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Effect of UV and EMS Mutation on *Aspergillus niger* in the Production of α-amylase

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Production of extracellular α- amylase enzyme by a filamentous fungus, Aspergillus niger was studied in rice submerged fermentation. The potential strain was successfully mutated by ultraviolet (UV) and ethyl methane sulphonate (EMS). High level of α- amylase activity was obtained by the mutant strains UV-3 and EMS-2. These strains exhibited 1.44 and 2.19 times increased production of α- amylase respectively than the wild strain, which reveals the potential use of mutant strains in the industries.

Key words: *Aspergillus niger,* α- amylase, EMS, UV, Mutation, Ascomycetous fungi.

Aspergillus niger is one of the most common ascomycetous fungus used in the large scale production of organic acids, enzymes etc. α- amylases are ectoenzymes produced by *A.niger* which hydrolyses complex starch molecule to give diverse product including dextrins and progressively smaller polymer composed of glucose unit. Alpha amylase enzyme is currently being used in a broad array of industrial applications including thinning and liquefaction of starch in alcohol, brewing and sugar industries as well as in processed food industry such as baking, high fructose corn syrup, textile, paper and distilling (Pandey *et al.,* 2000 & Maarel *et al.,* 2000), washing powders, textile designing, production of modified starches and hydrolysis of oil-field drilling fluids (Mc Tigu *et al.,* 1995 & UpaDek & Kottwitz, 1997). There have been many efforts to generate microorganisms with high ability to produce amylases that can degrade native starch more efficiently (Wu *et al.,* 2006). Although amylases can be obtained from several sources such as plants and animals, the enzymes from microbial sources generally to get industrial demand (Pandey *et al*., 2000). The hyphal mode of growth, tolerant to low water activity and high osmotic pressure conditions, make fungi most efficient for bioconversion of solid substrates (Raimbault, 1998). On the other hand, strain improvement has been achieved through

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mutation, selection, or genetic recombination. In many cases, mutations are harmful, but occasionally it may lead to a better-adapted organism to its environment with improved bio-catalytic performance.

The potential of a microorganism to mutate is an important property conferred by DNA, since it creates new variations in the gene pool. The challenge is to isolate those strains, which are true mutants that carry beneficial mutations (Parekh *et al*., 2000). UV and EMS are important inducers of strain mutations. The pyrimidines (Thymine and Cytosine) are especially sensitive to modifications by UV rays absorption. This may result in the production of thymine dimers that distort the DNA helix and block future replications (Sambrook *et al*., 2000). EMS is a powerful mutagen that induces point mutations in the DNA. The most common mutation induced is the GC to AT transition, although a small percentage of the mutations induced are deletions.

MATERIAL AND METHODS

Organism and media

Pure cultures of *A.niger* were obtained from the Department of Food Microbiology, Paddy Processing Research Center (PPRC), Thanjavur. The strains were maintained on Rose Bengal Agar medium at 30°C and inoculated on rice medium to estimate the α- amylase activity.

Development of UV & EMS mutants

To improve the amylase production through mutagenesis, two classical mutagenic agents UV irradiation and ethyl methane sulphonate (EMS) were used to obtain the mutants. *A.niger* conidia were suspended in 9 ml of distilled water and irradiated with UV radiation at a distance of 46.6 cm. Radiation intensity was delivered at 256μW/cm^2. The samples were incubated at different periods (10, 15, 20, 25 minutes). The UV treated samples were then inoculated in the rice substrate and named as UV-1, UV-2, UV-3, and UV-4.

A.niger broth culture was centrifuged at 5000 rpm for 15 minutes and the supernatant was removed. The cells were washed by resuspending in 5 ml of double distilled H_2O and centrifuged again at 5000 rpm for 15 minutes. The cells were resuspended in 5 ml of phosphate buffer and centrifuged as above and the supernatant was removed. Finally the cells were suspended in 1.7 ml of phosphate buffer and are transferred to glass culture tubes.

The culture tubes except the non-mutagenized control tube were added with 200μl of EMS (0.496g per 20 ml) and are incubated on a roller at 30°C for varying time points between 30, 35, 40, 45 minutes. At each time point, 8 ml of sterile 5% sodium thiosulfate was added to inactivate the EMS and stop the mutagenesis. Each cell suspension should contain 10 million cells per ml. The cell aliquot was saved at each time point to determine post mutagenesis cell viability. The cell suspension was then transferred to 15 ml conical tubes and centrifuged at 5000 rpm for 15 minutes. The supernatant was removed and resuspended the cells in 9 ml of sterile double distilled H_2O. The EMS treated samples were then inoculated in the rice substrate and named as EMS-1, EMS-2, EMS-3, EMS-4.

Sample inoculation

25gms of each culture sample was mixed well with 75 ml distilled water in a 100 ml Erlenmeyer conical flask and the suspension was incubated for about 10 hours. The sample suspension was then autoclaved at 15 lbs pressure for 15 minutes. The samples were inoculated with 10 ml of fungal spore suspension (*A.niger* both wild and different mutant) in an inoculation chamber and are incubated at room temperature.

Determination of α- amylase activity

An enzyme extract was prepared by mixing 3gm of sample with 50ml of diluted tween 80 (4:1). The sample was homogenized and then centrifuged in 4°C at 5000 rpm for 20 minutes. The centrifuged samples were filtered by whatmans filter paper no.41 and are incubated at 4°C. α- amylase activity was determined by using dinitro salicyclic acid method (Miller., 1959).

RESULTS AND DISCUSSION

Effect of UV & EMS mutation on α-amylase production in *Aspergillus niger*

The results of the enzymatic profile were given in (Table 1 & 2). Compared to the wild strain α- amylase activity was high in mutated

Table 1. α-Amylase activity in UV mutated *A. niger* (U/l)

A.niger	α- Amylase activity			
	First day	Third day	Fifth day	Seventh day
Wild	599.9	7410	2679.9	459.9
UV-1	619.9	7619.9	3179.9	459.9
UV-2	599.9	11259.9	2199.9	459.9
UV-3	619.9	17959.9	2299.9	419.9
UV-4	699.9	10599.9	1939.9	159.9

Table 2. α-Amylase activity in EMS mutated *A. niger* (U/l)

A.niger	α- Amylase activity			
	First day	Third day	Fifth day	Seventh day
Wild	599.9	7410	2679.9	459.9
EMS-1	699.9	14539.9	3179.9	259.9
EMS-2	919.9	23399.9	3219.9	419.9
EMS-3	1179.9	14019.9	3879.9	579.9
EMS-4	919.9	7939.9	3019.9	199.9

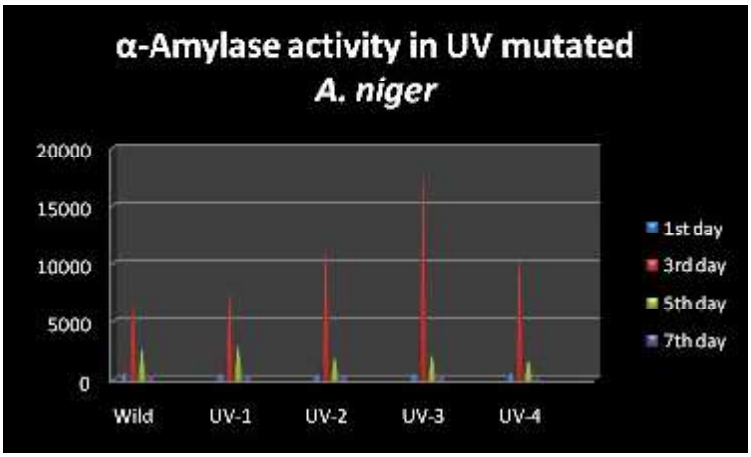

Fig. 1. α-Amylase activity in UV mutated *A. niger* (U/l)

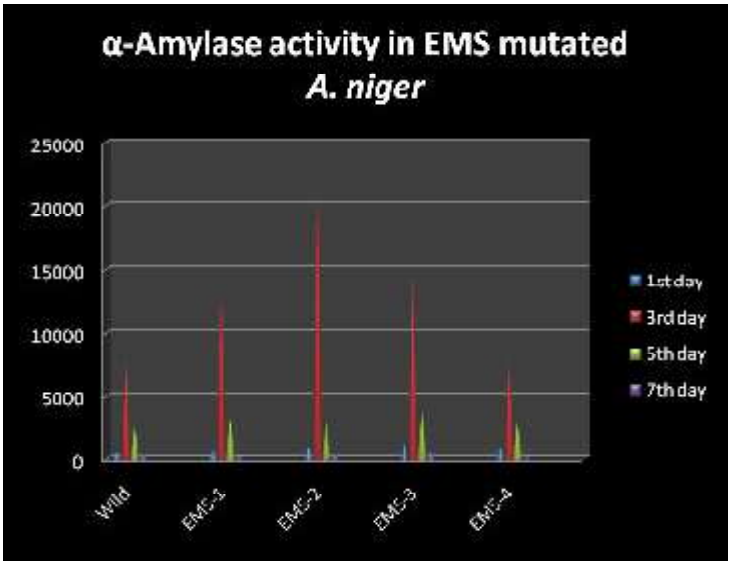

Fig. 2. α-Amylase activity in EMS mutated *A. niger* (U/l)

strain. Maximum amylase production was observed in *A.niger* at 3rd day. Compared to the wild strain α- amylase activity was increased 0.02 times in UV-1 and 0.52 times in the strain UV-2 and 1.44 times in the strain UV-3 and 0.43 times in the strain UV-4 (Table 1). Studies of production and stability studies revealed that myrosinase from the UV mutant strains of *Aspergillus sp* NR463U4 retained activity for 3.5 times longer than wild type at 30ºC (Butrindr *et al.,* 2004). Similar results were discussed by Soledad *et al.,* (2006) in the UV mutated strains GSI-SO67 and GSI-SO67 of *Aspergillus niger* which showed significantly increased level of mannase, xylase and cellulase production.

In EMS mutated strains, maximum amylase production was observed in *A.niger* at 3rd day (Fig. 2). Compared to the wild strain á-amylase production is increased 0.97 times in the strain EMS-1 and 2.19 times in EMS-2 and 0.90 times in EMS-3 and 0.071 times in EMS-4. After 3rd day incubation the α- amylase production was gradually decreased. Nuansri *et al.,* (2005) also reported that the EMS mutagenesis increases myrosinase activity in *Aspergillus sp.* NR4617E1. He showed that about 1.90 U/ml of myrosinase was produced in 36 hrs. Thus mutated strain was significantly increased their level of α-amylase production improving in this way in support of impending industrial use.

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