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Strain Improvement of *Aspergillus niger* for the Production of Lipase

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An indigenous strain of *Aspergillus niger* AU15 which produced lipase constitutively was used to produce mutants using ultraviolet and N-methyl-N-nitro-N-nitosoguanidine. A mutant strain with lipase productivity of 2-fold higher was obtained. Optimization studies were also carried out. Dextrose as carbon source, peptone and cornsteep liquor as nitrogen source were found to be optimal for maximum lipase production.

Key words: Lipase, *Aspergillus niger*, UV, NTG.

Extracellular lipase produced by microorganisms is being investigated for its potential application in various industrial processes like detergents, oil, fats and dairy, coupled with enormous therapeutic uses (1-3). The advantages of enzymatic hydrolysis of lipid substances and their biotechnological transformation over the conventional chemical process are less energy requiring and yield superior quality products (4). New applications, such as the resolution of racemic mixtures to produce optically active compounds, should also arise from the stereo-specific acting properties of some lipases (5).

Shu-Gai *et al* (6) reported a high lipase yield of 3.25 fold by a mutant of *Pseudomonas* sp. after the treatment with ultraviolet (UV) and N-methyl-N-nitro-N-nitosoguanidine (NTG). The aim of our investigation is to develop improved mutant-strains from a locally isolated strain of *Aspergillus niger* and to study the effect of different nutrients in the culture medium on the production of lipase.

Materials and Methods

Microorganism: Aspergillus niger strain AU15 which was isolated from an oil mill waste and exhibited good lipase activity was used (7). The strain was grown on potato dextrose agar (PDA) (8) at 28°C for 72 h and kept in the refrigerator at 4ºC until further use.

Mutagenisis by UV: The growth of 72 h old culture of AU15 was scrapped off in 5 mL sterile distilled water and diluted with 45 mL sterile distilled water containing Tween 80 (1:4000). Added sterile glass beads and shaked on a rotary shaker for 30 min to break the hyphal mycelium. The suspension was filtered through sterile cotton to remove the mycelium. The spore suspension was prepared in phosphate buffer (pH 7) containing 5×10^6 spores mL^{-1}.

Five mL of spore suspension was transferred aseptically into seven sterile petri dishes having a flat bottom. Exposed for different periods (0, 10, 20, 30, 40 and 60 min) to UV light at a distance of 26.5 cm away from the center of 40 watt Tup germicidal lamp. The UV lamp had about 90% of its radiations at 2540 – 2550°A. During the exposure the suspension was agitated by gently rotating the plate. After irradiation, the spore suspension of each plate was serially diluted by using potassium phosphate buffer (pH 7). One mL quantities of appropriate dilutions were plated on PDA and incubated at 28°C for 5 d. The colonies were selected on the basis of their morphological characters and given the code numbers AUV1 to AUV6 (Table 1).

Table 1. UV and NTG mutants and their enzyme activity

UV mutants		NTG mutants	
Isolate No.	Lipase activity (UL^{-1})	Isolate No.	Lipase activity (UL^{-1})
Parent		Parent	
AU15 (control)	2160	AUV4	3330
UV-mutant		NT-mutant	
AUV1	2220	ANT30	2990
AUV2	610	ANT60	3400
AUV3	1230	ANT90	4160
AUV4	3380	ANT120	3300
AUV5	2590	ANT150	2370
AUV6	980		

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Mutagenesis by NTG: The spore suspension of AUV 4 strain was prepared by using the phosphate buffer pH 6.5 as described earlier. To 9 mL of spore suspension, 1 mL of sterile solution of NTG (3 mg mL^{-1} PBS) was added. The reaction was allowed to proceed. Control was treated in the same way except that no NTG was added to the buffer. Samples were withdrawn from the reaction mixture at intervals of 30, 60, 90, 120 and 150 min and centrifuged for 10 min at 5000 rpm. The cells were washed three times with sterile water and resuspended in 10 mL sterile buffer. The samples were serially diluted in the same buffer and plated over PDA as mentioned earlier. The selected mutants were given the code numbers ANT30, ANT60, ANT90, ANT120 and ANT150 (Table 1). The mutants produced by both the agents were studied for their lipase activities.

Submerged fermentation: The growth on PDA slants was scrapped with 5 mL sterile distilled water and transferred into 250 mL Erlenmeyer flask containing 45 mL inoculum medium, which contained (gL^{-1}); glucose, 10; peptone, 20; NaCl, 3 and yeast extract, 5; and pH 6. The flasks were incubated at 28°C on rotary shaker (220 rpm) for 72 h for the development of inoculum. Five mL inoculum was added to 45 mL of production medium in 250 mL Erlenmeyer flask. The production medium contained (gL^{-1}); dextrose, 10; peptone, 20; yeast extract, 5; $(NH_4)_2SO_4$, 5; $MgSO_4.7H_2O$, 0.2; $FeSO_4.7H_2O$, 0.01; and NaCl, 5 and pH 6.5. The flasks were incubated at 28°C on rotary shaker (220 rpm) for 96 h. The samples were withdrawn and assayed for their lipase activity.

Effect of different carbon sources on the production of lipase by mutant ANT90: Different carbon sources (dextrose, mannitol, sucrose and lactose) were used to study their effect on the production of lipase using a promising mutant ANT90. These were added at 1% (w/v) level into the production medium. The fermentation and assay were carried out as described earlier.

Effect of different nitrogen sources on the production of lipase by mutant ANT90: Different nitrogen sources (cronsteep liquor, malt extract, tryptone, yeast extract, soyabean meal and peptone) were used to study their effect on the production of lipase using mutant ANT90. These were added at 2.5% (w/v) level to the production medium. The fermentation and assay were carried out as described earlier.

Lipase activity: Lipase activity in culture broth was determined by titrimetry using olive oil as substrate (9). One unit of lipase activity was defined as the amount of enzyme required to release 1 mM of fatty acid at 37°C under standard assay conditions (pH 7, reaction time 30 min). All the fermentations and assays were carried out in triplicate.

Results and Discussion

From the results in the Table 1, it is evident that UV induced mutant AUV4 showed the highest lipase activity (3380 UL^{-1}). It was 1.6 fold of the parent strain. Further mutagenesis of this strain by NTG treatment could yield many mutants. Examination of lipase activity of the selected mutants showed that the mutant ANT90 showed further improvement in lipase activity (4160 UL^{-1}) (Table 1). It was 2-fold more of the wild strain (AU15). Shu-Gui *et al* (6) reported an increase in lipase production of 3.25–fold by using a *Pseudomonas*-mutant of UV and NTG.

Among the different carbon sources examined, dextrose gave the highest enzyme activity (4360 UL^{-1}) followed by manntiol (3590 UL^{-1}) (Table 2). Among the different nitrogen sources, corn steep liquor and peptone showed the highest enzyme activity (4400 UL^{-1} and 4390 UL^{-1}), respectively (Table 2). Petrovic *et al* (10) reported maximum lipase production when glucose and peptone were incorporated in the production medium using *Penicillium roquefortii*. Other workers (11-13) employing *Candida lipolytica, Penicillium restrictum* and *Rhizopus rhizopodiformis*, reported maximum lipase production when peptone was used as a nitrogen source in the production medium. Waller and Comeau (14) reported that corn steep liquor when incorporated into the production medium gave good lipase activity using a novel strain of

Table 2. Effect of different carbon and nitrogen sources on lipase production by the mutant ANT 90

Carbon & Nitrogen source	Lipase activity (UL^{-1})
Carbon (1% w/v)	
Dextrose	4360±42
Mannitol	3590±34
Lactose	3201±35
Sucrose	3121±29
Nitrogen (2.5% w/v)	
Cornsteep liquor	4400±38
Peptone	4390±36
Malt extract	4212±39
Yeast extract	4130±34
Soyabean meal	2845±23
Tryptone	1398±19

Candida sp. Our results are in agreement with their findings.

Based on the results it can be concluded that a 2-fold higher yield of lipase was achieved with a mutant strain of *Aspergillus niger,* obtained by the treatment with UV and NTG. Further the supplementation of production medium with dextrose as carbon source and corn steep liquor or peptone as nitrogen source increased the lipase production.

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