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STUDIES OF LIPASE PRODUCTION CAPABILITY OF *Fusarium* SPECIES AND THEIR APPLICATION IN VEGETABLE OIL HYDROLYSIS

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ABSTRACT

During this study *F. solani, F. oxysporum* and *F. moniliformi* were screened for lipase activity. *F. moniliformi* shown highest lipolytic activity followed by *F. oxysporum* and *F. solani*. through the Tributyrin Clearing Zone. The fermentation was carried out in shake flask culture employing a liquid media than results found that all three fungal strains were shown lipase production. Effect of various factors on production of fungal lipase result were shown that carbon sourceTween-20, nitrogen source yeast extract, 2nd and 3rd day of incubation, temperature 35°C and 55°C, pH 4 and 7 supported the production of lipase activity. Mostly Co^{2+}, Cu^{2+}, Mn^{2+}, Hg^{2+} & Na^{+} act as enhancer of fungal lipase activity, only *F. solani* by Fe^{2+} and *F. oxysporum* Mg^{2+} and Zn^{2+} enhances lipase activity. In optimized conditions intracellular lipolytic activity were raised highly as compared extracellular and maximum activity found in *F. moniliformi* at pH-4 and 45°C. These properties are interesting aiming industrial application both in low pH and High temperature. Application of all fungal lipolytic activity was used for detection in hydrolysis of soybean, mustard and coconut oils results concluded that all fungal species have capability to hydrolyse soybean, mustard and coconut oil. Highest potential lipolytic activity for soyabean oil hydrolysis were obtained in *F. solani* and *F. moniliformi* for mustard and coconut oils. Potential of lipase to be applied in oil hydrolysis and also oil spillages or hydrocarbons in wastewater efficiently and its potential to be applied in production of biofuel from vegetable oil hydrolysis.

KEYWORDS: Lipase, *Fusarium* species, fermentation, temperature, screening, oil spillages

Increasing interest in isolating new enzymes and new enzyme-producing strains for their use in various fields due to increased awareness of environment and cost issues for major Research and Development. Among these enzymes, lipases, carboxylesterases, cellulases, xylanases, pectinases, amylases and proteases are some of the most important enzymes (Cherry J.R. and Fidantsef A.L., 2003] [Gotor-Fernández et. al., 2006][Pandey et. al., 2005]. Lipases provide improved worldwide attention due to their diverse industrial applications and versatility in nature. The world's enzyme demand is met by 12 major producers and 400 minor suppliers across the globe, 60% of the world's supply of industrial enzymes is from Europe alone and strikingly 75% of these industrial enzymes include lipases [Muralidhar et. al., 2001][Rao et. al., 1998]. Lipase is used to catalyze several unnatural and remarkable reactions in non-aqueous media that include bio-fuel production, production of value added products like esters, organic acids, food, beverage, cosmetics, and pharmaceutical materials [Kumar A. and Kanwar S.S., 2012]. Multi-faceted microbial lipases (glycerol ester hydrolases; EC 3.1.1.3) have an unsurpassed role in swiftly growing modern biotechnology [Pandey et. al., 1999]. Lipases are indispensable for the bioconversion of lipids (triacylglycerols) from one organism to another and within the organisms. In the past, fungal lipases gained significant attention in the industries due to their substrate specificity and stability under varied chemical and physical conditions. Fungal enzymes are extracellular in nature, and they can be extracted easily, which significantly reduces the cost and makes this source preferable over bacteria. Soil contaminated with spillage from the products of oil and dairy harbors fungal species, which have the potential to secrete lipases to degrade fats and oils. Herein, the strategies involved in the characterization of fungal lipases, capable of degrading fatty substances, are narrated with a focus on further applications. Oil spillages from these production points cause a hindrance in ensuring environmental hygiene due to the formation of clogs in drain pipes [Lemus G.R. and Lau A.K., 2002]. Cleanup and recovery of oil wastes is difficult and depends upon many factors, including the type of oil spilled, the temperature of water affecting evaporation, and biodegradation. Microbial degradation is one of the most important events to ameliorate oil pollution in the environment [Sztajer et. al., 1988] So, in the present study an attempt has been made to isolate potential fungal strain(s) which are capable of producing active lipase which can fit in the conditional processes. The lipase thus obtained was further analyzed in optimization conditions for their better production.

MATERIALS AND METHODS

Fungal Strains

Fungal Strains of *Fusarium solani, F. oxysporum, F. moniliformi,* were obtained from soil of "School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur" and the cultures were

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maintained on Potato Dextrose Agar (PDA) and Basal media.

Rapid screening of fungi for the production of lipase

Tributyrin agar medium was used for assay of enzyme productivity according to the tributyrin clearing zone technique [Elwan et. al., 1997]. Fungal colonies that have lipolytic activity showed clear zone.

Lipase production

Inoculums were prepared by inoculated fungal strains spores on experimented media to obtain standard concentration of approximately 1.2x 107 spores /ml. Prepared inoculums were grown in a modified form of fermentation medium according to this composition (g/L) 30-peptone, 2.0- KH_2PO_4, 0.5- $NaN0_3$, 0.5- KCl, 0.5-$MgSO_4.7H_20$, 1.0-$CaCl_2$, silicone MS Antifoam A - 40ppm and 20% (w/v) emulsified triglycerides [Fukumoto et. al., 1963]. Fermentation was done in an orbital incubator shaker (REMI, 9001:2000) at 25°C, pH- 4, and 50rpm for 3 days [Mirza et. al., 1983]. Protein was extracted and purification done by 80% ammonium sulphate precipitation method than total protein concentration estimated by Folin Lowry method at 660nm absorbance [Lowery et. al., 1951].

Lipase Assay

Lipase activity was assayed quantitatively using 2ml phosphate buffer taking 2 ml phosphate buffer pH 7.0, 1 ml 10% (w/v) emulsion of olive oil in gumacacia, 0.5 ml of 0.1M $CaCl_2$ and 1 ml enzyme solution and incubating the mixture at 37°C for one hour. The reaction was stopped by adding 5 ml mixture of ethanol: acetone (1:1) and the whole mixture was titrated against 0.05M NaOH using phenolphthalein as indicator. The blank used and the activity unites were [Kundu A.K. and Pal N., 1970]. "A unit of lipase activity was defined as the amount of enzyme that would liberate one micromole of free fatty acids per minute from the substrate under the assay conditions described."

Effect of Physico-Chemical Parameters on Lipase Production

Lipase production was optimized by altering various physicochemical and culture conditions observing the effect at 25^{o}C in Orbital Incubator Shaker (REMI, 9001:2000) at 50 rpm for appropriated incubation periods for 1-3 days.

Effect of Incubation periods:

The effect of incubation period on lipase production for *Fusarium* sps. were studied measuring the enzyme activity, growth at different incubation period (2-5) days at 25°C, 50 rpm and pH 7.

Effect of different Incubation pH and temperatures:

The effect of pH on lipase activity was determined by incubating 0.1ml of protein precipitate in 0.4 ml of appropriate buffers. To this, 0.5 ml of olive oil (1% w/v) was dissolved and the reaction mixture was incubated at 37°C for 5 minutes. The effect of temperature was determined by incubating 0.5 ml of proper diluted enzyme and 0.5 ml of olive oil (1% w/v in 0.2M sodium acetate buffer pH 4) for 5 minute at different temperatures (ranges from 25°C, 35°C, 45°C and 55°C).

Effect of Different media components:

The media can be classified into 3 categories that are carbon, nitrogen and effectors. Effect of various supplements on lipase production medium was studied by adding different carbon sources (glycerol and T-20), Nitrogen sources (peptone and yeast extract) as equivalent amount present in basal medium. (in mm)While the effect of activators and inhibitors like (NaCl, $CaCl_2$, $CoCl_2$, $ZnSO_4$, $MgCl_2$, $MnSO_4$, $CuSO_4$, $FeCl_2$, $HgCl_2$ and SDS) was determined by incubating 0.5 ml of olive oil (1% w/v in 0.2M sodium acetate buffer pH 4) at 37°C for 5 minutes.

Fungal Lipase Production in Optimized Conditions:

At optimized source of carbon, nitrogen, activator, inhibitor, pH, and temperature of the fermentation medium fermentation were done for all three fungal species and isolated protein used for checking lipase activity assay.

Application of Fungal Lipase in Various oil hydrolysis:

Various vegetable oils like (Soybean/Mustard/ Coconut oil) were used as source of carbon , nitrogen (Yeast Extract), activator, inhibitor, pH, and temperature of the fermentation medium was adjusted according to optimized conditions in an Orbital Incubator Shaker at 50 rpm for appropriated incubation periods. Composition of media was selected from (Kouker and Jaeger) with slightly modification (removing of agar and Rhodamine B from media). The hydrolysis of various oils was confirmed by lipase activity [Kouker G. and Jaeger K., 1987].

RESULTS AND DISCUSSION

Screening of Fungal Strains

In this study, nine fungal strains *Fusarium solani, F. oxysporum* and *F. moniliformi,* were screened for lipase production. Table (1) shown that all the tested strains, produced lipase with varying lipolytic activities. The most active lipolytic activities were observed in *F. oxysporum* (15.5mm) and after that *F. solani* (2.0mm) was shown average lipolytic activity. On the other hand, the least lipase productive organism was *F. moniliformi* (1.0mm). In previous studied in *Fusarium oxysporum* [Saad et. al., 2005] and Gopinath and coworkers, revealed the following fungi as potential candidates that secrete enzymes lipases, *Absidia corymbifera, Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans, Aspergillus terreus, Cunninghamella verticillata, Curvularia pallescens, Fusarium oxysporum, Geotrichum candidum*, *Mucor racemosus*,*Penicillium citrinum*, *Penicillium frequentans*, *Rhizopus stolonifer*, and *Trichoderma viride*[Gopinath et. al., 2005].

Figure 1: Shown all three *Fusarium sps. F. moniliformi, F. oxysporum* and *Fusarium solani*

Table 1: Screening of liapase producing fungal species Diameter of Tributyrin clreaing zone by lipolysis activity

Fungal Lipase	Average of Growth of Fungal Sps. in Trybutyrin Assay Media (in mm)	Average of Growth of Fungal Sps. In Trybutyrin Assay Media+ Hydrolysis	Diameter of Tributyrin clreaing zone
F. solani	4.0	6.0	2.0
F. oxysporum	7.0	21.5	15.5
F. moniliformi	13.0	14.0	1.0

Estimation of Total protein Produced by Fermentation of three fungal species:

In this study, three fungal strains *F. moniliformi* (0.73 mg/ml), *F. oxysporium* (0.46 mg/ml) and *F. solani* (0.86 mg/ml) were compared with standard protein estimation method of Lowry to estimate the production of total protein during fermentation. Table (2) shown that all the tested strains, produced protein in varies concentration.

Table 2: Estimation of various fungal protein concentrations by folin Lowery method

Fungal sps.	Protein Concentration (in mg/ml)	Fungal sps.
F. solani	0.73	*F. solani*
F. oxysporum	0.46	*F. oxysporum*
F. moniliformi	0.86	*F. moniliformi*

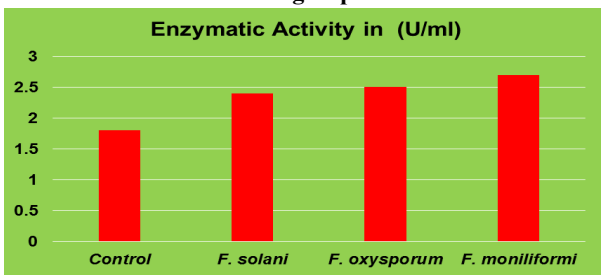
Production Fungal Lipase in fermentation medium:

In this study, lipase activity assay were performed to determine the lipase production and these results was compared with control, and found that all three fungal strains were shown lipase activity in (Table 3). Lipase production of fungal strains observed reading were (in U/ml) *F. moniliformi* (2.7), *F. oxysporium* (2.5) and *F. solani* (2.4).[Naz S. and Jadhav S.K., 2015]

Table 3: Production of fungal lipases in fermentation medium through various fungal species

Various Fungal sps.	Enzymatic Activity in (U/ml)
Control	1.8
F. solani	2.4
F. oxysporum	2.5
F. moniliformi	2.7

Graph 1: Production of fungal lipases through various fungal species



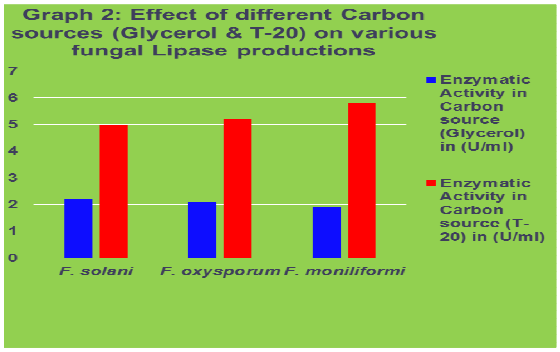
Effect of Physio- Chemical Parameters

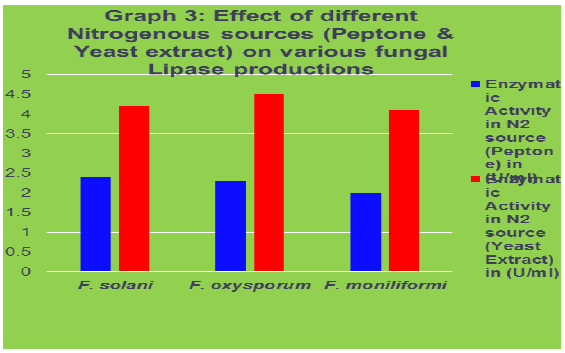
Effect of different Carbon sources (Glycerol & T-20)

Due to glycerol and Tween-20 effects on lipase production of fungal strains observed reading were (in U/ml) *F. moniliformi* (1.9 & 5.8), *F. oxysporium* (2.1 & 5.2) and *F. solani* (2.2 & 5.0) (Table 4). Result was shown that both carbon supplement media support the production of lipase activity, but the highest lipase activity was observed on Tween-20 source of carbon, whereas glycerol showed moderate effects. Previously studies was reported optimum activity in *Fusarium oxysporum* KG-13, *Fusarium spps* KG-15, were also showing very nice production profile with an average when T-20 as a carbon source [Ghosh et. al., 1996]. Previously results revealed that use of glycerol as main carbon source rather than glucose or lipid supported mycelial growth and lipase production [Naz S. and Jadhav S.K., 2015][Michael et. al., 1999].

Table 4: Effect of different Carbon sources (Glycerol & T-20) on various Intra-cellular fungal Lipase productions

Lipase enzyme of Fungal sps.	Enzymatic Activity in Carbon source (Glycerol) in (U/ml)	Enzymatic Activity in Carbon source (T-20) in (U/ml)
F. solani	2.2	5.0
F. oxysporum	2.1	5.2
F. moniliformi	1.9	5.8





Effect of different Nitrogenous sources (Peptone & Yeast extract)

Effect of various nitrogen sources such as Peptone & Yeast extract (equimolar amounts) were shown in (Table 5). Result were found that addition of yeast extract the maximum lipase production, whereas peptone support the on the biosynthesis of lipase. Due to peptone & yeast extract effects on lipase production of fungal strains observed reading were (in U/ml) *F. moniliformi* (2.0 & 4.1), *F. oxysporium* (2.1 & 4.5) and *F. solani* (2.4 & 4.2). Maximum productivity of the enzyme in *Aspergillus flavus* and *Candida rugosa* with yeast extract [Kamariah et. al., 1999]. Previously studied revealed the same results as present studies [Naz S. and Jadhav S.K., 2015][Burkert et. al., 2004].

Table 5: Effect of different Nitrogenous sources (Peptone & Yeast extract) on various Intra-cellular fungal Lipase productions

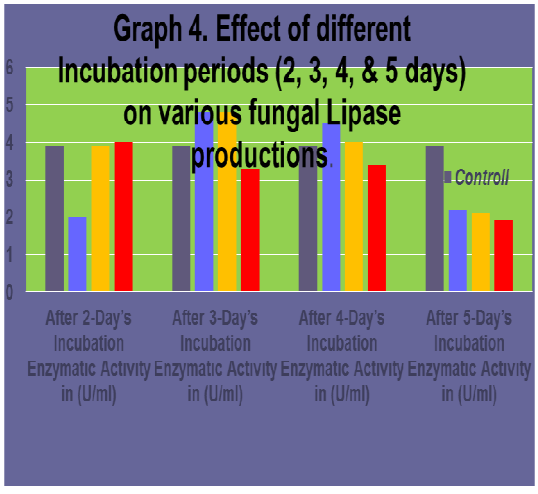
Lipase enzyme of Fungal sps.	Enzymatic Activity in N_2 source (Peptone) in (U/ml)	Enzymatic Activity in N_2 source (Yeast Extract) in (U/ml)
F. solani	2.4	4.2
F. oxysporum	2.3	4.5
F. moniliformi	2.0	4.1

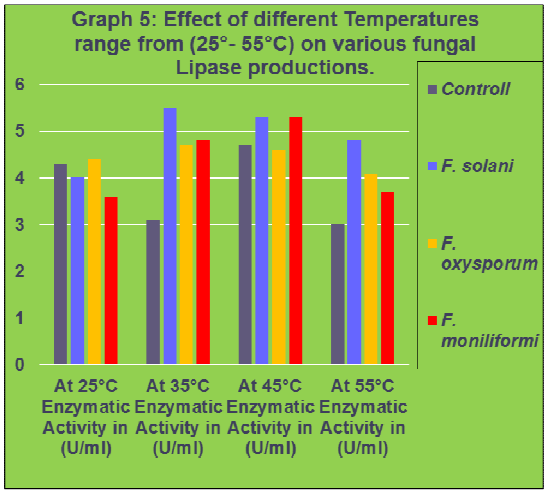
Effect of different Incubation periods (2, 3, 4, & 5 days)

Results shown that *F. oxysporium* and *F. solani* (4.8) were produced maximum lipase activity (inU/ml) at 3rd day of incubation (Table 6), but with the increasing the day of incubation lipase production was reduced and there was no activity found in these three fungal species whereas *F. moniliformi* (4.0) produced maximum lipase activity (in U/ml) at 2nd day of incubation. Previous studies reported that lipase yield was maximum in 2-3 days of incubation in *Fusarium* sp. [Iftikhar et. al., 2012].

Table 6: **Effect of different Incubation periods (2, 3, 4, & 5 days) on various Extra-cellular fungal Lipase productions**

Lipase enzyme of Fungal sps.	After 2-Day's Incubation Enzymatic Activity in (U/ml)	After 3-Day's Incubation Enzymatic Activity in (U/ml)	After 4-Day's Incubation Enzymatic Activity in (U/ml)	After 5-Day's Incubation Enzymatic Activity in (U/ml)
Controll	3.9	3.9	3.9	3.9
F. solani	2.0	4.8	4.5	2.2
F. oxysporum	3.9	4.8	4.0	2.1
F. moniliformi	4.0	3.3	3.4	1.9





Effect of different Temperatures range from (25°C, 35°C, 45°C, & 55°C)

The optimum temperature for lipase activity (in U/ml) of *F. oxysporium* and *F. solani* (5.5 and 4.7) were 35°C than lipase activity which was decreased with the increasing temperature. Whereas *F. moniliformi* (5.3) shown maximum lipase activity at 45°C (Table 7). Previous was achieved at 30°C and the production dropped on both sides of this temperature application Higher temperatures appreciably proved to be deleterious for enzyme production. This decrease in the lipase activity might be due to the fact that the enzyme denatured at higher temperatures. The optimum growth temperature for lipase production in this study is inline with the findings of [Iftikhar *et al.*] whereas in some *Fusarrium* sp. the optimal temperature and was 45°C and 5.5, respectively. The enzyme had a remarkable stability in a pH range of 4.0-8.0 [Facchini et. al., 2015]. Therefore, 35 and 45°C was optimized according to *Fusarium* sps. for further studies.

Table 7: Effect of different Temperatures range from (25°C, 35°C, 45°C, & 55°C) on various Intra-cellular fungal Lipase productions

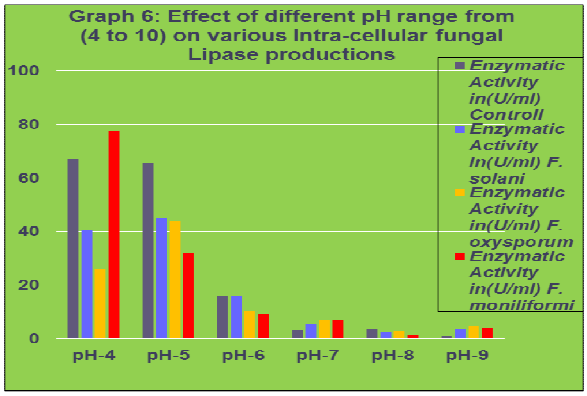
Fungal sps.	At 25°C Enzymatic Activity in (U/ml)	At 35°C Enzymatic Activity in (U/ml)	At 45°C Enzymatic Activity in (U/ml)	At 55°C Enzymatic Activity in (U/ml)
Controll	4.3	3.1	4.7	3.0
F. solani	4.0	5.5	5.3	4.8
F. oxysporum	4.4	4.7	4.6	4.1
F. moniliformi	3.6	4.8	5.3	3.7

Effect of different Incubation pH (4-10)

Table (8) results shown that there were no lipase activity found in pH range from (4-6) only pH-4 was optimum for *F. moniliformi* (77.6). Optimum pH for maximum lipase activity was found pH-9 for *F. oxysporum* (4.5) pH- 7 for *F. solani* (5.6) as compare to controll, whereas pH-10 also support lipase production in all 3 *Fusarium* species. In previous study indicate that the initial pH of the growth medium is also important for lipase production [Naz S. and Jadhav S.K., 2015].

Table 8: Effect of different pH range from (4 to 10) on various Intra-cellular fungal Lipase productions

Fungal sps.	**Enzymatic Activity in(U/ml)**			
	Controll	***F. solani***	***F. oxysporum***	***F. moniliformi***
pH-4	67.0	40.6	26.0	77.6
pH-5	65.5	45.1	44.1	32.1
pH-6	16.1	16.1	10.1	9.2
pH-7	3.1	5.6	6.6	6.8
pH-8	3.7	2.4	2.8	1.6
pH-9	1.0	3.5	4.5	3.8
pH-10	3.5	5.6	6.6	4.1



Effect of activators and inhibitors in Lipase Activity

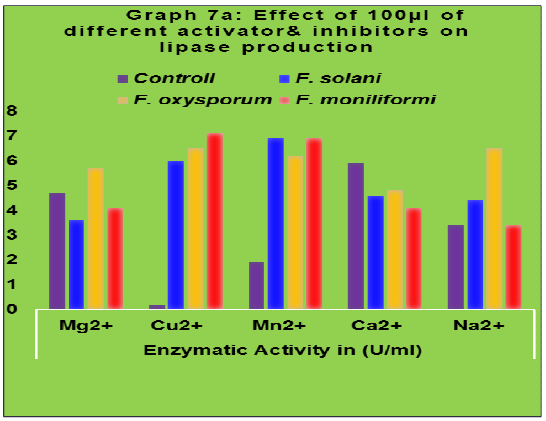
The effect of activators and inhibitors with respect to control shown that mostly Co^{2+}, Cu^{2+}, Mn^{2+}, Hg^{2+} and Na^{+} act as enhancer of all fungal species lipase activity, whereas Fe^{2+} for *F. solani* and Mg^{2+} and Zn^{2+} for *F. oxysporum* act as enhancer of lipolytic activity. Due to Ca^{2+} there was no enhancements in lipase activity Table (9a and b). Previous study indicated that lipolytic activity was enhanced by Mg^{2+}, Zn^{2+}, Co^{2+} and Mn^{2+}, but was inhibited by Cu^{2+}, Ba^{2+} and Hg^{2+}, while Ca^{2+} had no effect, while SDS had negligible effects on enzymatic activity, which not completely supported present result [Ghosh et. al., 1996].

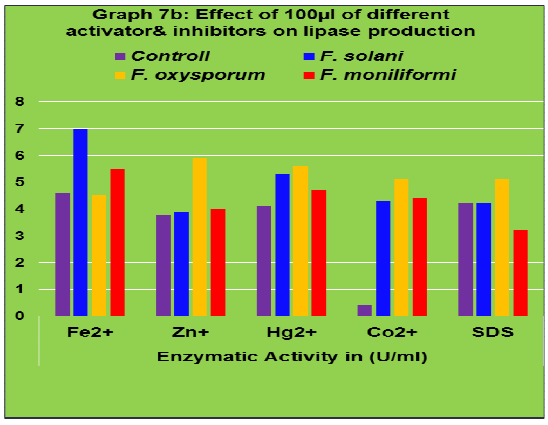
Table 9(a): Effect of 100µl of different Activators & Inhibitors on Lipase production

Fungal sps.	Enzymatic Activity in (U/ml)				
	Mg^{2+}	Cu^{2+}	Mn^{2+}	Ca^{2+}	Na^{2+}
Controll	4.7	0.2	1.9	5.9	3.4
F. solani	3.6	6.0	6.9	4.6	4.4
F. oxysporum	5.7	6.5	6.2	4.8	6.5
F. moniliformi	4.1	7.1	6.9	4.1	3.4

Table 9(b): Effect of 100µl of different Activators & Inhibitors

Fungal sps.	Enzymatic Activity in (U/ml)				
	Fe^{2+}	Zn^{+}	Hg^{2+}	Co^{2+}	SDS
Controll	4.6	3.8	4.1	0.4	4.2
F. solani	7.0	3.9	5.3	4.3	4.2
F. oxysporum	4.5	5.9	5.6	5.1	5.1
F. moniliformi	5.5	4.0	4.7	4.4	3.2



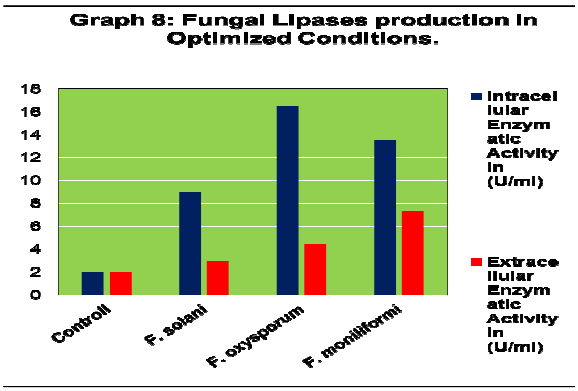


Production of various fungal Lipases in Optimized Conditions

Intra and extracellular lipase production of fungal strains in optimized condition were observed (in U/ml) *F. moniliformi* (13.5 and 7.3), *F. oxysporum* (16.5 and 4.5) and *F. solani* (9.0 and 3.0) (Table 10). Result shown that during optimized condition both intra and extracellular lipase production were high but intracellular production were more as compare to extracellular production.

Table 10: Production of various Intra and Extra-cellular fungal Lipases in Optimized Conditions.

Lipase enzyme of Fungal sps.	Intracellular Enzymatic Activity in (U/ml)	Extracellular Enzymatic Activity in (U/ml)
Controll	2.0	2.0
F. solani	9.0	3.0
F. oxysporum	16.5	4.5
F. moniliformi	13.5	7.3



Fungal Lipase Lipolytic activity in Vegetable oil Hydrolysis

Various vegetable oils (Soybean/Mustard/ Coconut oil) were used as source of carbon. During optimized conditions of all three fungus lipolytic activity (U/ml) were confirmed by hydrolysis of various oils. Maximum intracellular lipolytic activities were obtained as compare to extracellular lipolytic activity in soyabean oil hydrolysis, whereas in Mustard and Coconut oils extracellular lipolytic activity were high. For soyabean oil hydrolysis *F. solani* (11.12), were shown maximum intracellular lipolytic activity whereas in mustard and coconut oils *Fusarium moniliformi* (6.05 and 6.15) extracellular lipolytic activity were maximum (Table 11A, B, C). This study revealed that all fungal strains have capability to hydrolyzed soybean, mustard and coconut oil, it also shown that lipase demonstrated to be a potential enzyme to be applied in oil hydrolysis, releasing a great yield of fatty acids [Kavitha et. al., 1997]. The present results were accordance with D'Annibale *et al*., [D'Annibale et. al., 2006] as in his work every oil has an inducing effect (5-7-fold increase).

Figure 2: Fungal strains Lipolytic activity in Vegetable Oil (Soyabean) Hydrolysis.

Table 11(A): Fungal strains Lipolytic activity in Vegetable Oil (Soyabean) Hydrolysis.

Lipase enzyme of various fungal sps.	Intracellular Lipolytic activity in Coconut Oil (U/ml)	Extracellular Lipolytic activity in Coconut Oil (U/ml)
Controll	2.90	2.90
Fusarium solani	4.25	3.95
Fusarium oxysporum	3.25	3.95
Fusarium moniliformi	6.05	6.15

Table 11(B): Fungal strains Lipolytic activity in Vegetable Oil (Mustard) Hydrolysis.

Lipase enzyme of various fungal sps.	Intracellular Lipolytic activity in Soyabean Oil (U/ml)	Extracellular Lipolytic activity in Soyabean Oil (U/ml)
Control	2.9 0	2.9
Fusarium solani	11.12	5.28
Fusarium oxysporum	7.07	6.53
Fusarium moniliformi	7.75	6.65

Table 11(C): Fungal strains Lipolytic activity in Vegetable Oil (Coconut) Hydrolysis.

Lipase enzyme of various fungal sps.	Intracellular Lipolytic activity in Mustard Oil (U/ml)	Extracellular Lipolytic activity in Mustard Oil (U/ml)
Control	2.90	2.90
Fusarium solani	3.20	4.10
Fusarium oxysporum	5.74	3.40
Fusarium moniliformi	5.80	6.05

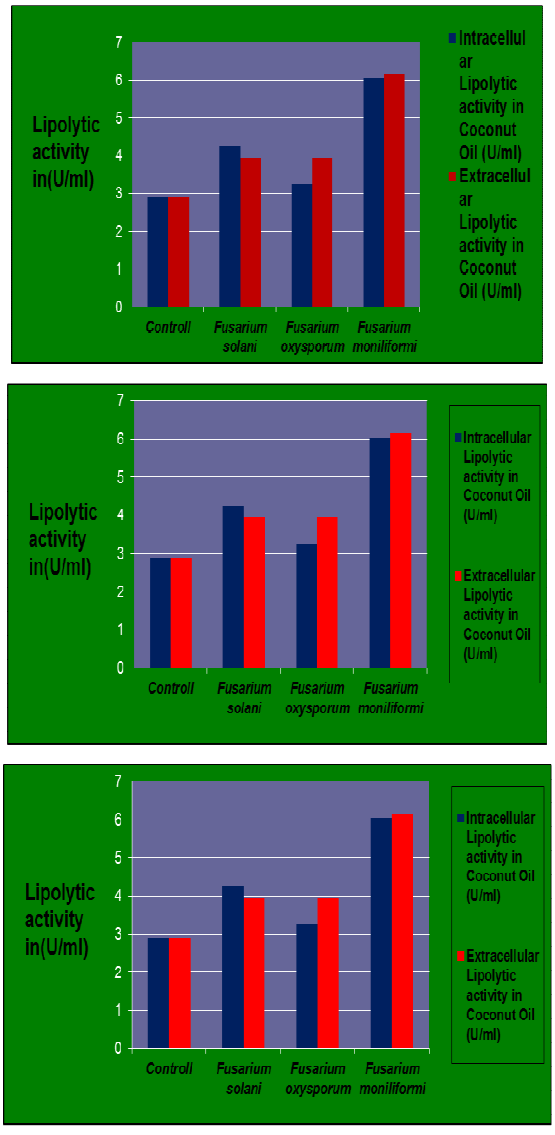


Figure 9(a, b, c): Fungal strains Lipolytic activity in Vegetable Oil Hydrolysis.

CONCLUSION

Lipases are ubiquitous enzymes of considerable physiological significance and industrial potential. Lipases catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids, in contrast to esterases. Microbial lipases are currently receiving much attention with the rapid development of enzyme technology due to their multifold properties. During present study all three fungal species *F. moniliformi*, *F. oxysporum* and *F. solani* have ability to produced lipase enzyme in tributyrin agar medium and in modified form of fermentation medium intracellular. The fermentation conditions such as carbon sources, nitrogenous sources, incubation time, pH, temperature, activator and inhibitors were optimized for all three fungal species. In optimized conditions intracellular lipolytic activity were increased highly as compared extracellular lipolytic activity and maximum activity found in *F. moniliformi* at pH-4 and 45°C. These properties are interesting aiming industrial application both in low pH range and High temperature condition. Application of all fungal lipolytic activity was used for detection in hydrolysis of soybean, mustard and coconut oils. Study were concluded that they all fungal species have capability to hydrolyse soybean, mustard and coconut oil hydrocarbons. Highest potential lipolytic activity for soyabean oil hydrolysis were obtained in *F. solani* and *Fusarium moniliformi* for mustard and coconut oils. lipase demonstrated to be a potential enzyme to be applied in oil hydrolysis, releasing a great yield of fatty acids and also have ability to hydrolyze other oil spillages and reduce fat/oil particles in wastewater efficiently, in approximately 3-fold and its potential to be applied in production of biofuel from vegetable oil hydrolysis. *Fusarium moniliformi* due to their highest lipolytic activity at (pH-4 and 45°C) extreme condition can be uses for butyric acid production from milk fat. Lipases are important products on the manufacture of coatings, adhesives, biofuels, surfactants, specially lubricating oils, shampoos and other personal care products. For future prospect lipase quantity and quality can be more enhance by either produce genetically manipulate microorganisms or gene.

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