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Strain improvement of Phosphate solubilizing fungal strains for the production of lipase

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Abstract: A laboratory study was conducted to isolate, investigate and enhance the phosphate solubilization efficiency of certain isolated fungal strains viz., *Aspergillus niger, Aspergillus fumigatus, Penicillium sp*. These fungal strains were isolated from the rhizosphere of paddy fields in Tamilnadu, India and screened for phosphate solubilization. The enhancement of Lipase activity of these strains was studied through mutagenesis: UV, sodium azide and ethyl methane sulphonate. The mutant ANuv50 exhibited 32.72% increased efficiency for Lipase activity in the presence of sucrose as a carbon source and 91.49% increased efficiency for Lipase activity in the presence of olive oil as a carbon source compared to the wild strains. Similarly, the Lipase activity by sodium azide mutant (ANsa90) was 61.11% higher in the presence of sucrose as a carbon source, 107.60% higher in the presence of olive oil as a carbon source and EMS mutant (ANems150) was 137.65% higher in the presence of sucrose as a carbon source, 98.93% higher in the presence of olive oil as a carbon source than the wild strain. The efficient strains were further studied for the effect of carbon and nitrogen source on Lipase activity. Significance of the findings is discussed in detail.

Keywords: Phosphate solubilizing fungi, Mutagenesis, Enhancement, Mutant strains, Lipase activity
Abbreviation: SA: Sodium azide, EMS: Ethyl methane sulphonate, UV: Ultra Violet, ANsa: Sodium azide treated *Aspergillus niger,* AFsa: Sodium azide treated *Aspergillus fumigatus,* PEsa: Sodium azide treated *Penicillium.* ANems: Ethyl methane sulphonate treated *Aspergillus niger,* AFems: Ethyl methane sulphonate treated *Aspergillus fumigatus,* PEems: Ethyl methane sulphonate treated *Penicillium,* ANuv: Ultra Violet treated *Aspergillus niger,* AFuv: Ultra Violet treated *Aspergillus fumigatus* and PEuv: Ultra Violet treated *Penicillium.*

INTRODUCTION

Lipases hydrolyze triacylglycerols to fatty acids, di-acylglycerols, monoacyl glycerols and glycerol and under certain conditions, catalyze reverse reactions such as esterification and transesterification [1-2]. In the last decades, the interest in microbial lipase production has increased. Due to the versatility of the molecular structure and catalytic properties, these enzymes have potential application in different industrial sectors such as food, waste water treatment, cosmetics, oleochemical, pharmaceutics, detergents [1] and in the fuel sector, which applies lipase as catalyst for synthesis of esters and for transesterification of the oil for the production of biodiesel [3-5].

Lipolytic enzymes such as lipases can be found in animals[6], plants [7] and microorganisms [8-10]. The advantages of enzymatic hydrolysis of lipid substances and their biotechnological transformation over the conventional chemical process are less energy requirement and superb quality of the derived products. Bacterial lipases are glycoprotein, but some extracellular bacterial lipases are lipoproteins. Most of the bacterial lipases reported so far are constitutive and are non-specific in their substrate specificity and a few bacterial lipases are thermostable[11].

Lipase in general and this enzyme from fungi in particular, have a long array of industrial application in the product and process of detergents, oil, fats and dairy coupled with enormous therapeutic uses. Fungi are the important enzyme producers since their enzymes are produced extracellularly [12].

Ellaiah *et al.* [13] isolated mutant strains of *Aspergillus* by exposing the parent using ultra-violet and N-methyl, N-nitro, N-nitroso guanidine. Lipase activity of these mutants were studied and compared with wild type strains. The effect of different carbon and nitrogen sources were also studied on the mutant which produce high lipase activity.

Bapiraju *et al.* [14] investigated the lipase activity of *Rhizopus* species isolated from coconut oil mill waste. They improved the lipase activity of the strain by natural selection and random mutagenesis by UV and N-methyl, N-nitro, N-nitroso guanidine. However, not much work has been carried on lipase activity of mutated *Aspergillus* and *Penicillium*

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species. Hence the present study has been undertaken to reveal the efficacy of lipase activity by mutated strains isolated from paddy soils.

MATERIALS AND METHODS

Microorganisms and isolation

The fungal strains were isolated from rhizosphere soils of Paddy field, Mannachanallur, one of the largest paddy producing taluk located in Tamil Nadu State, India. The soil samples were screened for phosphate solubilizing fungi in Pikovskaya's agar medium by conventional dilution plate count method. The fungal strains showing halo formation around their colonies were isolated and grown on Sabouraud agar slants at 27ºC for 3 days and kept in the refrigerator at 4^0C until further use [13].

Identification

The fungal cultures were identified based on the colony morphology and spore structure [15].

Random mutational studies

Mutagenesis by UV

72 hrs old fungal cultures were scraped off from agar slants and suspended in 5ml sterile distilled water and then diluted with 45 ml of sterile distilled water containing Tween 80 (1:4000). Sterile glass beads were added to the suspension and kept on rotary shaker for 30 min to break the hyphal mycelium. The suspension was filtered to remove the mycelium. The spore suspension was prepared in phosphate buffer (pH7.0) containing 10^6 spores per ml. Five ml quantities of the spore suspension were transferred aseptically into sterile petri dishes and exposed to UV light (2600 A^o) at a distance of 15cm away from the center of the Germicidal lamp for various time intervals (10, 20, 30, 40, 50, 60, 70 and 80 min). The suspension was agitated by gently rotating the plates in between the time intervals [13].

The UV exposed spore suspensions were stored in dark for overnight to avoid photo reactivation. After overnight incubation, irradiated spore suspensions were serially diluted by using phosphate buffer (pH 7.0) and plated on Sabouraud's Dextrose agar medium. The plates were incubated for 5 days at 27°C. The colonies were selected on the basis of their morphological characters and were given the code numbers ANuv30, ANuv40, ANuv50, ANuv60, AFuv30, AFuv40, AFuv50, AFuv60, PEuv30, PEuv40, PEuv50 and PEuv60.

Mutagenesis by Chemicals

Chemical mutagenesis was performed using Sodium azide and Ethyl methane sulphonate (EMS) for the strain improvement of phosphate solubilizers. Spore suspensions of fungal strains were prepared by using phosphate buffer pH 7.0 as described earlier [13]. To 9 ml of each spore suspension, 1 ml of sterile solution of Sodium azide (250 μg ml^{-1} in phosphate buffer) was added. Similar procedure was adopted for EMS (150 μg ml^{-1} in phosphate buffer). The reaction was allowed to proceed. Control tube was also kept without any chemical mutagen. Samples were withdrawn from the reaction mixture at an interval of 30, 60, 90, 120 and 150 min. and centrifuged for 10 min. at 5000 rpm. The cells were washed three times with sterile distilled water and again re-suspended in 10 ml sterile buffer. The samples were serially diluted with the same buffer and plated on Sabouraud's Dextrose agar medium. The selected sodium azide treated mutants were given the code numbers ANsa30, ANsa60, ANsa90, ANsa120, AFsa30, AFsa60, AFsa90, AFsa120, PEsa60, PEsa90, PEsa120 and PEsa150.

The EMS treated mutants were given the code numbers ANems30, ANems60, ANems90, ANems120, AFems30, AFems60, AFems90, AFems120, PEems60, PEems90, PEems120 and PEems150 [14].

Submerged fermentation

Inoculation

The pure fungal culture from Sabouraud agar slant was transferred to Sabouraud agar plates. After four days incubation at 27°C, two 8mm disc were cut from all three fungal plates and inoculated into 100ml Czapek dox broth containing 1% olive oil as inductor and also 10% olive oil in 100ml Czapek dox broth instead of sucrose. The flasks were incubated at 27° C. Uninoculated Czapek dox broth served as a control in each case. Each experiment was done in triplicate set.

Lipase assay

Growth medium was withdrawn aseptically at three days interval from each flask and centrifuged at 3000rpm for 15 minutes and the supernatant was collected and used for lipase activity [16].

The enzyme solution was prepared by mixing the supernatant solution with 1ml of olive oil and 3% gum Arabic in 250ml conical flask and the contents were emulsified using magnetic stirrer for 5 minutes at top speed.

After emulsification, 10ml of enzyme solution was taken in a flask followed by addition of 20ml of double distilled water and 5ml of 0.1M phosphate buffer (pH 7). This mixture was kept on rotary shaker for 30 minutes at 120rpm. Simultaneously the same protocol was followed except for the enzyme solution and this was treated as a control. After 30 minutes a drop of 1% alcoholic phenolphthalein solution was added and titrated against 0.02 N NaOH till the appearance of pale pink colour.

Lipase activity

One unit of lipase activity was defined as the amount of enzyme required to release one μ mol of free fatty acid in one min under standard assay conditions.

$$\text{Lipase activity} = \frac{\text{Volume of NaOH consumed} \times \text{Normality of NaOH} \times 1000}{\text{Time of incubation} \times \text{Volume of enzyme solution expressed as Unit/ Substrate}}$$

Effect of different Carbon Sources

To study the effect of different carbon sources on lipase production, the carbon source of Czapek dox broth i.e. Sucrose was replaced by Lactose, Glucose and Mannitol. The fungal and bacterial cultures were inoculated and incubated as described above. After incubation Lipase assay was carried out as described earlier with Sucrose, Lactose, Glucose and Mannitol as a carbon source and 1% olive oil as a inductor in Czapek dox broth.

Effect of different Nitrogen Sources

To study the effect of different nitrogen sources on lipase production, the Nitrogen source of Czapek dox broth i.e. Sodium nitrate was replaced by Potassium nitrate, Ammonium sulphate and urea. 1% olive oil was used as a inductor in Czapek dox broth. The fungal and bacterial cultures were inoculated and incubated by above described inoculation methods and incubation temperature. After incubation Lipase assay was carried out as described earlier.

RESULTS AND DISCUSSION

The efficacy of Lipase activity of fungal strains treated by ultraviolet rays, sodium azide and ethyl methane sulphonate (EMS) are presented in the Tables 1 to 3, 4 to 6 and 7 to 9 respectively. The influence of different carbon and nitrogen sources on phosphate solubilization of fungal mutants are reported in the Tables 10 and 11.

Table 1. Efficacy of Lipase activity of UV treated *Aspergillus niger*

UV treated *Aspergillus niger* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
ANuv30	1.86±0.08	0.49±0.04	14.81	4.26
ANuv40	1.95±0.06	0.62±0.08	20.37	31.91
ANuv50	2.15±0.05	0.90±0.06	32.72	91.49
ANuv60	1.89±0.05	0.60±0.04	16.66	27.66
Wild type	1.62±0.07	0.47±0.04		

*Mean value ± Standard Deviation of triplicates

Table 2. Efficacy of Lipase activity of UV treated *Aspergillus fumigatus*

UV treated *Aspergillus fumigatus* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
AFuv30	1.22±0.09	0.33±0.04	20.79	22.22
AFuv40	1.39±0.08	0.38±0.04	37.62	40.74
AFuv50	2.09±0.09	0.60±0.04	106.93	122.22
AFuv60	1.75±0.06	0.56±0.07	73.26	107.40
Wild type	1.01±0.07	0.27±0.04		

*Mean value ± Standard Deviation of triplicates

Table 3. Efficacy of Lipase activity of UV treated *Penicillium* sp

UV treated *Penicillum* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PEuv30	1.28±0.06	0.48±0.05	6.67	4.35
PEuv40	1.43±0.06	0.73±0.04	19.17	58.70
PEuv50	1.62±0.08	0.69±0.06	35.00	50.00
PEuv60	1.69±0.08	0.85±0.04	40.83	84.78
Wild type	1.20±0.10	0.46±0.04		

*Mean value ± Standard Deviation of triplicates

Table 4. Efficacy of lipase activity of Sodium azide treated *Aspergillus niger*

Sodium azide treated *Aspergillus niger* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
ANsa30	1.90±0.08	0.51±0.07	17.28	8.51
ANsa60	2.09±0.05	0.68±0.05	29.01	44.68
ANsa90	2.61±0.07	0.98±0.04	61.11	108.61
ANsa120	1.85±0.06	0.63±0.03	14.19	34.04
Wild type	1.62±0.07	0.47±0.04		

*Mean value ± Standard Deviation of triplicates±

Table 5. Efficacy of lipase activity of Sodium azide treated *Aspergillus fumigatus*

Sodium azide treated *Aspergillus fumigatus* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
AFsa30	1.45±0.05	0.41±0.04	43.56	51.85
AFsa60	1.25±0.05	0.38±0.06	23.76	40.74
AFsa90	1.22±0.07	0.30±0.03	20.79	11.11
AFsa120	1.60±0.07	0.44±0.07	58.42	62.96
Wild type	1.01±0.07	0.27±0.04		

*Mean value ± Standard Deviation of triplicates±

Table 6. Efficacy of lipase activity of Sodium azide treated *Penicillium* sp.

Sodium azide treated *Penicillilum* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PEsa30	1.56±0.06	0.48±0.04	30.00	4.35
PEsa60	1.43±0.04	0.69±0.06	19.17	50.00
PEsa90	1.76±0.08	0.73±0.04	46.67	58.70
PEsa120	2.01±0.07	0.99±0.03	67.50	115.22
Wild type	1.20±0.10	0.46±0.04		

*Mean value ± Standard Deviation of triplicates

Table 7. Efficacy of lipase activity of Ethyl methane sulphonate treated *Aspergillus niger*

EMS treated *Aspergillus niger* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
ANems60	1.90±0.09	0.62±0.06	17.28	31.91
ANems90	2.09±0.10	0.77±0.07	29.01	63.83
ANems120	3.10±0.08	0.87±0.05	91.35	85.11
ANems150	3.85±0.07	0.94±0.03	137.65	100.00
Wild type	1.62±0.07	0.47±0.04		

*Mean value ± Standard Deviation of triplicates

Table 8. Efficacy of lipase activity of Ethyl methane sulphonate treated *Aspergillus fumigatus*

EMS treated *Aspergillus fumigatus* strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
AFems60	1.85±0.05	0.33±0.05	83.17	22.22
AFems90	2.26±0.09	0.40±0.04	123.76	48.15
AFems120	2.76±0.08	0.45±0.06	173.26	66.17
AFems150	2.60±0.09	0.41±0.03	157.43	51.85
Wild type	1.01±0.07	0.27±0.04		

*Mean value ± Standard Deviation of triplicates

Table 9. Efficacy of lipase activity of Ethyl methane sulphonate treated *Penicillium* sp.

EMS treated *Penicillium* sp strains	Lipase activity after 96 hrs(Unit/g of substrate)*		Increased % of Lipase activity	
	Sucrose medium	Olive oil medium	Sucrose medium	Olive oil medium
PEems60	1.64±0.06	0.50±0.03	36.67	8.70
PEems90	1.79±0.05	0.58±0.03	49.17	26.09
PEems120	1.89±0.04	0.63±0.04	57.50	36.96
PEems150	1.97±0.08	0.77±0.06	64.17	67.39
Wild type	1.20±0.10	0.46±0.04		

*Mean value ± Standard Deviation of triplicates

Table 10. Efficacy of lipase activity of fungal strains grown on different Carbon sources

Carbon sources vs ANems120 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*	Carbon sources vs AFems120 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*	Carbon sources vs ANems150 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*
Glucose	2.94±0.06	Glucose	2.54±0.05	Glucose	2.44±0.03
Sucrose	3.10±0.08	Sucrose	2.76±0.08	Sucrose	2.61±0.07
Mannitol	1.85±0.07	Mannitol	1.69±0.07	Mannitol	1.98±0.07
Lactose	1.65±0.09	Lactose	1.25±0.07	Lactose	1.34±0.09

*Mean value ± Standard Deviation of triplicates

Table 11. Efficacy of lipase activity of fungal strains grown on different Nitrogen sources

Nitrogen sources vs ANems120 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*	Nitrogen sources vs AFems120 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*	Nitrogen sources vs ANems150 Strain	Lipase activity after 96 hrs (Unit/g of substrate)*
Sodium nitrate	3.10±0.08	Sodium nitrate	2.76±0.08	Sodium nitrate	2.61±0.07
Potassium nitrate	2.85±0.05	Potassium nitrate	2.67±0.09	Potassium nitrate	2.24±0.06
Ammonium sulphate	2.01±0.05	Ammonium sulphate	2.13±0.10	Ammonium sulphate	1.98±0.08
Urea	1.55±0.07	Urea	1.54±0.06	Urea	1.27±0.08

*Mean value ± Standard Deviation of triplicates

The Lipase production capacity of isolated fungal cultures were analysed by inoculating the isolates in Czapek's dox broth enriched with 1% Olive oil as a inducer and the same were inoculated in Czapek's dox broth replaced with 10% Olive oil as carbon source for Lipase production. A number of reports are available for the addition of inducer to induce the production of Lipases. Salihu *et al.*[17] utilized Olive oil and Palm oil as an inducer. Olive oil by Rifaat *et al.* [18]; Hosseinpour *et al.* [19]; Cocunut oil by Rani and Panneerselvam [12] and different lipid sources namely Olive oil, Coconut oil, Groundnut oil, Triacetin, Tributyrin and Surfactants namely Tween 20, Tween 40, Triton –X was used for acceleration of Lipase by Pogaku *et al.* [11]. It was suggested that the Lipase formed might be derived from carbohydrates in the medium with small quantities of lipid material as inducer [20, 21].

Rani and Panneerselvam [12] showed that *Aspergillus fumigatus, A. terreus, Penicillium chrysogenum, P. funiculosum and Fusarium moniliforme* were selected as the highest Lipase producers. In the present study, the Lipase production efficacy of the wild strains was observed in the descending order of *Aspergillus niger* >*Penicillium* sp. > *Aspergillus fumigatus*. Juichiro *et al.* [22] reported that several strains of *Rhizopus* and *Aspergillus niger* as most potent Lipase formers. Successful isolation of the enzyme could be made from the bran-koji culture of *Aspergillus niger*. Ogundero [23] stated that *A. fumigatus* and *A. nidulans* were able to degrade vegetable oils and triglycerides.

Lawrence *et al.* [24] showed that the Lipase activity was considerably increased by nutritional and physical conditions from *Pseudomonad*. Suzuki *et al.* [25] used the Olive oil as a carbon source for microbial growth and Lipase production by *Pseudomonas* sp. *Ray et al.* [16] reported the Lipase activity of the isolated bacterial strain, *Corynebacterium* sp. was increased 2.3 fold by mutagenic technique. This was agreed with our present study, since all mutant strains tested were found to produce more Lipase activity, when compared to wild strains. ANems150 was the best strain for Lipase production among the chemical mutated fungal strains followed by ANems120, AFems120, Ansa90, AFems150, AFems90, ANsa60, PEsa120 and PEems150 grown in Sucrose containing medium. Ellaiah *et al.* [13] isolated a fungal strain, which produced Lipase constitutively. The isolated fungal strain was used to produce mutants using physical and chemical agents and the mutant strain with Lipase productivity of 2-fold higher was obtained. Bapiraju *et al.* [14] reported that the the UV and chemical treated fungal strains of *Rhizopus* sp. showed 133% to 232% higher than the wild strains. Caob and Zhanga [26] reported that the Lipase production of UV and chemical treated *Pseudomonas* mutant was 3.25 fold higher than the wild strain.

The present study stated that the mutant strain ANsa90, AFuv50 and PEsa120 showed 2 fold and PEuv40, PEsa90 and AFsa120 showed 1.5 fold enhanced productivity of enzyme, over the wild strain. This is in agreement with findings of Mahadik *et al.* [27], who reported that Mutant strains of *Aspergillus niger* showed seven to five fold enhanced productivity of Lipase, over the wild strain. Mala *et al.* [28] isolated UV and nitrous acid derived mutants of *A. niger* selected on media containing bile salts. Nitrous acid mutants exhibited increased efficiency of Lipase production compared with wild strain. Mutation alters the genotype of microorganisms, when it expresses that leads to alter the character or death of microorganisms. The ultra-violet radiation forms thymine dimer in gene sequence. But the photolyase enzymes present in living system break the thymine dimer and correct it. The increasing exposure time to UV radiation may form the thymine dimer in gene sequence that code photolyase enzyme. In this situation the thymine dimer can not be broken by the enzyme of living system [29,30]. The chemical agents such as Sodium Azide, Ethyl Methane Sulphonate may alter the gene sequence by altering

the base pairs. The azide ion alters the structure of cytosine such that it forms hydrogen bonds with adenine, rather than guanine. This produces a cytosine to thymine transition. Ethyl Methane Sulphonate is a strong mutagenic agent. It alkylates N7 of Guanine and severely alters the base pairing.

In the present study, all the strains, grown in 10% Olive oil containing medium showed poorest Lipase activity. This may be due to the inoculated strains failiure to utilize more 10% Olive oil as a carbon source for their growth and subsequently reduction in Lipase activity. This was in agreement with Mahadik *et al.* [27] who stated, higher concentration of oil in the medium did not help Lipase production in the case of mutant. On the other hand, Sucrose, Lactose, Mannitol and Glucose may support the growth initially and the inducer 1% Olive oil induced the inoculated strains for more Lipase production in later stages. Many researchers have reported the positive effect of sugars on Lipase production by *Aspergillus niger* [31], by *Rhizopus chinensis* [32] and by *Rhizopus oryzae* [33]. On the contrary of present study, Muralidhar *et al.* [34] stated that their experimental results indicate that Olive oil is a better carbon source for Lipase production by *Candida cylindraces* compared to Glucose. This is supported by Dalmau *et al.*, [35]; Gordillo *et al.*, [36] for *Candida rugosa.* Optimization of the quantity of Olive oil in the fermentation medium along with the other nutrients resulting in the rise in enzyme production also contradicts the fact that large quantities of Olive oil decrease the Lipase activity by *Candida rugosa* [37].

Nahas [38] reported that Carbohydrates were good sources of carbon for growth but low Lipase production was obtained by *Rhizopus oligoporus*. Lipase production was strongly repressed by higher concentration of Glucose. The present study revealed that fungal culture utilizing Olive oil as a carbon source exhibited lowest Lipase producing activity. This result was not in agreement with those of Paul and Carles [39]. Falony *et al.* [40] reported that the *A. niger* strain showed more Lipase activity among the test strains used. The production of Lipase was more significant in culture medium added with lipids as the carbon source than in the culture medium without lipids. Previous works on the physiology of Lipase production showed that the mechanisms regulating biosynthesis vary widely in different microorganisms. Results obtained with *Calvatia* [41], *Rhizopus* [33], *Aspergillus* [42], and *Rhodotorula* [43] showed that Lipase production seems to be constitutive and independent of the addition of lipid substrates to the culture medium, although their presence enhanced the level of Lipase activity produced. On the other hand [44,45], lipid substrates are necessary for Lipase production by *Geotrichium candidum* and *Fusarium oxysporum* respectively and also, carbohydrates can act as repressors of its biosynthesis.

In the present study, Lipase production efficacy of mutant fungal strains (ANems120, AFems120 and ANems150) were demonstrated in the presence of various carbon and nitrogen sources. The effect of different carbon sources on Lipase production efficiency of fungal strains were found to be in the ascending order Mannitol < Lactose < Glucose < Sucrose. In present study *Aspergillus niger,* and *Penicillium* sp., utilized Sucrose as a carbon source exhibited highest Lipase producing activities, these results are in agreement with those of Susumu and Yashio [46]. Elliah *et al.* [13] reported that the *Aspergillus niger* utilized dextrose as carbon source for highest Lipase activity. The Lipase production was decreased in the order of Sucrose < Lactose < Mannitol < Dextrose by the mutant strain of *Aspergillus niger*. These results were in contrary to the present study.

Petrovic *et al.* [47] reported maximum Lipase production when Glucose and peptone were incorporated in the production medium using *Penicillium roquefortii*. In the present study, Glucose favoured the production of Lipase. This is in contrary with Mahadik *et al.* [27] who reported that there was no increase in enzyme levels when mutant UV-10 was grown in medium supplemented with Glucose. However, the addition of Glucose in the medium resulted in increased levels of Lipase production by wild strain, *Aspergillus niger*. The maximum Lipase activity was found with the combination of Sucrose with Olive oil inducer and Glucose with Olive oil inducer in the present study. But the repressive effect of Glucose with fatty oil is reported by Dalmau *et al.* [35] for *Candida rugosa,* Nahas [38] for *Rhizopus oligosporus*, Baillargeon *et al.* [48] for *Geotrichium candidum*, and Rapp [45] for *Fusarium oxysporum.* but differ from those obtained for *C. rugosa* by Chang *et al.* [49]. Cordova [50] reported that the carbon sources such as Glucose, Fructose, Glycerol, Xylose, Sucrose and Lactose are probably utilized before Lipase production, there were little or no differences with these substrates as carbon source.

Damaso *et al.*[4] reported that the *Aspergillus niger* was found to be the best Lipase producer. Soap stock was the best substrate and inducer compare to Olive oil and they also explained the repressive effect of Olive oil on Lipase production. Coca *et al.* [51] stated that the *Aspergillus niger* and *Aspergillus fumigatus* were the best Lipase producer in the medium containing Olive oil as a carbon source. This is in contrary with our present results. The present study revealed that *Aspergillus fumigatus* utilizing Mannitol and Lactose as carbon sources and exhibiting lowest Lipase producing activity. In case of Sucrose and Glucose exhibited highest Lipase producing activity.

Kakde and Chavan. [52] found that carbon sources like Fructose and Sucrose induced Lipase activity while Lactose, Starch and Carboxyl Methyl Cellulose inhibited Lipase activity by *Penicillium chrysogenum.* Nitrogen sources like Nitrate, Nitrite, Amide, Ammonium, and Protein showed its effect on Lipase enzyme of fungi. Casein and Peptone which are organic forms stimulated maximum Lipase enzyme production of storage fungi. Both the species of *Penicillium* viz. *Penicillium notatum* and *Penicillium chrysogenum* showed maximum extracellular Lipase activity in presence of Casein and Peptone. Urea which is an amide form and Ammonium Phosphate which is an ammonium form hampered the extracellular Lipase enzyme production of fungi. These reports were found to be similar to our present study results. Chavan and Kakde, [53] studied that the Lipase enzyme activity of storage fungi under the influence of carbon and nitrogen sources. They found that carbon sources as like Fructose and Sucrose induces Lipase activity while Starch, Lactose and Carboxyl Methyl Cellulose inhibit Lipase activity. Nitrogen sources as like Nitrate, Nitrite, Amide, Ammonium, and Protein affect in different ways on Lipase enzyme of

fungi. The results of the present study revealed that the Lactose and Urea was a poor carbon and nitrogen sources respectively in the tested fungal strains like ANems120, AFems120 and ANems150.

The effect of different nitrogen sources on Lipase production efficiency of bacterial and fungal strains was found to be in the ascending order Urea <Ammonium Sulphate < Potassium Nitrate < Sodium nitrite in the present study. This was in accordance with Lima *et al.* [54], who stated that the Lipase activity by *Penicillium* sp. was higher when the medium containing only the inorganic nitrogen sources. In the presence of Olive oil and Ammonium Sulphate produced less mycelial growth subsequently less Lipase activity. But the Ammonium Sulphate and Potassium Nitrate combination produced higher Lipase activity in short fermentation time. Other workers [55-57] employed *Candida lipolytica, Penicillium restrictum* and *Rhizopus* sp. reported maximum Lipase production, when organic nitrogen was used as a nitrogen source. Waller and Comeau [58] reported that the incorporation of corn steep liquor into the production medium gave good Lipase activity by *Candida* sp. Rodriguez *et al.* [59] reported that Ammonium Sulphate, Ammonium Nitrate and Sodium Nitrate except Urea reduced the production of Lipase activity compared to the initial medium containing yeast extract. With Urea, the activity was around six times higher. A similar observation has been reported using *Penicillium* sp. [60]. On the other hand, Lima *et al.* [51] found that Lipase production in *Penicillium* sp. was stimulated using Ammonium Sulphate.

CONCLUSION

Many microorganisms are able to produce Lipase in liquid broth containing different oil substrate as a inducer. The present study concluded that the treatment using physical and chemical mutagenic agents increased the Lipase production efficacy of the fungal strains. This study also strengthened the idea that addition of carbon and nitrogen sources favors the Lipase production to a certain extent.

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