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AUGMENTED TRIGLYCEROL ACYLHYDROLASE UNITS BY OPTIMIZATION AND CONVENTIONAL BREEDING OF *ASPERGILLUS* STRAIN (MBL-1612)

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Abstract

The present investigation describes the production potential of a morphologically identified isolate of an ascomycetous fungus *i.e., Aspergillus* sp. (MBL-1612) that was verified by DNA barcoding marker like 18S rRNA gene sequence. Later, the sequence of *Aspergillus* sp. (MBL-1612) was registered with Accession No. KM924435 in the public nucleotide library (genbank) of NCBI. The selected hyper producer of *Aspergillus* sp. (MBL-1612) strain was then subjected to physical and chemical mutagenic treatments in order to improve its lipolytic potential. Proximate analysis proved mustard meal as the best substrate with the maximum extracellular lipases activity of 9.73 UmL^{-1} (wild) and 16.87 UmL^{-1} (mutant). The optimum conditions for the best lipase production through solid substrate fermentation were established by using 10 gram substrate, 1.0mL inoculum level at 35^{o}C after 96 h at pH of 6.5. Finally, the maximum production by mutant strain of *Aspergillus* sp. was achieved when olive oil was used at 1% (149.43 %).

Key Words: Fermentation, *Aspergillus,* lipase, bioprocessing, barcoding

Introduction

Enzymes are biological catalysts and very precise in their action. Today these biomolecules are substituting chemical applications in industries and reimbursing sustainability. Enzymes are biological catalysts and very precise in their action. Lipases are fats and oil hydrolyzing enzymes. Microbial lipases are of more important because of high yield, high catalytic activity, less production and processing cost. Moreover, microorganisms can be easily manipulated for required characteristics. Fungal lipases are considered better for their easy handling during fermentation and low cost extraction. Lipases form an integral part of the industries ranging from food, dairy, pharmaceuticals, agrochemical and detergents to oleo-chemicals, tea industries, cosmetics, leather and in several bioremediation processes (Patil *et al*., 2011).

Because of the vast applications, newer microbes are to be screened for production of lipases. Quantitative enhancement requires strain improvement and medium optimization for the overproduction of the enzyme as the quantities produced by wild strains are usually too low (Iftikhar *et al*., 2014). Gamma irradiations may cause some beneficial mutations to the genes of cells through the DNA repair mechanisms within cells (Ellaiah *et al*., 2002). The organisms are normally grown in a complex nutrient medium containing carbon (oil, sugars, mixed carbon sources), nitrogen and phosphorous sources and mineral salts. Compounds such as olive oil, oleic acid and span 80 seem to play an essential role in lipase synthesis (Manikandan, 2004).

The objective of the present work was to isolate indigenous fungal flora for maximum lipases potential and identification through DNA barcoding markers and finally strain improvement through mutagenesis. Moreover, the wild strain of *Aspergillus* sp. (MBL-1612) was exploited for improving its lipolytic potential using solid state fermentation technique. Further, the results of the proximate analysis of the basal substrate were also aimed to be helpful in designing the medium for commercial production of lipases. The results can also be helpful for establishment of industrial unit of lipases in order to reduce the economic industrial burden on country.

Materials and Methods

Microorganism: A purified culture of *Aspergillus* sp. (MBL-1612) was obtained from the laboratory of Mycology & Biotechnology, Department of Botany, Government College University, Faisalabad. The culture was revived after regular intervals using 4% potato dextrose agar medium (Iftikhar *et al*., 2014).

DNA barcoding studies: The morphologically identified fungal hyper lipase producer was exploited for extraction of gDNA (genomic DNA) as described by van Kan *et al.,* (1991). The extracted gDNA was sent to Macrogen, Korea (http:// www.macrogen.com) for sequencing with 18S universal primers. The sequences were aligned and removed any ambiguity, if present. The sequence was stored in the public nucleotide database of NCBI (https://www.ncbi.nlm.nih.gov/genbank/).

Physical mutagenesis: Gamma irradiation was used for enhanced lipolytic potential of the fungal hyper producer (Estrella *et al*., 1999).

Chemical mutagenesis: Chemical mutagenesis for enhanced lipases production was carried out using various mutagens *i.e.,* nitrous acid, sodium azide and ethyl methane sulphonate (EMS) (Ellaiah *et al*., 2002).

Proximate analysis: In the present study, residues of different agro-industrial byproducts were investigated for improved lipases production. Meals were analyzed according

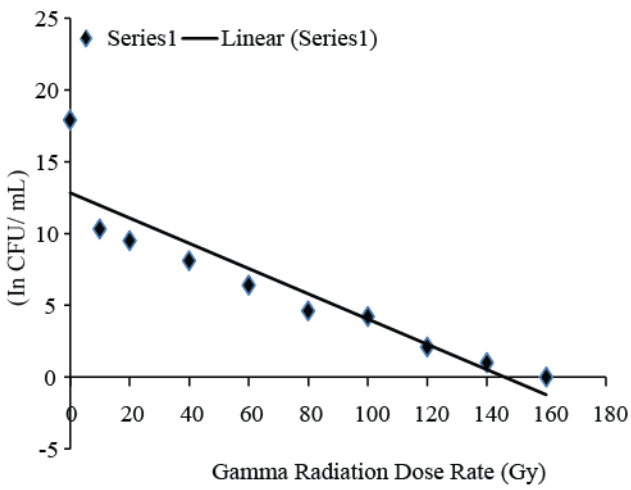
to their respective procedures described in The American Association of Cereal Chemists (Anon., 2000).

Production of extracellular lipase: Fungal strain was subjected to solid state fermentation method for production and extraction of lipases as devised by Kempka *et al*., (2008).

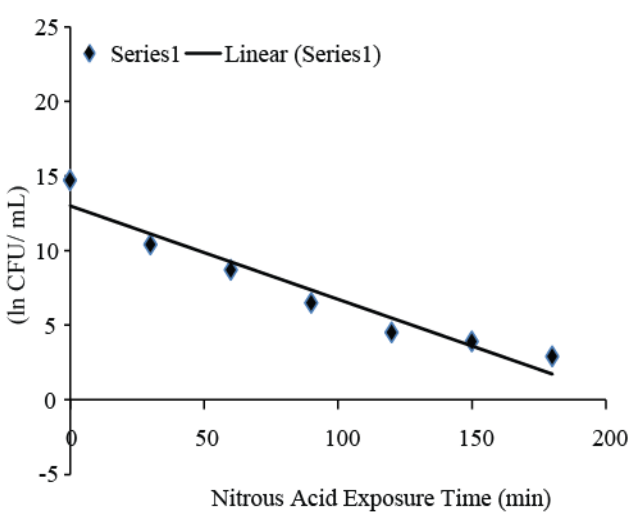
Results and Discussions

Molecular studies of hyper-producer: In order to identify the fungus on genetic basis, 18S rRNA gene sequence is being widely utilized as a molecular marker. The whole mass of mycelium from the colony was used to extract gDNA. For sequence based identification, 18S rRNA gene was targeted. The two universal primers ITS1 and ITS4 were used as reported by Iftikhar *et al*., (2014). Obtained sequence was stored in genbank with the Accession No.KM924435. This sequence analysis corresponds with *Aspergillus* sp.

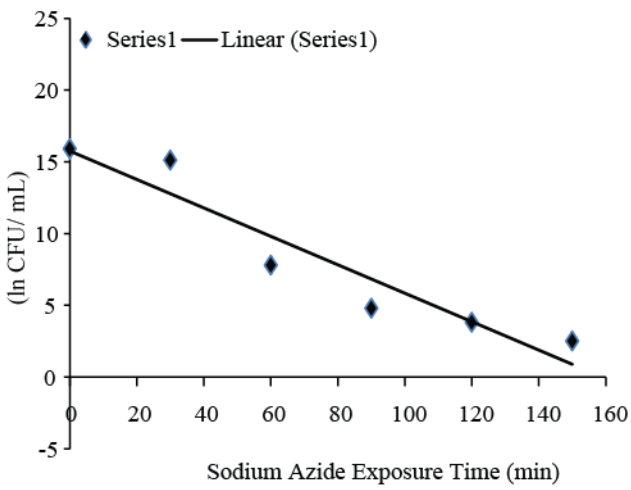
Identification of hyperproducer: A hyper producer of *Aspergillus* sp. (MBL-1612) was exposed to physical (gamma radiation) and chemical mutagenesis (nitrous acid, sodium azide and EMS). Kill curves were formulated Figs. 1(a, b, c & d). There was 140.05 % increased extracellular lipase yield after 120 Gy of mutagenic treatment with gamma radiations as shown in Table. 1. This production was greater than previously reported activity (127%) by *Aspergillus* sp. after 120 Gy, when 95 % death of cells was observed and complete after 160 Gy (Estrella *et al*., 1999; Ellaiah *et al*., 2002). Pimentel *et al*., (1994) also reported Gamma radiation mutagenesis for improvement of extracellular lipase production by *Penicillium citrinum*. He reported 157 % increase extracellular lipases production at 120 Gy dose. Mala *et al*., (2001) reported that mutant strains of *Aspergillus niger* showed five to seven fold enhanced productivity of lipase over the wild strain. Therefore, hyper producer strain of *Aspergillus* sp. [MBL- 1612 $^{\text{Gamma-5}}$(120 Gy)] was used for further studies.



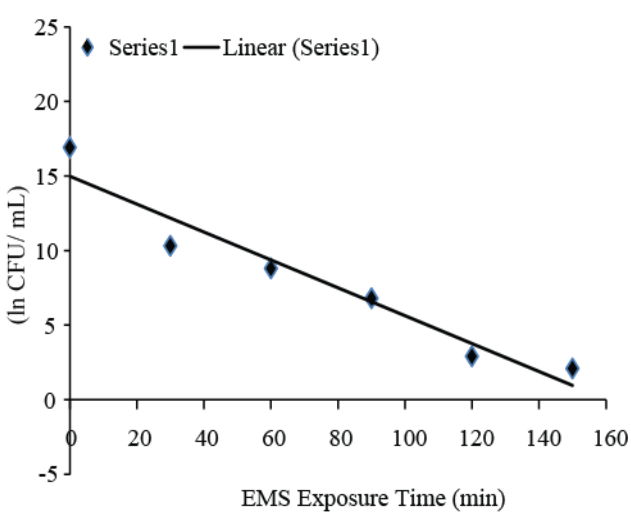
Survival %= 2.1/17.9*100=11.73%
Kill %= 100-11.73= 88.27%
Fig. 1(a). Dose plot of gamma radiation to formulate the kill curve



Survival%= 2.9/14.7*100=19.72%
Kill%=100-19.72=80.28%
Fig. 1(b). Dose plot of nitrous acid to formulate the kill curve



Survival%= 2.5/15.9*100=15.72%
Kill%= 100-15.72=84.28%
Fig. 1(c). Dose plot of sodium azide to formulate the kill curve



Survival%= 2.1/16.9*100=12.42%
Kill %= 100-12.42=87.58%
Fig. 1(d). Dose plot of EMS to formulate the kill curve

Table 1. Activity of extracellular lipase produced by various potent mutants of *Aspergillus* sp. (MBL-1612) using solid state fermentation technique.

Strain improvement by gamma Irradiation treatment				Strain improvement by nitrous acid treatment			
Wild/mutant	**Zone size (mm)**	**Lipase Activity (UmL^{-1})**	**% Increase or decrease in activity**	**Wild/mutant**	**Zone size (mm)**	**Lipase Activity (UmL^{-1})**	**% Increase or decrease in activity**
Wild	7	7.14	100				
Mutants $MBL^{Gamma-1}_{(120\ Gy)}$	12	8.12	113.72	Mutants $MBL^{NA-1}_{(180\ Min)}$	11	7.56	105.88
$MBL^{Gamma-2}_{(120\ Gy)}$	11	9.85	137.95	$MBL^{NA-2}_{(180\ Min)}$	12	7.43	104.06
$MBL^{Gamma-3}_{(120\ Gy)}$	7	8.94	125.21	$MBL^{NA-3}_{(180\ Min)}$	10	8.32	116.52
$MBL^{Gamma-4}_{(120\ Gy)}$	13	8.89	124.50	$MBL^{NA-4}_{(180\ Min)}$	8	7.24	101.40
$MBL^{Gamma-5}_{(120\ Gy)}$	14	10.00	140.05	$MBL^{NA-5}_{(180\ Min)}$	10	7.86	110.08
$MBL^{Gamma-6}_{(120\ Gy)}$	10	7.12	99.71	$MBL^{NA-6}_{(180\ Min)}$	13	7.32	102.52
$MBL^{Gamma-7}_{(120\ Gy)}$	9	9.65	135.15	$MBL^{NA-7}_{(180\ Min)}$	14	6.98	97.75
$MBL^{Gamma-8}_{(120\ Gy)}$	8	9.50	133.05	$MBL^{NA-8}_{(180\ Min)}$	13	7.34	102.80
$MBL^{Gamma-9}_{(120\ Gy)}$	15	9.23	129.27	$MBL^{NA-9}_{(180\ Min)}$	16	6.57	92.01
$MBL^{Gamma-10}_{(120\ Gy)}$	16	7.97	111.62	$MBL^{NA-10}_{(180\ Min)}$	15	7.78	108.96
Strain improvement by sodium azide treatment				**Strain improvement by EMS treatment**			
Wild/mutant	**Zone size (mm**	**Lipase Activity (UmL^{-1})**	**% Increase or decrease in activity**	**Wild/mutant**	**Zone size (mm)**	**Lipase Activity (UmL^{-1})**	**% Increase or decrease in activity**
Mutants $MBL^{SA-1}_{(150\ Min)}$	13	7.92	110.92	Mutants $MBL^{EMS-1}_{(150\ Min)}$	12	7.21	100.98
$MBL^{SA-2}_{(150\ Min)}$	15	9.45	132.35	$MBL^{EMS-2}_{(150\ Min)}$	16	9.26	129.69
$MBL^{SA-3}_{(150\ Min)}$	9	8.81	123.38	$MBL^{EMS-3}_{(150\ Min)}$	10	7.63	106.86
$MBL^{SA-4}_{(150\ Min)}$	8	8.34	116.80	$MBL^{EMS-4}_{(150\ Min)}$	9	8.34	116.80
$MBL^{SA-5}_{(150\ Min)}$	10	7.86	110.08	$MBL^{EMS-5}_{(150\ Min)}$	13	7.45	104.34
$MBL^{SA-6}_{(150\ Min)}$	12	8.56	119.88	$MBL^{EMS-6}_{(150\ Min)}$	14	8.87	124.22
$MBL^{SA-7}_{(150\ Min)}$	9	8.1	113.44	$MBL^{EMS-7}_{(150\ Min)}$	12	8.65	121.14
$MBL^{SA-8}_{(150\ Min)}$	11	7.95	111.34	$MBL^{EMS-8}_{(150\ Min)}$	11	8.92	124.92
$MBL^{SA-9}_{(150\ Min)}$	14	9.23	129.27	$MBL^{EMS-9}_{(150\ Min)}$	15	9.2	128.85
$MBL^{SA-10}_{(150Min)}$	11	9.21	128.99	$MBL^{EMS-10}_{(540\ Min)}$	8	6.43	90.05

Table 2. Proximate Analysis of agro-industrial byproducts before and after the fermentation of by wild and mutant strain of *Aspergillus* sp. (MBL-1612) through Solid state fermentation.

Sr#	Substrate before and after fermented by wild and mutant strain	Substrate (g)	Moisture (%)	Fat (%)	Ash (%)	Fiber (%)	Protein (%)
	Unfermented meal		7.75	9.65	6.65	11.34	38.21
1	Wild fermented meal	Coconut meal	8.21	6.98	7.94	12.23	40.45
	Mutant fermented meal		8.45	6.11	8.44	13.45	43.23
	Unfermented meal		8.21	5.45	6.25	17.12	18.21
2	Wild fermented meal	Sunflower oil seed cake	9.21	4.19	5.66	18.24	20.34
	Mutant fermented meal		9.45	3.23	4.21	18.56	21.46
	Unfermented meal		9.32	7.67	11.87	7.87	17.65
3	Wild fermented meal	Sesame oil seed cake	10.21	6.67	10.86	8.21	19.35
	Mutant fermented meal		10.96	4.21	9.65	9.23	22.36
	Unfermented meal		7.76	6.67	5.98	6.32	40.32
4	Wild fermented meal	Soyabean oil seed cake	8.79	5.52	4.78	7.76	42.24
	Mutant fermented meal		9.21	4.23	3.12	8.32	48.73
	Unfermented meal		6.34	2.45	14.54	15.56	10.34
5	Wild fermented meal	Rice Husk	7.21	2.34	11.34	16.65	12.48
	Mutant fermented meal		7.55	1.74	10.97	17.64	16.74
	Unfermented meal		7.85	5.34	5.21	12.23	25.34
6	Wild fermented meal	Almond oil seed cake	8.77	4.32	4.21	12.72	27.3
	Mutant fermented meal		8.7	3.42	3.32	10.97	30.21
	Unfermented meal		2.12	3.45	3.21	17.85	31.23
7	Wild fermented meal	wheat bran	2.98	2.72	2.91	18.25	33.24
	Mutant fermented meal		3.12	2.21	2.53	20.34	35.56
	Unfermented meal		8.24	12.34	6.61	6.34	39.45
8	Wild fermented meal	Mustard oil seed cake	9.21	11.45	5.21	6.45	42.21
	Mutant fermented meal		9.76	11.16	5.21	6.45	46.23

Proximate analysis: In this study, eight different agricultural byproducts *i.e.,* coconut meal, sesame meal, sunflower meal, almond meal, soybean meal, mustard meal, wheat bran and rice husk were trested to determine the ingredients composition before and after the fermentation by wild and mutant strains of *Aspergillus* sp. [MBL- 1612 $^{Gamma\text{-}5}$(120 Gy)] through SSF for enriched enzymatic potential (Table 2). Results depicted that soybean meal and mustard meal are protein enriched agro-industrial byproducts. Mustard seed cake with protein profile 39.45% before fermentation, 42.21% and 46.23% after fermentation by wild and mutant strains respectively through SSF was the preferred source due to its abundant and inexpensive availability and maximum fat contents. Increased protein level after fermentation may be attributed to efficient bioconversion of highly polymerized carbohydrates into microbial proteins. Likewise, Mitra *et al*., (1996) reported that filamentous fungi by SSF convert cassava to a protein enriched animal feed with protein content as high as 14.32% from the initial 1.28%. Although maximum fats were found in mustard oil seed cake before fermentation as *i.e.,* 12.34% and after the fermentation of 48 hours *i.e.,*11.45% and 11.16% by wild and mutant strain of *Aspergillus* sp. [MBL- 1612 $^{Gamma\text{-}5}$(120 Gy)] (Table 2). Slight increase in the crude fiber contents was observed after 48 h of fermentation in wild and mutant fermented oil seed cake of mustard (Table 2). It may be attributed to the utilization of easily digestible soluble carbohydrates by the growing fungus, leaving the indigestible fiber content high as reported by Singh *et al*., (1990). Ingredient profile indicates mustard meal as good source of nutrients for microbial processes especially for nitrogen and carbon values, similar reported by Lomascolo *et al.,* (2012). .

Optimization of other cultural conditions for the production of extracellular lipase

Effect of agro-industrial by-product: Next step was to select an appropriate agro-industrial byproduct as best substrate for maximum lipase biosynthesis through SSF by

the axenic strain of *Aspergillus* sp. (MBL-1612) in comparison to the mutant hyper producer. Many researchers validate agro-industrial wastes as better substrates for filamentous fungi. In this experiment, among all the tested agro-industrial by-products mustard meal gave significantly high extracellular enzyme activity *i.e.,* 9.73 UmL^{-1} and 16.87 UmL^{-1} by wild and mutant respectively that may be attributed to its comparatively high fat contents based on proximate analysis. Increase in the specific activity of the enzyme by wild and mutant strain of the fungi was also observed (Fig. 2). Hence, it was considered that other substrates may not fulfill the nutritional needs of the organism likewise. Our results correspond to results of Amin *et al*., (2014). Similarly, Sethi *et al*., (2013) results mustard oil cake (MoC) as the best substrate for extracellular lipase production. Thence, mustard meal was optimized as the best source of nutrition for enhanced enzyme production by wild and mutant strain of *Aspergillus* sp. (MBL-1612) and selected for further studies.

Effect of substrate concentration: Substrate concentration plays key role in order to support lipase production. Various concentration levels of mustard meal were tested to optimize the lipolytic potential of wild and mutant strains of *Aspergillus* sp. (MBL-1612) through SSF (Fig. 3). Among all the tested substrate levels, 10 g substrate level gave the highest units of extracellular lipase by both strains after 48 hours incubation period. Similar results were reported by other researchers, (Amin *et al*., 2014) and it might be justified as, at high substrate concentration levels inoculum level becomes inadequately less. As the size of substrate was increased, the production of lipases was observed almost steady. Therefore, 10g of substrate level was optimized for further studies.

Effect of type and size of inoculum: High extracellular lipase production also attributed to suitable type and size of inoculum. Two types of inoculum *i.e.,* vegetative and spore inoculum were investigated for maximum lipase activity by *Aspergillus* sp. (MBL-1612) through SSF (Fig. 4). Results indicate higher extracellular lipase yield when spore inoculum was used. Various inoculum sizes ranging from 0.5-5mL with an interval of 0.5mL were also tested (Fig. 5). The higher extracellular lipase activity was obtained by wild and mutant, when 1.0 mL level of inoculum was used. Though current findings are not in agreement with the previous observations of (Imandi *et al*., 2010), where 2mL of inoculum supported maximum enzyme production. Our results are in line with reports of Iftikhar *et al*., 2014. Finally, 1mL of inoculum was selected for further studies.

Type of moistening agent, Volume of the diluent, pH and type of extractant: Type of moistening agents (diluents) also plays an imperative role in the production of lipase. Volume of diluent, pH of diluent and type of extractant as well influence extracellular lipase yield, by wild and mutant strains of *Aspergillus* sp. (MBL-1612) (Figs. 6, 7 & 8). As far as the volume of the diluent is concerned, it was 10mL of diluent which supported maximized production of enzyme (Fig. 6). It might be due the fact that triacylglycerol acylhydrolase production was decreased at very higher moisture content which may be attributed to decrease in porosity and hence decrease in gaseous exchange leading to suboptimal growth and less enzyme production as directed by Silman *et al*., (1979). Less lipase activity was observed at lower moisture content may be attributed to decrease in the solubility of nutrients of the substrate which lowers the degree of imbibing and creates higher water tension as suggested by Guerra *et al*., (2003). The present investigation corresponds to the results of Mateus et al. (2009). Regarding pH of diluent, it was observed that pH 6.5 supported maximized enzyme production (Fig. 7). Though contrasting reports for higher enzyme production are also available like at pH of 5 (Gutarra *et al*., 2009; (Iftikhar *et al*., 2015) and 6.5 by *Rhizopus chinensis.* Further, phosphate buffer as an extractant supported highest lipase production as shown in (Fig. 8). Other extractants showed comparatively less enzyme activity as than phosphate buffer. Therefore, optimized conditions regarding volume of diluent, initial pH of diluent and extractant were 10mL with pH of 6.5 and phosphate buffer (pH 7) as extractant respectively were optimized and used for further studies.

Effect of Incubation temperature and incubation hours on the lipase production: Incubation temperature and rate of fermentation plays significant role in lipase production from *Aspergillus* sp. (MBL-1612) as shown in Figs. 9 & 10. Maximum units were obtained at 35^{o}C (Fig. 9). While, on the other hand 96h of incubation period supported maximized lipases production both from wild and the mutant strains (Fig. 10). Our results correspond to the investigations of (Amin *et al*., 2014) who demonstrated highest lipase activity (684.02 U/gds) after 96 h of reaction. Likewise, Sethi *et al*., (2013) also reported, mustard meal was the best substrate for extracellular lipase production after 96h incubation. While above or below this time interval comparatively lower production of enzyme was observed. It might be due to the exhaustion of nutrients in substrate, which resulted in the inactivation of enzyme or it may be suggested after prolonged rate of fermentation some proteases produced that were responsible for gradual decrease in lipase activity. Therefore, incubation temperature of 35°C and Incubation period of 96 h were used for further studies.

Effect of carbon and nitrogen sources and additional oils on the production of lipase: Additives to the basal substrate found much significant for hyper production of triacylglycerol acylhydrolase by *Aspergillus* sp. (MBL-1612). Hence, different carbon sources, nitrogen sources and additional oils at 1% concentration level were investigated in order to observe their effect on enzyme production followed by fungal growth Figs. (11, 12 & 13). Lactose, ammonium sulphate and olive oil flavored hyper production of extracellular lipases *i.e.,* 17.68 UmL^{-1} (wild) and 26.42 UmL^{-1} (mutant). It might be due to the fact that lipases production is greatly affected by type and concentration of additives (Hosseinpour *et al*., 2011). It was observed that fungi can efficiently use carbohydrates as carbon source. In order to observe their effect on triacylglycerol acylhydrolase production culture medium was supplemented with such carbon sources. Results

revealed that among all the carbon sources used lactose showed better activity as shown in (Fig. 11). Similarly, Sethi *et al*., (2013) reported lactose as best carbon source for hyper lipase activity in mustard seed oil cake medium. Similar results were obtained by Amin *et al*., (2014) who also found lactose as better carbon source for lipase yield.

Different organic and inorganic nitrogen sources are frequently used for hyper lipase production. For hyper production of lipases basal medium was supplemented with various organic and inorganic nitrogen sources. Among all used nitrogen sources ammonium sulphate gave maximum lipase activity and urea showed least activity (Fig. 12). Likewise, Malilas *et al*., (2013) reported maximum yield of lipase when 1% of olive oil and 1% of $(NH4)_2SO_4$ was employed during SmF. Khayati *et al*., (2013) also reported lactose and ammonium sulfate significantly enhanced the lipase production. Oils or other inducers generally found ideal supplements for lipase production. A number of oils were used as inducer in the experiment but olive oil was found most favorable to enhance lipase activity (Fig. 13). A number of reports in favour of our work previously exist. Silva *et al*., (2005) also revealed maximum lipase activity when olive oil was used as inducer. Therefore, Lactose, ammonium sulphate and olive oil at 1% were proved for enhanced lipase production by wild and mutant strain of *Aspergillus* sp. (MBL-1612).

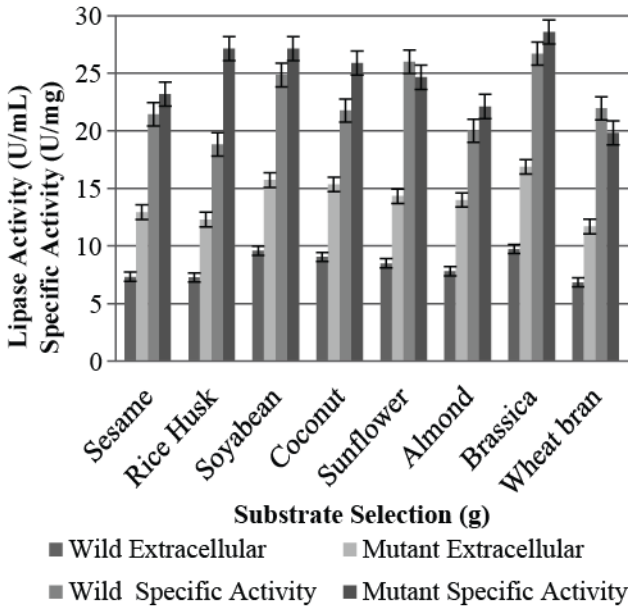


Fig. 2. Effect of the substrate on the production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

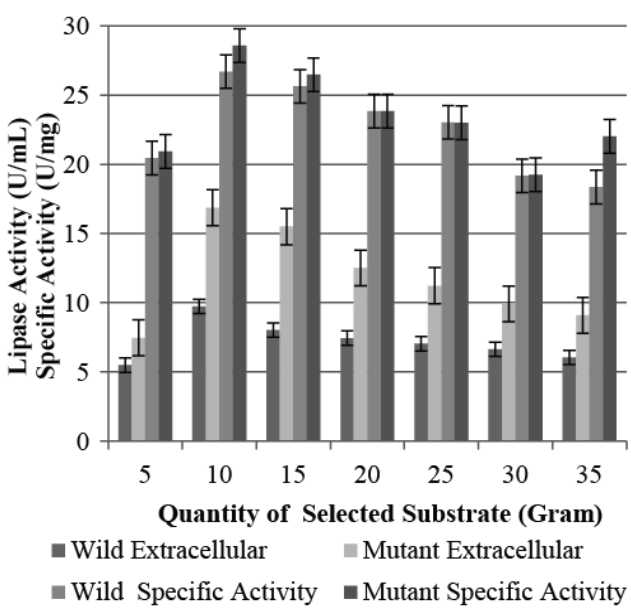


Fig. 3. Effect of the substrate concentration on the production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

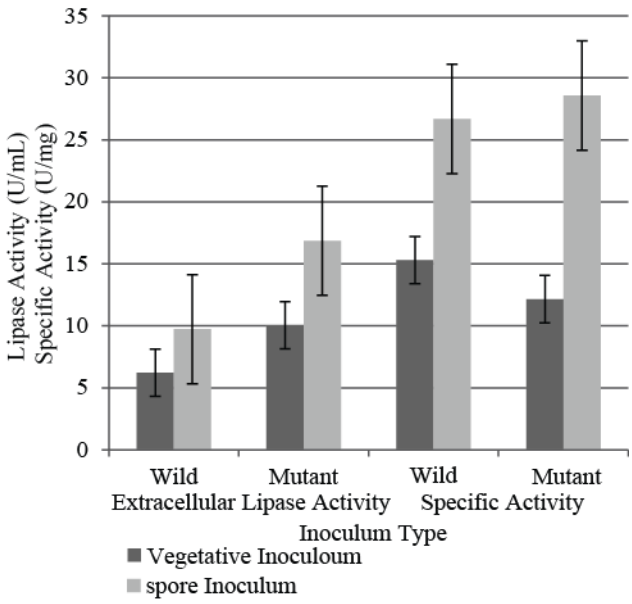


Fig. 4. Effect of type of inoculum on the production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

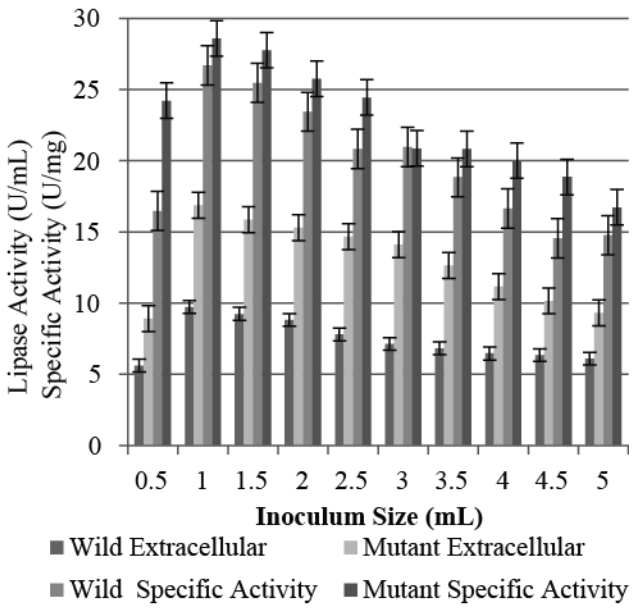


Fig. 5. Effect of size of inoculum on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

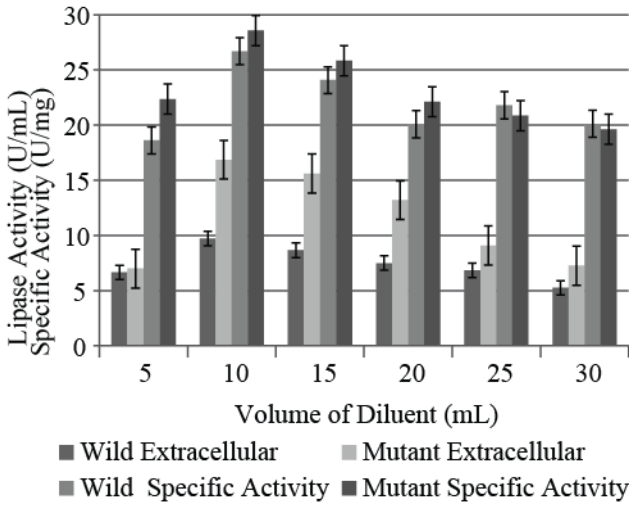


Fig. 6. Effect of volume of the diluent on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

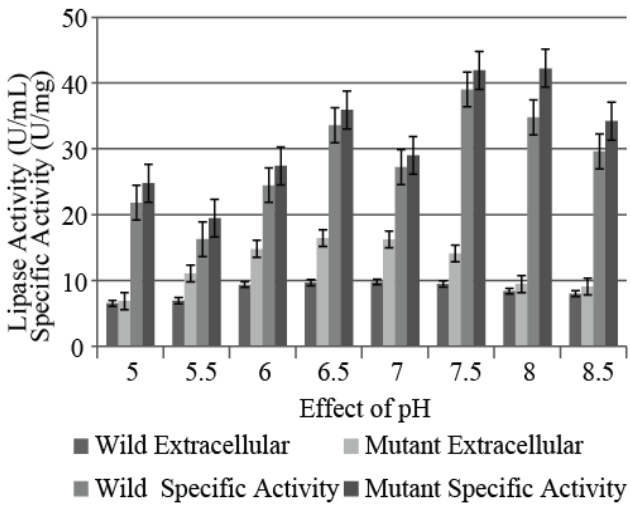


Fig. 7. Effect of initial pH on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

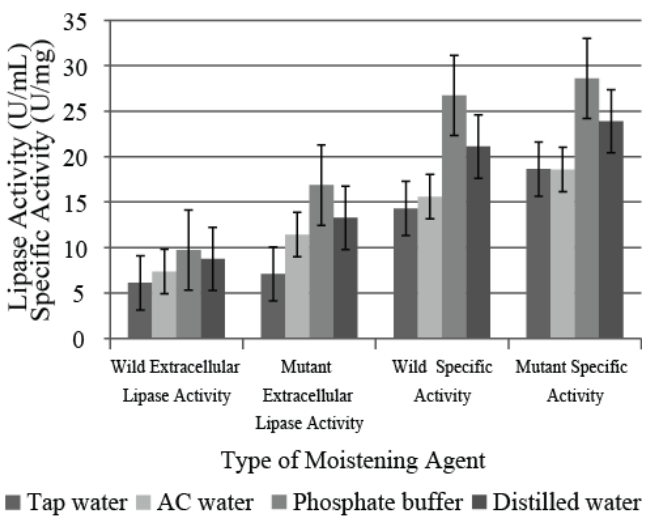


Fig. 8. Effect of type of moistening agents on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

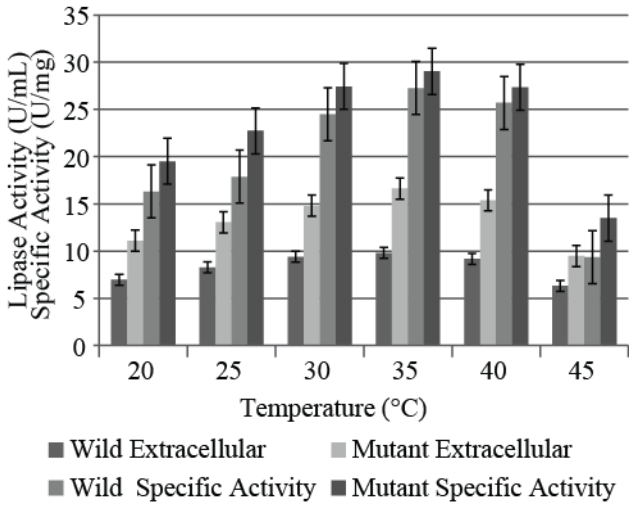


Fig. 9. Effect incubation temperature on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

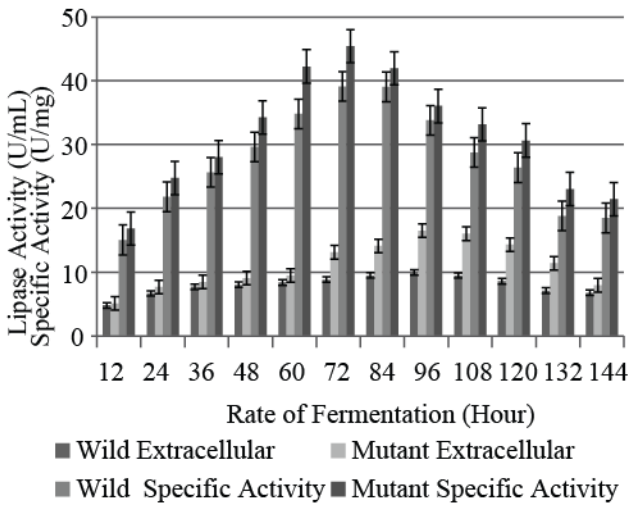


Fig. 10. Effect of incubation period on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

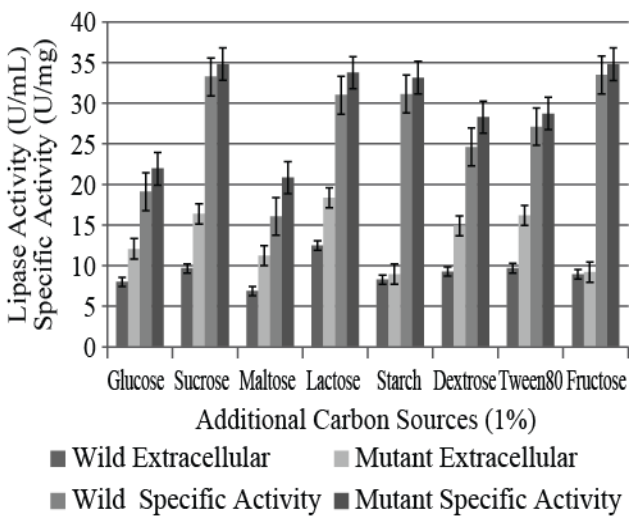


Fig. 11. Effect of additional carbon sources on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

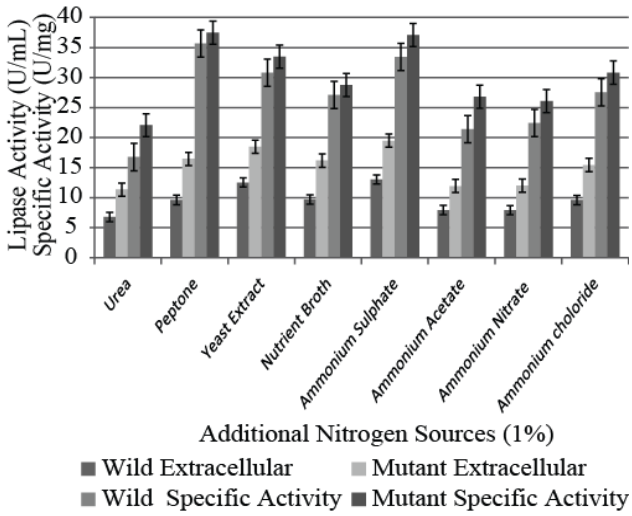


Fig. 12. Effect of additional nitrogen sources on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

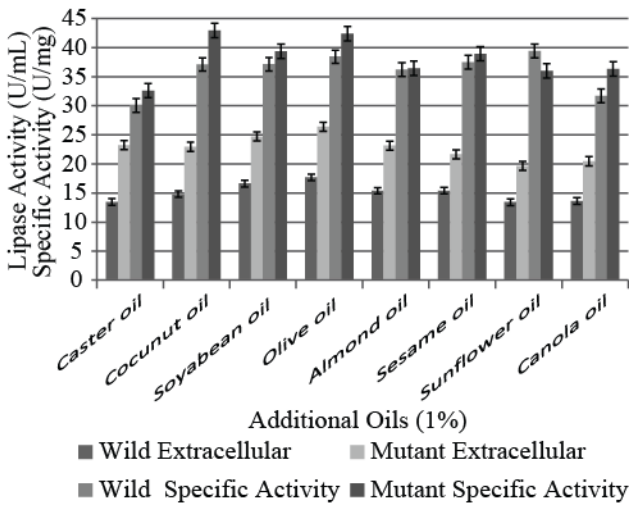


Fig. 13. Effect of additional oils sources on production of extracellular lipases & specific activity by wild & mutant strain of *Aspergillus* sp. (MBL-1612) through SSF

Conclusions: From the current findings, it is concluded that sequence of *Aspergillus* sp. (MBL-1612) which wasregistered in NCBI database could be useful as a reference for identification of fungi. Present work is one of few studies in which proximate analysis of agro-industrial byproducts was investigated. It will help to design an industrial scale optimized medium and is able to identify the byproducts that can interchangeably be used for lipases production.

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References

Amin, M., H.N. Bhatti, M. Zuber, I.A. Bhatti, M. Asgher. 2014. Potential use of agricultural wastes for the production of lipase by *Aspergillus melleus* under solid state fermentation. *J. Animal Plant Sci.,* 24(5): 1430-1437.

Anonymous. 2000. AACC (The American Association of Cereal Chemists). Approved Methods of American Association of Cereal Chemists. *Am. Assoc. Cereal Chem.,* Inc St Paul Minnesota, USA.

Ellaiah, P., T. Prabhakar, B. Ramakrishna A.T. Taleb and K. Adinarayana. 2002. Strain improvement of *Aspergillus niger* for the production of lipase. *Indian J. Microbiol.,* 42:151-153.

Estrella, C.M., R. Diez, B.M. Suarez, B.M.A. Monozon and R.J.L. Tudela. 1999. Comparative in-vitro activity of voriconazole (UK.109, 496) and other six antifungal agents against clinical isolates of *Scedosporium prolificans* and *Scedosporium apiospermum*. *J. Antimicrob Chemother*, 43:149-151.

Guerra, P.N., T.A. Agrasar, L.C. Macias and L. Pastrana. 2003. Main characteristics and applications of solid substrate fermentation: *Electronic J. Environ. Agri. & Food Chem.*, 2(3): 343-350.

Gutarra, M.L.E., M.G. Godoy, F. Maugeri, M.I. Rodrigues, D.M.G. Freire and L.R. Castilho. 2009. Production of an acidic and thermostable lipase of the mesophilic fungus *Penicillium simplicissimum* by solid-state fermentation. *Biores. Technol.*, 100(21): 5249-5254.

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. Sci. Res.*, 7(3): 362-366.

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. J. Biosci.,* 7(2): 178-186.

Iftikhar, T., M. Niaz, M.Z. Haider, Sidra, D.R. Nayyab. R. Abdullah and M.A. Zia. 2014. Morpho-molecular identification of a novel *Aspergillus* spp. and its cultural optimization for lipases production. *Pak. J. Bot*., 46(6): 2297-2304.

Imandi, S.B., S.K. Karanam and H.R. Garapati. 2010. Optimization of process parameters for the production of lipase in solid state fermentation by *Yarrowia lipolytica* from Niger Seed Oil Cake *(Guizotia Abyssinica*). *J. Microbial & Biochem. Technol.*, 2: 28-33.

Kempka, A.P., N.L. Lipke, T.L.F. Pinheiro, S. Menoncin, V. 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 verrucosum* in solid-state fermentation. *Bioprocess Biosystems Engineering*, 31(2): 119-125.

Khayati, G., H.G. Gilani and M. Kazemi. 2013. The Effect of olive cake types on lipase production by isolated *Rhizopus* sp. and process statistical optimization. *J. Bio. Sci. Biotech*., 2(1):45-55.

Lomascolo, A., E. Uzan-Boukhris, J.C. Sigoillot and F. Fine. 2012. Rapeseed and sunflower meal: a review on biotechnology status and challenges. *Appl. Microbiol. & Biotechnol.,* 95: 1105-1114.

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

Malilas, W., S.W. Kang, S.B. Kim, H.Y. Yoo, W. Chulalaksananukul and S.W. Kim. 2013. Lipase from *Penicillium camembertii* KCCM 11268: Optimization of solid state fermentation and application to biodiesel production. *Korean J. Chem. Eng*., 30: 405-412.

Manikandan, K. 2004. *Optimization of media for lipase production by Bacillus sphaericus.* M Tech Thesis, Annamalai University, Annamalai Nagar.

Mateus, G.G., L.E. Melissa, V. Gutarra, P.F. Shayany, V.B. Juliana, L.T.M. Olga, M.G.F. Denise. 2009. Use of a low cost methodology for bio detoxification of castor bean waste and lipase production. *Enz. & Microbial Technol.*, 44: 317-322.

Mitra, P., R. Chakraborty and V. Chandra. 1996. Production of proteolytic enzymes by solid-state fermentation. *J. Sci. & Indus. Res.,* 55:439-442.

Patil, K.J., M.Z. Chopda and R.T. Mahajan. 2011. Lipase biodiversity. *Ind. J. Sci. Tech*., 4(8): 971-982.

Pimentel, M.C., N. Krieger, L.C. Coelho, J.O. Fontana, E.H. Melo, W.M. Ledingham and F.J.L. Lima. 1994. Lipase from a Brazilian strain of *Penicillium citrinum*. *App. Biochem. Biotechnol*., 49: 59-74.

Sethi, B.K., J.R. Rout, R. Das, P.K. Nanda and S.L. Sahoo. 2013. Lipase production by *Aspergillus terreus* using mustard seed oil cake as a carbon source. *Ann. Microbiol.*, 63(1): 241-252.

Silman, R.W., M.F. Conway, R.A. Anderson and E.B. Bagley. 1979. Production of aflatoxin in corn by large scale solid state fermentation process. *Biotechnol. & Bioeng.*, 21**(**10): 1799-1808.

Silva, W.O.B., S. Mitidieri, A. Schrank and M.H. Vainstein. 2005. Production and extraction of an extracellular lipase from the entomopathogenic fungus *Metarhizium anisopliae*. *Process Biochem.,* 40: 321-326.

Singh, K., C.J. Linden, E.J. Johnson and P.R. Tengerdy.1990. Bioconversion of wheat straw to animal feed by solid substrate fermentation or ensiling. *Indian J. Microbiol.,* 30(2): 201-208.

van Kan, J.A.L., G.F.J.M. Van Den Ackerveken and P.J.G.M. De Wit. 1991. Cloning and characterization of cDNA of avirolence gene *avr9* of the fungal pathogen *Cladosporium fulvum*, causal agent of tomato leaf mold. *Mol. Plant-Microbe Interactions,* 4:52-59.

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