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UV mutagenesis of *Aspergillus niger* for enzyme production in submerged fermentation

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Abstract: The present study was conducted to improve the enzyme production from *Aspergillus niger* using UV mutation. Submerged fermentation was carried out in 250ml Erlenmeyer flask using Vogel's media at 30°C for six days. Results of this study revealed that UV-mutation enhanced CMCase activity up to two times while FPase activity up to three times as compared to the parental strain. For avicelase, xylanase and fungal biomass production, UV radiation has slight effect as compared to parental strain.

Key words: UV-mutagensis, *Aspergillus niger*, enzyme production, submerged fermentation.
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INTRODUCTION

Today the major prospect of biotechnology is the production of enzymes by using various strains of bacteria and fungi in submerged and solid state fermentation. For commercial production of enzymes filamentous fungi are mostly preferred because the enzymes produced by these fungi are more efficient as compared to those obtained from yeast and bacteria[1]. For efficient enzyme production strains can be improved by mutagenesis which is successful method. This process is mostly trial and error process involving laborious steps of procedures in performance [2,3]. For improved yield of cellulase production from different fungi by treating it with chemicals such as ethyl methyl sulphonate (EMS), N – methyl – N – nitro – Nnitrosoguanidine (MNNG) and (HNO_2) nitrous acid[4-6]. Sub-lethal concentration of mutagens in fungus can increases the rate of enzyme production[7]. Ultraviolet (UV) and gamma (γ) irradiations can also be used to obtain mutants yielding higher cellulase production from conidia of thermophilic fungi[8,9]. In this research article we reported here the effect of different exposure time on *Aspergillus niger* for different enzymes production in submerged fermentation.

MATERIALS AND METHODS

Microorganism

Aspergillus niger was obtained from Fermentation Biotechnology Lab of Food & Biotechnology Research Center, PCSIR laboratories complex Lahore and used as a source of microorganism in all the experiments. It was maintained on PDA slants and stored at 4°C for further use.

UV mutagenesis

Spore suspension was prepared in serial dilution method from 5days old culture slant one ml of 10^6 dilution was poured in petri plate and placed under UV lamp (240nm) at a 10cm distance up to 60 minutes with regular interval of five minutes. After irradiation of spores, were cultured on PDA plates incubated at 30±1°C for five to seven days until sporulation of fungal culture. Mutagenised colonies were screened by analyzing enzyme activity after fermentation batch. Best enzyme producing strains were selected for further study.

Preparation of conidial suspension

Inoculum was prepared by adding sterilized distilled water into the 5-day old slant. With the help of inoculating loop the mycelia was mixed and one ml ($1x10^8$) of spore suspension was used as inoculum. Inoculum size was measured with heamacytometer as described by Sharma[10].

Fermentation methodology

For enzyme production strain of *Aspergillus niger* was grown on Vogel's media. 25ml of Vogel's media was placed in 250ml Erlenmeyer flask supplemented with 0.5% carboxymethyl cellulose. The flask was cotton plugged and then autoclaved at 121°C for 15 min. After sterilization, media was inoculated with 2% spore suspension and incubated at 30°C with agitation speed of 120rpm for five days. After termination of fermentation period the culture filtrate was centrifuged at 8000 rpm for 10 min at 4°C to remove unwanted particles and spores. The supernatants obtained after centrifugation were used as the crude extracellular enzyme source.

Determination of enzyme activities

Different enzyme like, CMCase, FPase, Xylanase and Avicelase were determined by incubating the 0.5ml of culture filtrate with 0.5ml of

appropriate substrates (CMC, filter paper 1.5x6cm strip, xylan and avicel in citrate buffer pH 5.0) incubated at 50°C for 15min. After incubation the reaction was stopped by the addition of 1.5ml of DNS followed by incubating for 10 min in boiling water bath. The sugars released were measured at 550nm spectrophotometrically[11]. Total proteins were determined by the method described by Lowery et al[12]. Glucose produced was estimated by Millers[11] method.

Estimation of dry cell mass (DCM)

Dry cell mass was determined by filtering the culture broth through preweighed Whatman filter paper No. 1. Mycelium was thoroughly washed with tap water and dried in an oven at 105°C for 2 h. The dry cell mass was weighed and calculated as g/l by subtracting the initial weight from the final weight.

RESULTS AND DISCUSSION

In the present study an attempt was made to improve the yield of enzymes production from *Aspergillus niger* by UV mutation and grown under submerged fermentation conditions. The results of this study revealed improved production of enzymes as compared to wild. Figure 1 described the production of CMCase and FPase under submerged fermentation for six days. Parent strain produces CMCase activities of 1.8±0.08IU and FPase activity of 0.92±0.03IU while the mutagenised strain produces three times more production of FPase (2.5±0.06IU) and two fold increase in CMCase (2.6 ± 0.04IU) production as compared to the parental strain with UV exposiure time of 45min.

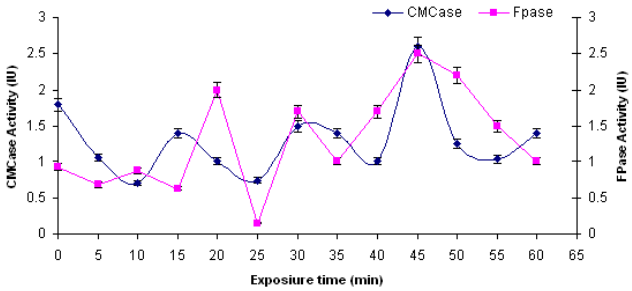

Figure 1: Effect of different UV radiation exposiure time on CMCase and FPase production by *Aspergillus niger* in submerged fermentation for six days at 30°C. Error bars indicated SD among triplicates.

When Avicelase and Xylanase activities were compared there was slight difference in both parental and mutagenised strains as shown in figure 2. Avicelase activity of parental strain was 1.7±0.03IU while the mutagenised strain with 45min of UV radiation shows maximum activity of Avicelase 1.95±0.05IU which is not as much higher as compared to Parental (0 min). The same situation was observed in case of Xylanase activities. Parental (0 min) strain produces 17.5±1.02IU while he mutagenised strain with 25 min of UV radiation exposiure show maximum activity (21.5±1.1 IU) of Xylanase. By increasing the UV radiation exposiure time there was decline in enzymes, glucose, total proteins and fungal biomass production was observed.

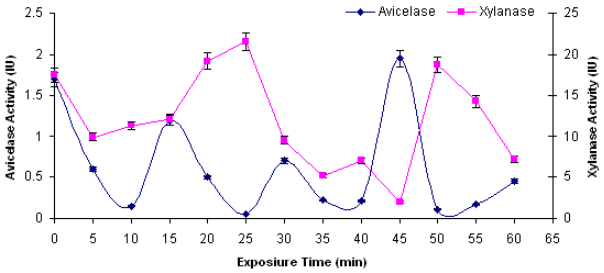

Figure 2: Effect of different UV radiation exposiure time on Avicelase and Xylanase production by *Aspergillus niger* in submerged fermentation for six days at 30°C. Error bars indicated SD among triplicates.

Figure 3 showed the fungal biomass, total proteins and glucose production produced after fermentation of six days using Vogel's media. Highest biomass (2.8±0.4g/L) production was observed with 15 min of UV exposiure time as compared to parental (0 time) strain producing biomass of 1.2±0.2g/L. By changing the time duration of UV irradiation fungal biomass production was reduced. When total proteins and glucose production was checked, it was observed that when the strain was subjected to UV irradiation there was decline in production was observed. It might be due to the increased exposure time which resulted in back mutation[13]. By increasing the exposure time up to 20min amylase activity was decreased to zero[14].

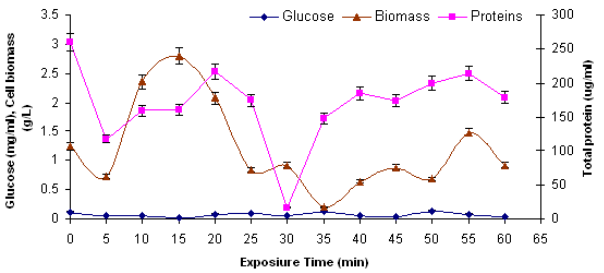

Figure 3: Effect of different UV radiation exposiure time on glucose, total proteins and fungal biomass production by *Aspergillus niger* in submerged fermentation for six days at 30°C. Error bars indicated SD among triplicates.

By inducing mutation in the wild strain, the yield of enzyme (glucoamylase) can be increased to many fold[15,16]. Liu et al[17] produced that mutant strains of *Aspergillus* sp. by combined mutation, and produced amyloglucosidase which was capable of converting maltodextrins into glucose.

Prabakaran et al[18] isolated three fungal strains from sugarcane field, and then subjected to UV mutation for highest enzyme activates production. Among the three isolated and mutated strains, highest production of cellulases was observed by *Penicillium chrysogenum* with UV exposure time of 5 minutes. Bapiraju et al[19] reported that *Rhizops* sp. Mutated by UV showed highest lipase activities as compared to parent strain.

For strain mutation UV rays are important inducers. Main effect of this light is to modify the structure of pyrimidine (cytosine and thyamine) causing the formation of thyamine dimmer which distort the structure of DNA heliex and block the further replication process[20]. In most cases UV mutation are very harmful but at sometime it may lead to better adaptation of an organism to its environment with improved biocatalytic performance.

Sandana et al[21] have reported lipase production 2.53 times more by UV mutants than the parental strain of *Aspergillus niger*. Navalainen et al [22] have reported that *Aspergillus* strains can be treated with UV irradiations or chemicals such as N-methyl, N-nitro, N-nitrosoguanidine, dimethyl sulphate, EMS, ethidium bromide and nitrous acid to induce mutation for the improvement of amyloglucosidase production.

According to Agrawal et al[23] UV radiation was a potent mutagen. UV irradiation was found to be best for the improvement of strains like *Aspergillus niger* for maximum production of various enzymes[24]. UV mutagenic strains of *Aspergillus niger* have the ability to produce 156% more lipase production as compared to the wild strains. UV treated strains of *Aspergillus niger* UAM-GS1 increases the production of hemicellulolytic and cellulolytic activites[25].

Suntornsuk and Hang[26] have reported that the strain improvement in *Rhizopus oryzae* by UV, resulted in the production of more glucoamylase by a mutant than the parent strain. Some workers[27,28] obtained mutants, which produced cellulase enzymes with 5-fold increase in β-glucosidase and one fold increase in both CMCase and FPase, through chemical mutagens treatments.

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