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Screening of lipase producing Fungi and Catalytic Activity from Molasses Culture Medium

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Abstract: In present study, some fungal species *Aspergillus fumigatus, Aspergillus niger,* and *Mucor geophillus* grown on agro-industrial by product 2.5% and 5% molasses contained mineral medium as a carbon source to enhance economic, cheapest attractiveness in submerged culture condition of commercially important enzyme lipase. These three species of fungi were screened on the basis of production of greater yield of lipase. It was evaluated maximum production of lipase 44.56 Units/ml by *Mucor geophillus*, when it was grown on 5% molasses mineral medium at 72 hours, which was found dominated as compared to other used species of fungi. Final pH, reducing sugar and total protein correlation with the yield of lipase were also checked from the fermented medium.

Keywords: Lipase, Molasses, *Aspergillus fumigatus, Aspergillus niger, Mucor geophillus*, submerged fermentation.

1. INTRODUCTION

The exponential increase the application of lipases in various fields in the last few decades demand extension in both qualitative improvement and quantitative enhancement. Quantitative and qualitative production of lipase can be enhanced or improved by using agro-industrial residues or by product as substrate by submerged fermentation by wild strains (Casthilo *et al.,* 2000; Haq *et al.,* 2009; Iftikhar *et al.,* 2010; Iftikhar *et al.,* 2010).

Lipases or triacylglycerol acylester hydrolases (E.C.3.1.1.3) are carboxylesterases which catalyze the hydrolysis triacylglycerol and the synthesis of esters formed from glycerol and long chain fatty acids in heterogeneous reactions at the interface of water soluble and water insoluble systems and in organic solvents. Furthermore, Lipases also catalyze the hydrolysis and transesterification, synthesis of ester bond, enantioselectivity and other characteristic properties like substrate specificity, stereospecificity and ragiospecificity. Enzymatic hydrolysis of triacylglycerol and these characteristic properties have accounted for a marked increase and the advantage of product quality and less energetic demand in industrial scale. The inverse processes of glyceride synthesis and transesterification or biotransformation have great importance especially in food, detegent and cosmetics industry, as well as make them popular for biotechnological applications in oil processing, organic synthesis and production of surfactants and preparation of enantiomerically pure pharmaceuticals (Paiva *et al.,* 2000; Shimizu and Nakano, 2003; Park *et al.,* 2005; Gupta *et al.,* 2007; Grbavcic *et al.,* 2007; Franken *et al.,* 2009).

In this regard, the purpose of this study to increase the production of industrial important enzyme lipase by some wild fungal species using agro-industrial by product (molasses) as a low cost substrate in submerged culture condition that can may cause the decrease of industrial scale production cost.

2. MATERIAL AND METHODS:

Isolation of micro organisms:

Different wild species of fungi such as *Aspergillus fumigatus, Aspergillus niger* and *Mucor geophillus* were isolated from soil of University of Sindh, Jamshoro and grown in Enzyme and Fermentation Biotechnology research Laboratory, Institute of Biotechnology and Genetic Engineering .The Stock Culture was maintained on Czepaks agar as described previously (Naqvi and Dahot 1998). The sterilized slants were inoculated with required species of fungi. After inoculation the slants were incubated at 27°C to obtain luxuriant growth. The isolation and maintenance of pure culture of lypolytic microbes was done from soil sampling as described by (Alexandar, 1977).

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Culture medium:-The following ingredients were used for the preparation of culture medium as reported by Burrel *et al.,* (1966). The fermentation medium composed of (g/L) of KH_2PO_4, 1.0; $MgSO_4$. $7H_2O$, 0.2; $CuSO_4$.$5H_2O$, 0.0023; $FeSO_4.7H_2O$, 0.0063; $ZnSo_4$.$7H_2O$, 0.0011; $MnCl_2.4H_2O$, 0.0035; $CaCl_2.2H_2O$, 0.0467; NH_4NO_3, 2.4 was used for the production of Lipase . The initial pH of the medium was maintained at 6.0.

Preparation of Spore suspension. The surface of stock culture of *Aspergillus fumigatus, Aspergillus niger,* and *Mucor geophillus* was gently rubbed with sterilized wire loop and 10 ml of sterilized distilled water was added. The spore suspension was further diluted to 100 ml with sterilized water. (Schwermann, 1994).

Sterilization of fermented medium: Molasses was diluted with the concentration of 2.5% and 5% the slurry was autoclaved at 1.5 kg /cm^2 for 30 minutes. The autoclaved slurry was cooled at room temperature; sterilized molasses medium was incorporated in to the fermentation mineral medium as a carbon and energy source for the growth of microorganisms.

Cultivation condition:-50 ml 2.5% and 5% sugar cane molasses containing fermentation medium was taken in 250 ml conical flask and the initial pH of the medium was adjusted at 6.0. Flasks were plugged with cotton wool and autoclaved for 20 minutes at 1.5 kg /cm^2. The Sterilized media cooled at room temperature and were inoculated with 1.0 ml of wild species of fungi such as *Aspergillus fumigatus, Aspergillus niger* and *Mucor geophillus* spores. These flasks were incubated in an orbital cooled shaking incubator (gallenkamp) at 30±2ºC. The culture broth was separated from biomass after an interval of 24 hours cultivation time through Whatman No: 1 filter paper

Biomass estimation: After filter of culture broth, the pre-weighed filter paper containing biomass was washed with distilled water and dried at 105-110°C in an oven till complete dry weight obtained.

Lipase assay: Lipase activity was measured by the olive oil method (Dahot and Memon, 1987; AOAC, 1965). The reaction mixture containing 2.5 ml of substrate (10% olive oil emulsion with 2% gum Arabic in 0.2 M sodium phosphate buffer) and 2.5 ml enzyme solution was incubated at 37°C for 1 hour in shaking incubator at 200 rev. / min. The reaction was stopped by the addition of 5 ml of methanol: chloroform (2: 1 v/v).

A unit of lipase activity was defined as the amount of enzyme required releasing one micro mole of free fatty acids per hour under the assay conditions.

Determination of reducing sugars and Total protein: The reducing sugar concentration from fermented medium was determined by Dinitrosalicylic acid (DNS) method (Miller, 1959) and the results were calculated from glucose standard curve at 450 nm.

The total protein contents were also determined from culture medium by Lowery et al, (1951) method, using bovine serum albumin as a standard at 750 nm.

3. RESULTS AND DISCUSSION

Molasses is known as a best fermentation medium because it contains higher amount of total sugar and reducing sugars and other nutrients. The ratio of total sugar and reducing sugar reflects the average degree of polymerization of sugar moieties in solution. It is important that this ratio is close to 1.0 (Vallander and Eriksion, 1985). Because it contain 50% sugars, which is much higher than others medium. Sugar is necessary content for growth of fungi and synthesis of lipase. In this study, molasses were diluted with 2.5% & 5% concentration for growth of fungi and synthesis of lipase. The aim of present investigation to explore suitable specie of wild fungi and utilization of agro-industrial by product of sugar cane as a carbon source for the production of greater yield of lipase.

Aspergillus fumigatus start to produce lipase from 24 hours up to 72 hours approximately 41.16 and 41.00 U /ml and turned to decrease when it was grown in 2.5% & 5% molasses mineral medium. The final pH values of culture broth were observed increasing in order to alkaline side in both cases. According to data presented in **(Fig.1 and 2)** correlation can be seen between growth of biomass and lipase activity, which is noted at 72 hours. The utilization of reducing sugar contents by *Aspergillus fumigatus* decreases with the increasing of time during the fermentation process. The concentration of protein remains nearly constant through out the fermentation process.

Aspergillus fumigatus was replace with *Aspergillus niger* for the production of lipase with the utilization of same medium which have used previously, *Aspergillus niger* secreted the yield of lipase and mycelial mass less than *A. fumigatus* (39 units/ml and 39.97 Units / ml) in both substrates at 72 hours through this study, and results are

presented in **(Fig.3 and 4)**. The concentration of reducing sugars, protein, and mycelial mass decreased with the increased of time in both culture mediums. The final pH of both fermented mediums was noted towards the acidic side from the adjusted initial pH 6.0 may be due to the production of some organic acids during the fermentation (Davies, 1963).

Mucor geophillus produced maximum yield of lipase approximately 43.68 and 44.56 Units/ml at 30±2°C in 2.5% and 5% molasses mineral medium at 72 hours incubation time respectively as shown in **(Fig. 5 and 6)**. It was also noted that the consumption of reducing sugar and total protein contents secretion are increasing with the increasing of time during the submerged fermentation condition. While final pH of culture broth turned the acidic side, whereas growth of biomass rapidly increased through out the fermentation period. The greater amount of lipase was synthesized by *Mucor geophillus* when grown on 5% molasses mineral medium in comparison to other used fungal species by submerged culture condition as represented in **(Fig.7)**. Various workers have reported the less amount of lipase production than our reported data. Puthli *et al.,* (2006), Benjamin and Pandey, (1995) reported separately the low amount of lipase 1.84 $U.mL^{-1}$ after 66 hours and 12.55 $U.mL^{-1}$ after 72 hours secreted by *Candida rugosa*. Mahadik *et al.,* (2004) also produced less amounts of lipase yield 4.0 $U.mL^{-1}$ lipase and 13 $U.mL^{-1}$ by *Aspergillus niger* and*Penicillium restrictum* in submerged fermentation condition While Pinheriro *et al.,* (2008) studied on lipase production from *Penicillium verrucosum* and obtained maximal enzyme yield 3.22 $U.mL^{-1}$ at 96 hours and 2.63 $U.mL^{-1}$ at 72 hours in conventional and industrial fermentation medium respectively. Furthermore, *Mucor griseocyanus* produced 0.113 $IU.ml^{-1}$ in optimized fermentation condition (Armas *et al.,* 2008). Whilst in the present study *M. geophillus* secreted 44.56 $U.mL^{-1}$ lipase activity at 72 hours that is higher in comparison to reported worker who evaluate lipase production by different fungal species.

4. CONCLUSION:

It is concluded that highest production of lipase (approximately 44.56 Units / ml) was recorded by *Mucor geophillus*, when it was grown on 5% molasses at 72 hours in comparison to other species of fungi. On the basis of higher production of lipase, it is suggested that the 5% molasses is the best and cheapest source for the synthesis of lipase and *Mucor geophillus* is the best producer of lipase in comparison to other species of fungi.

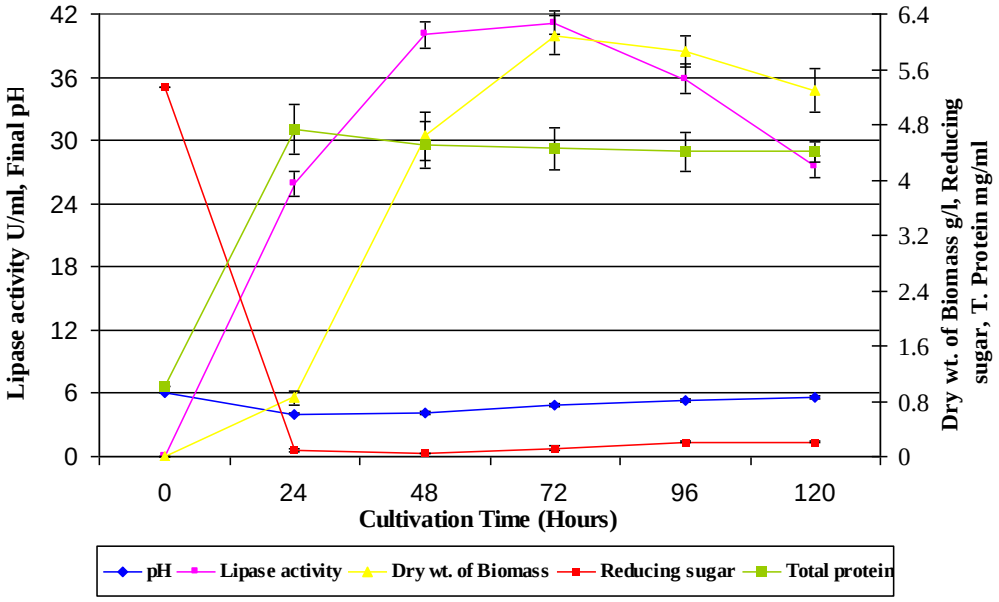


Fig.1: Lipase production by *Aspergillus fumigatus* grown on 2.5 % molasses mineral medium at 30±2°C

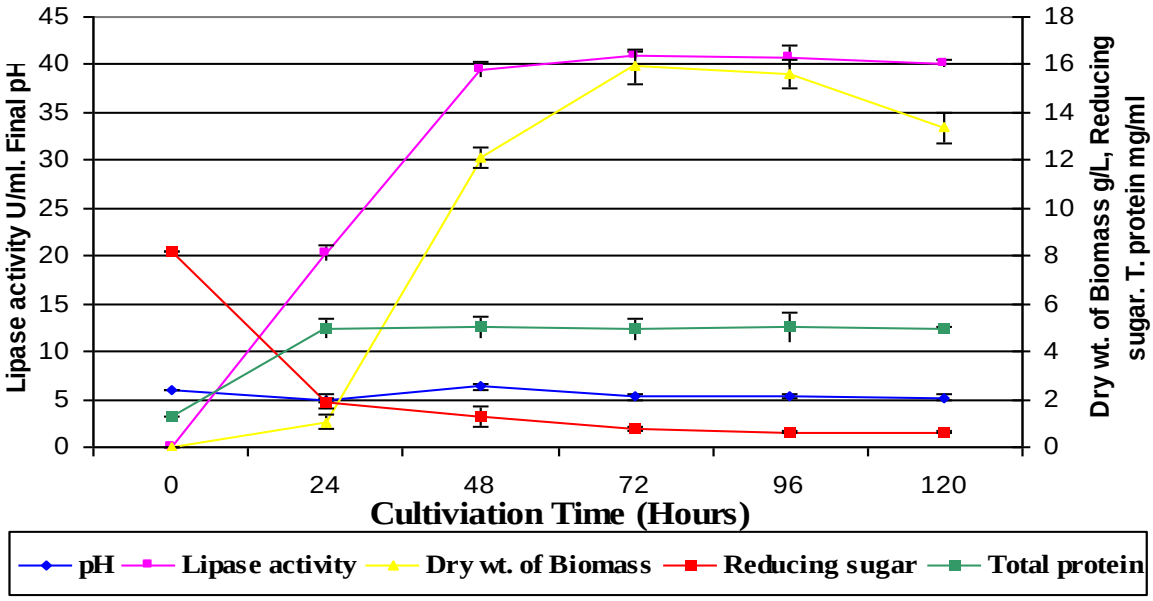


Fig-2: production by *Aspergillus fumigatus* won on 5 % molasses mineral medium at 30±2°C

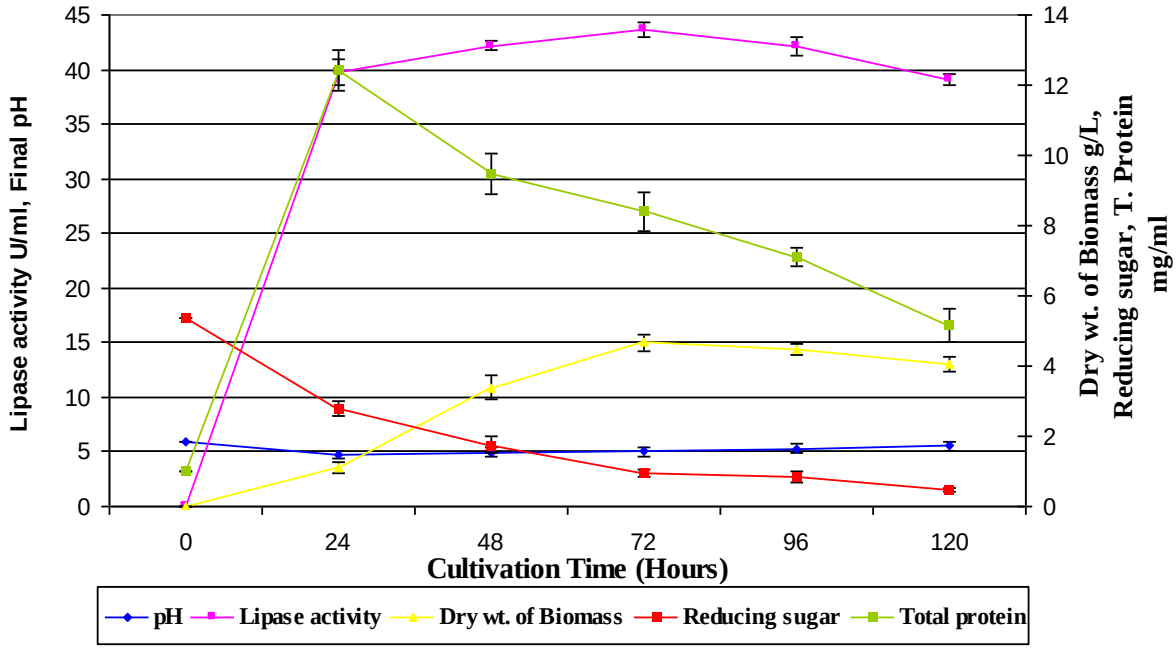


Fig.3: Lipase production by *Aspergillus niger* grown on 2.5 % molasses mineral medium at 30

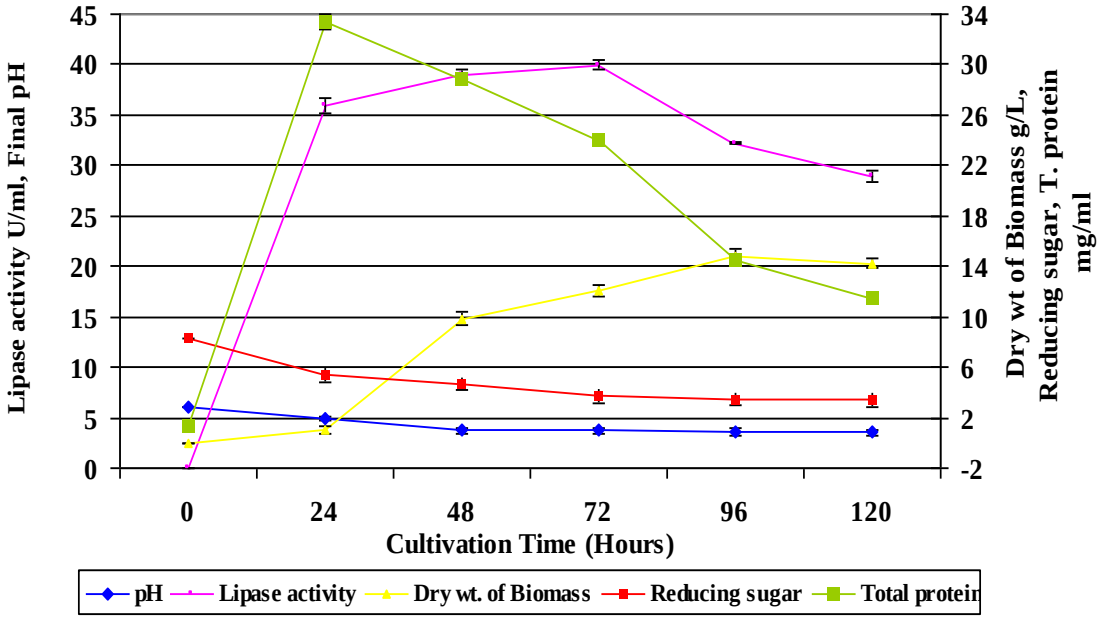


Fig.4: Lipase production by *Aspergillus niger* grown on 5 % molasses mineral medium at 30±2°C

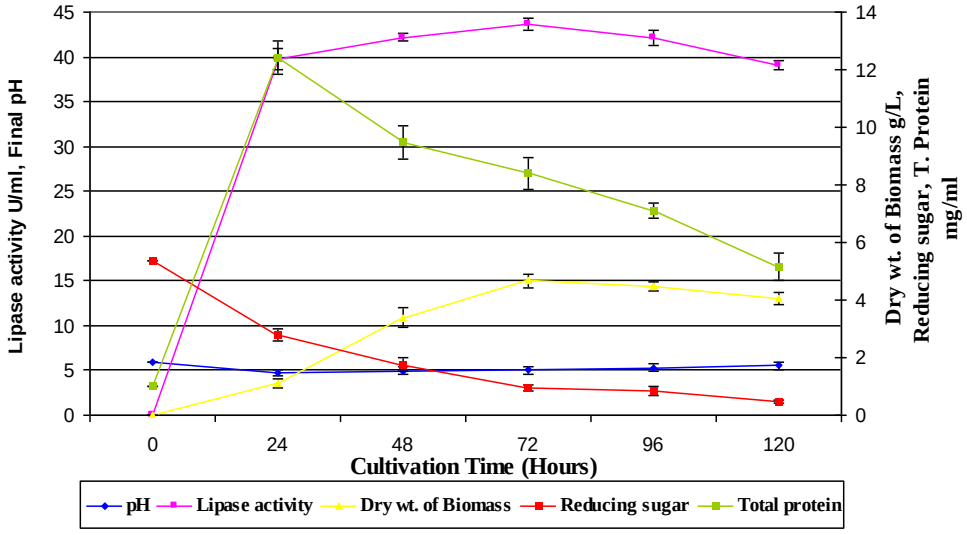


Fig.5: Lipase production by *Mucor geophillus* grown on 2.5 % molasses mineral medium at 30±2°C

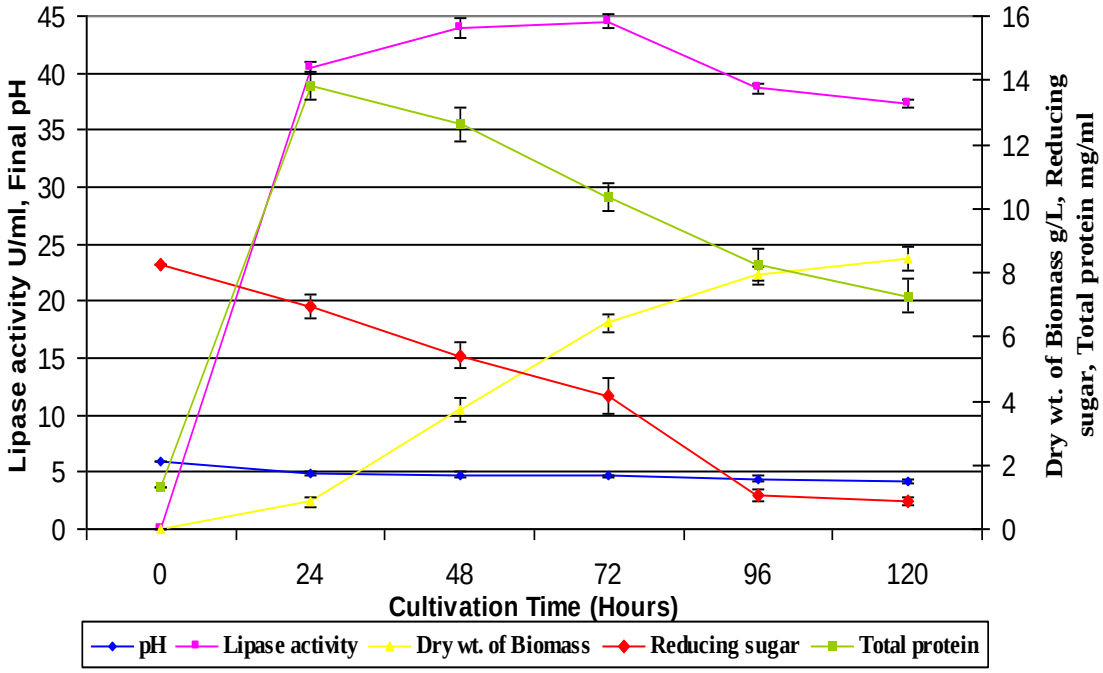


Fig. 6: Lipase production by *Mucor geophillus* grown on 5 % molasses mineral medium at 30±2°C

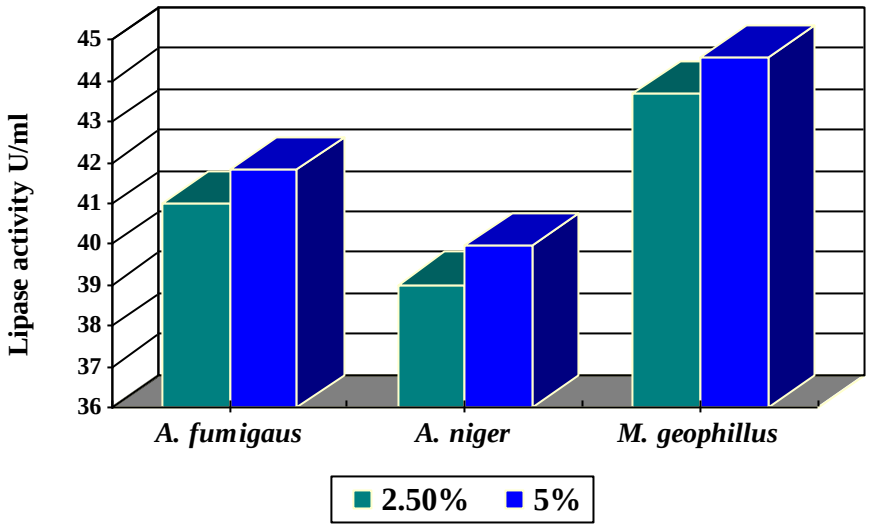


Fig.7: Comparison of lipase production rate by different fungi grown on molasses mineral medium for 72 hours at 30±2°C

REFERENCES:

Alexandar, (1977) Introduction to soil Microbiology, Second edition. Publication by John Wiley and sons, New york, London, Sydney and Toronto. 152–156 .

Armas J. C., J.C.D. Mendoza and J.L.M. Hernandez (2008) *Mucor griseocyanus* Lipase: Production, Characterization and Study of Some Catalytic Properties of the Immobilised Enzyme. Food Technol. Biotechnol. 46 **(2):** 195–201.

Association of official Agricultural chemists (1965) "Official Methods of analysis" , 10th ed . AOAC, Washington D .C. 423Pp.

Benjamin S. and Pandey A. (1995) Optimization of liquid media for lipase production by *Candida rugosa*. Bioresource Technol. 55 **(2):** 167-170.

Burrel R. G., C. W. Clayton M. F. Gallegly and V. D., Lilly (1966) Factors affecting the antigenicity of the mycelium of three species of *Phytophthora*. Phytopathalogy, **(56):** 422Pp.

characterization of triacylglycerol in several oils containing gamma linolenic acid. Biosci. Biotechnol. Biochem. **(67):** 60-67

Casthilo L.., R. Polato C.M.S. Baruque E.A., Sant'Anna G.L., and D.M.G. Freire (2000) Economic analysis of lipase production by *Penicillium restrictum* in solid state and submerged fermentations. Biochemical Eng. J. 4 (**3):** 239-247.

Dahot M. U. and A. R. Memon (1987) Properties of Moringa deffera seed lipase. Pak. J. Sci. Ind. Res. **(30):** 832–835.

Davies R., (1963) In: Biochemistry of Industrial Microorganisms. C. Rainbow and A.H. Rose, editors, Academic press, New York, 68Pp.

Franken, L.P.G., N.S., Marcon, H., Treichel, D., Oliveira, D.M. G., Freire, C. D, Destain and J. V., Oliverira, (2009) Effect of treatment with compressed propane on lipases hydrolytic activity. Food and Bioproc. Technol., 3 **(4):** 511-520.

Grbavcic, S. Z., S. I. Dimitrijevic-Brankovic, D. I. Bezbradica, S. S.Siler-Marinkovic, and Z. D. Knezevic, (2007) Effect of fermentation conditions on lipase production by *Candida utilis*. J. Serbian Chemical Society, 72 **(8–9):** 757–765.

Gupta, N., V. Shai, and R. Gupta, (2007) Alkaline lipase from a novel strain Burkholderia multivorans: Statistical medium optimization and production in a bioreactor. Process Biochem., 42 **(2):** 518–526.

Haq, I.U., S. Ali, A. Saleem and M.M. Javed. (2009) Mutagenesis of *Bacillus lichniformis* through ethyl methyl sulphonate for alpha amylase production. *Pak. J. Bot*., 41**(3):** 1489-1498.

Iftikhar, T., M. Niaz, M.A. Zia and I.U. Haq. (2010) Production of extracellular lipases by *Rhizopus oligosporus* in a stirred fermentor. *Braz. J. Microbiol.*, **(41):** 1124-1132.

Iftikhar, T., M. Niaz, S.Q. Abbas, M.A. Zia, I. Ashraf, K.J. Lee and I.U. Haq. (2010) Mutation induced enhanced biosynthesis of lipases by *Rhizopus oligosporus* var. *microsporus*. *Pak. J. Bot*., 42 **(2):** 1235-1249.

Lowery O. H., N. J. Rosebrough A. L. Farr and R. J. Randall (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265–275

Mahadik N.D. (2004) Production of acidic lipase by a mutant *Aspergillus niger NCIM 1207* in submerged fermentation. Proc. Biochem. 39 **(12):** 2031-2034.

Miller G. L., (1959) Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. **(31):** 426-429

Naqvi S. H, Ahmed and D. M. Umar. (1998). Production of lipase by *Aspergillus niger* grown on pretreated sugar cane waste. Sci. Int. **10**(2): 139–141.

Paiva A.L, V. M. Balcao and F.X. Malcata (2000) Kinetics and mechanisms of reactions catalyzed by

immobilized lipases, Enzyme and Microbial Technology. **(27)**: 187-204.

Park, H., K. Lee, Y. Chi, , and S. Jeong, (2005) Effects of methanol on the catalytic properties of porcine pancreatic lipase. J. Microbiol. Biotechnol., 15 **(2):** 296–301.

Pinheriro T.F., S. Menocin N.M Domingues D. Oliveria H. Treichel M. Diluccio and D.M.G. Freire (2008) Production and partial characterization of lipase from *Penicillium verrucosum* obtained by submerged fermentation of conventional and industrial media. Cienc. Technol. Aliment. **(28):** 444-450.

Puthli M.S., V.K. Rathod and A.B. Pandit (2006) Optimization of lipase in a triple impeller bioreactor. Biochem. Eng. J. 27 **(3):** 287-294.

Schwermann B., K. Pfau B. Lilensiek M. Schleyer T. Fischer and E. P. Baker (1994) Purification, Properties and Structural Aspects of a thermoacidophilic ℵ-Amylase from *Alicyclobacillus Acidocaldarius* Atcc 27009Eur. J. Biochem. **(226):** 981–991

Shimizu S. and M. Nakano (2003). Structural Vallander L. and Erikson K. E. (1985) Enzymatic saccharification of pretreated wheat straw. Biotec. Bioeng. 27 **(5):** 650-659