjusted at 9.0. Maximum lipase production was reported at pH 5. (Ahmad *et al.,* 2014; Iftikhar *et al*., 2015). Our results are in accordance with the earlier reports (Kamini *et al*., 1998; Mahadik *et al*., 2002; Adham & Ahmed, 2009). Therefore, pH 9 was selected for further studies.

Incubation temperature is a key parameter as far as the fermentation is concerned. The highest lipase activity obtained at 30^{o}C, which is in line with the other researchers (Kamini *et al.*, 1998; Mahadik *et al*., 2002; Saxena *et al.,* 2003a). The change of temperature resulted decrease in the productivity of both extra and intracellular lipases. The high temperature has inhibitory effect on the growth of microorganism and the productivity of enzymes (Maia *et al.,* 2001). The other workers also reported 30^{o}C as optimum incubation temperature (Elwan *et al.,* 1986; Korn & Fujio, 1997). Therefore, 30^{o}C is optimized for further studies.

Size of inoculum also has great effect on the production of lipase. 1mL of inoculum size showed maximum lipases activity in case of mutant because sufficient amount of mycelium produced for the enzyme biosynthesis. Our results resembled with the (Teng & Xu, 2007; Ushio *et al*., 1996). As the maximum production of enzyme was achieved in the presence of 1mL of inoculum, that's why it was selected for further studies.

Different metal ions influence the enzyme production to great extent. In the present studies various metal ions were tested with regard to their effect on lipases production. Ca^{+2} exhibited the best lipases activity at 1mM concentration. Our results are similar with the reports of Sidhu *et al*., (1998) and Simons *et al*., (1998). Adham & Ahmed (2009) also reported that Ca^{+2} supported highest lipase yield. Therefore, Ca^{+2} at 1mM concentration were optimized for enhanced lipases potential of microorganism.

Conclusion

In the present study a native fungal strain of *A. niger* (MBL-33) was subjected to nitrous acid mutagenic treatment @ the dose rate of 0.1M. A potent mutant of *A. niger* (MBL-33) with an increased activity of 423 % over the wild strain was obtained after screening out of fifty mutants. Medium M-7 supported highest output of enzyme by both wild and mutant strains. The optimum conditions for the best lipases activity were attained by using 1.0 mL inoculum level at 30^{o}C after 32h at pH of 9. Sucrose & peptone were the best additional carbon & nitrogen source @ 1% concentration respectively. Calcium (Ca^{2+}) proved the best metal ion as it supports the maximum lipases production by wild (24.52±0.37^{a} U/mL) and mutant (98.21±0.24^{a} U/mL) strains at the concentration of 1mM. It will help in designing industrial scale medium that can interchangeably be used for lipases production.

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References

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

Ahmad I., Z.U. Islam, Z. Yu and M.M. Javed. 2014. Propagation of *Aspergillus niger* in Stirred Fermentor for the production of Glucose Oxidase. *J. Pure & App. Microbiol.,* 8(2): 1735-1742.

Banerjee, M., I. Sengputa and S.K. Majumdar. 1985. Lipase Production by *Hansenula anomala var. Schnegii*. *Food Sci. Technol.*, 22: 137-139.

Bapiraju, K.V.V.S.N., Sujatha, P. Ellaiah and T. Ramana. 2004. Mutation induced enhanced biosynthesis of lipases. *Afr. J. Biotechnol.,* 3(11): 618-621.

Benjamin, S. and A. Pandey. 1997. Enhancement of lipase production during repeated batch culture using immobilized *Candida rugosa. Process biochem.*, 32: 437-440.

Carlton, B.C. and A.J. Brown. 1981. Gene mutation. In: *Manual of methods for General Bacteriology*. (Ed.): Gerhardt. *Am. Soc. for Microbiol.*, pp. 222-242.

Chahinian, H., G. Vanot, A. Ibrik, N. Rugani, L. Sarda and L.C. Comeau. 2000. Production of extracellular lipases by *Penicillium cyclopium* purification and characterization of partial acylglycerol lipase. *Biosci. Biotechnol. Biochem*., 64:215-22.

Chunhua, Y., X. Jiali and T. Tianwei. 2002. Lipase production by immobilized *Rhizopus arrhizus* cells and enzymatic synthesis of monoglyceride. *Process Eng.,* 2(6): 534-538.

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

Corzo, G. and S. Revah. 1999. Production and characteristics of the lipase from *Yarrowia lipolytica* 681. *Bioresour Technol.*, 70:173-80.

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

Elwan, S.H., M.S. Ammar and M.K. Moussallamy. 1986. Identity and lipase productivity of *Penicillium chrysogenum*. *Egyptian. J. Microbiol.,* 21:143-154.

Gromada, A. and J. Fiedurek. 1997. Selective isolation of *Aspergillus niger* mutants with enhanced glucose oxidase production. *J. Appl. Microbiol.*, 82: 648-652.

Hasan, F., A.A. Shah, S. Javed and A. Hameed. 2010. Enzymes used in detergents: Lipases. *Afr. J. Biotechnol.,* 9(31): 4836-4844.

Hatzinikolaou, G.D., J.B. Macris, P. Christakopolous, D. Kekos, N.F. Kolisis and G. Fountoukidis. 1996. Methods in Microbiology. *Biotechnol. Lett.,* 18: 547-552.

Herrgard, S., J.C. Gibasand and S. Subramaniam. 2000. Role of electrostatic network of residues in enzymatic action of *Rhizomucormehei* lipase family. *Biochem.*, 39: 2921-2930.

Holker, U., M. Hofer and J. Lenz. 2004. Biotechnological advantages of laboratory scale solid state fermentation with fungi. *App. Micro. Biotechnol.*, 64(2): 175-86.

Hosseinpour, M.N, G.D. Najafpour, H. Younesi and M. Khorrami. 2011. Submerged culture studies for lipase production by *Aspergillus niger* NCIM 584 on soya flour, middle-east. *J. Scientific Res.,* 7(3): 362-366.

Houde, A., A. Kademi and D. Leblanc. 2004. Lipases and their industrial applications: an overview. *App. Biochem. Biotechnol.*, 118: 155-170.

Iftikhar T., R. Abdullah, M. Iqtedar, A. Kaleem, M. Aftab, M. Niaz, Sidra, B. Tabassum and H. Majeed. 2015. Production of lipases by *Alternaria* sp. (MBL 2810) through optimization of environmental conditions using submerged fermentation technique. *Int. Biosci.,* 7(2):178-186.

Iwai, M. and Y. Tsujisaka. 1984. In: *Lipases*, (Eds.): B. Borgstrom and H.L. Brockman, *Elsevier Science*, pp. 443-466.

Jianghua, Li., W.U. Michen and W.U. Xianzhang. 2000. Fermentation technology of alkaline lipase produced by *Penicillium cyclopium* PG37. *Wuxi Qinggong Daxue Xuebao*., 19(2): 100-104.

Kamini, N., J. Mala and P. Puvanakrishanan. 1998. Lipase production from *Aspergillus niger* by solid state fermenation using gingelly oil cake. *Process Biochem.*, 33(5): 505-511.

Kashmiri, M.A., A. Ahmad and B.W. But. 2006. Production, purification and partial characterization of lipase from *Trichodermaviride*. *Afr. J. Biotechnol.*, 5(10): 878-882.

Kaushik, R., S. Saran, J. Isar and R.K. Saxena. 2006. Statistical optimization of medium components and growth conditions by response surface methodology to enhance lipase production by *Aspergillus carneus. J. Mol. Cat. B, Enzymatic.,* 40: 121-126.

Kempka, A.P., N.L. Lipke, T.L.F. Pinheiro, S. Menoncin, H. Treichel, D.M.G. Freire. M.D. Luccio and D. Oliveira. 2008. Response surface method to optimize the production and characterization of lipase from *Penicillium verrocosum* in solid state fermentation. *Biopr. Biosys. Engr.,* 31(2): 119-125.

Khattab, A.A. and W.A. Bazarra. 2005. Screening mutagenesis and protoplast fusion of *Aspergillus niger* for the enhancement of extracellular glucose oxidase production. *J. Ind. Microbiol*., 36(3): 199-205.

Korn, M.S. and Y. Fujio. 1997. Effect on the degree of maceration of soybean fermented by *Rhizopus* strains. *J. Fac. Agric.*, 41(3-4): 231-237.

Licia, M.P., M.R. Mario and R.C. Guillermo. 2006. Calalytic properties of lipase extracts from *Aspergillus niger*. *Food Technol. & Biotechnol.*, 44: 247-252.

Mahadik, N.D., U.S. Runtambekar, K.B. Bastawde, J.M. Khire and D.V. Gokhale. 2002. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Bioch.,* 38: 715-721.

Maia, M.M.D., A. Heasely, D.E. Camargo, M.M. Morais, E.H.M. Melo, J.M.A. Morais, W.M. Ledingham and F.J.L. Lima. 2001. Effect of culture conditions on lipase production by *Fusarium solani* in batch fermentation. *Bioresource Technol.,* 76: 23-27.

Mala, J.G.S., N.R. Kamini and R. Puvanakrishnan. 2001. Strain improvement of *Aspergillus niger* for enhanced lipase production. *J. Gen. Appl. Microbiol.*, 47: 181-186.

Peter, R., 1995. Production, regulation and some properties of lipase activity from *Fusarium oxysporium. Enzyme and Microbial. Technol*., 17: 283-883.

Rajeshkumar, J. and M.H. Ilyas. 2011. Strain improvement of Phosphate solubilizing fungal strains for the production of lipase. *Int. Multidisciplinary Res. J.,* 1(7): 15-23.

Rhodes, P.M. and P.F. Stanbury. 1997. *Applied Microbial Physiology*. A Practical Approach, Oxford University Press, USA, 0199635781.

Salihu, A., M.Z. Alam, M.I. Abdul Karim and H.M. Salleh. 2012. Lipase production: An insight in the utilization of renewable agricultural residues. *Resources, Conservation and Recycling,* 58: 36-44.

Saxena, R.K., W.S. Davidson, A. Sheoran and B. Giri. 2003a. Purification and characterization of alkaline thermostable lipase from *Aspergillus carneus*. *Process Biochem.*, 39: 239-247

Sexena, R.K., A. Sheoran, B. Giri and W.S. Davidson. 2003b. Purification strategies for microbial lipases. *J. Microbiol. Methods.*, 52: 1-18.

Shah, K.R., P.M. Patel and S.A. Buatt. 2007. Lipase Production by *Bacillus* Sp. under different physio-chemical conditions. *J. Cell and Tissue Res.*, 7(1): 913-916.

Sharma, R., Y. Chisti, U. Chand and Banerjee. 2001. Production, purification, characterization and applications of lipases. *Biotechnol Adv.,* 19: 627-662.

Sidhu, P., R. Sharma, S.K. Soni and J.K. Gupta.1998. Production of extracellular alkaline lipase by a new thermophilic *Bacillus* sp. *Folia. J. Microbiol.*, 43: 51-4.

Simons, J.W., V.M.D. Kampen, S. Riel, F. Gotz, M.R. Egmond and H.M. Verheij. 1998. Cloning, purification and characterization of the lipase from *Staphylococcus epidermidis*-comparison of the substrate selectivity with those of other microbial lipases. *Eur. J. Biochem*., 253: 675-83.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods *7th Edition, Ames, Iowa State University Press*: ISBN 0-81381560-6.

Socole, R.C. and S.P.L. Vandenbergh. 2003. Overview of applied solid-state fermentation in Brazil. *Biochem. Eng. J.*, 13: 205-218.

Sztajar, H. and I. Maliszewska. 1989. The effect of culture conditions on lipolytic productivity of *Penicillium citrinum*. *Biotechnol Lett.*, 11: 895-898.

Sztajar, H., I. Maliszewska and J. Wieczorek. 1988. Production of exogeneous lipases by bacteria, fungi, and actinomycetes. *Enzyme Microb Technol*., 10(8): 492-497.

Teng, Y. and Y. Xu. 2007. Culture condition improvement for whole cell lipase production in submerged fermentation by *Rhizopus chinensis* using statistical method. *Bioresource Technol.,* 99: 3900-3907.

Ushio, K., T. Hirata, K. Yoshida, M. Sakaue, C. Hirose and T. Suzuki. 1996. Super inducer for induction of thermostable lipase production by *Pseudomonas* species NT-163 and other *Pseudomonas* like bacteria. *Biotechnol. Techniques*, 10(4): 267-272.

Valeria, M.G.L., L. Krieger, M.I.M. Sarquis, A.D. Mitchell, P.L. Ramos and D.J. Fontana. 2003. Effect of Nitrogen and Carbon Sources on Lipase Production by *Penicillium aurantiogriseum*. *Food Technol. Biotechnol*., 41(2): 105-110.

Vargas, G.D.L.P., H. Treichel, D. DE Oliveira, S.C. Beneti, D.M.G. Freire and M.D. Luccio. 2008. Optimization of lipase production by *penicillium simplicissimum* in soybean meal. *J. Chem. Technol. & Biotechnol*., 83: 47-54.

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