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Differences in enzyme cellulase production by *Chaetomium globosum* as a effect of UV induce mutagenesis

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ABSTRACT

The current research paperdeals with studies designed to assess the effect of UV induce mutagenesis on enzyme cellulase production by *Chaetomium globosum*and time of UV exposure at which maximum cellulase production take place. Culture of *Cheatomium globosum* expose to UV for different time interval i.e. 10min; 20min; 30min; 40min; 50min; 60min and 0min(control). The cellulase production increases from 10min. to 20min. and decline from 30min. to 60min. The maximum cellulase production was seen at 20min.

Key words: Cellulase, UV induced, *Chaetomium*

INTRODUCTION

Cellulases are a group of hydrolytic enzymes capable of hydrolysing cellulose to smaller sugar components like glucose units. Cellulolytic enzymes play an important role in nature's biodegradation processes where plant lignocellulosic material is efficiently degraded by cellulolytic fungi and bacteria. In industry, these celluloytic enzymes have found novel applications in the production and processing of chemicals, foods and manufactured goods such as paper, rayon and cellophane and the preparation of plant protoplasts in genetic research (Kader *et al* 1999).

There are different kinds of stresses influence the individual's physiological and chemical mechanism such stresses like heat, cold, flood, drought, salinity, UV radiation, chemicals, Abscisic acid, heavy metal and water deficit. Organism strives to survive stress condition by adjusting their gene expression pattern or metabolic activities (Sakpal, 2008). In one of the studies on enzyme cellulase production *in Aspergillus fumigatus, Penicillium chrysogenum and Verticillium terrestre,* isolated from sugarcane field soil, revealed the extracellular production

of amylase, cellulase and lipase was achieved by developing mutants after exposure to UV light (Prabakaran*et al;* 2009). Induced mutations are thought to arise as a result of enzymatic processes utilizing DNA damage as a substrate. In addition, such strains are UV sensitive, X-ray sensitive, and recombination deficient in varying degrees (Jeffrey F; Lemontt, 1970).

UV-induced mutation in fungi, UV-sensitive strains has been selected on the assumption that UV mutagenesis might be related to dark repair of lethal damage. In this study, an attempt was taken to produce mutants of *Chaetomium globosum* by exposing to UV radiation for evaluation of cellulase activities compared to the control and also to evaluate whether it has any considerable effect for the production of such enzymes.

MATERIAL AND METHODS

The effect of cellulosic substrates on the production of extra-cellular cellulases and their cellulolytic activity in *Chaetomium globosum* has been studied in shake flask cultures.

1. Collection of culture :
Culture of *Chaetomium globosum* procured from laboratory of SIES College of arts, commerce, and science.

2. Preparation and Pouring of Culture media:
The different culture media used for proper isolation and growth of *Chaetomium globosum* were follows; Czapek'sDox Agar with Cellulose (Bagool, 1982), Czapek'sDox Agar with Filter paper strips (Subba Rao, 1977), Reese Medium (Mandels& Weber, 1969) . Here cellulose was used as a Carbone source.

3. Inoculation of *Chaetomiumglobosum*:
Pure culture of *chaetomiun globosum* was inoculated into the test tube with media and incubated at room temperature for 9 days. After 9 days, grown culture in test tubes was scrapped lightly by sterile nichrome loop and added 15 ml autoclaved distilled water in it. Water with culture was transferred into new sterilized test tubes which was further well shaken using vortex mixer with addition of tween 80 to obtained uniform spore suspension. This uniform spore suspension was used as inoculum. Seven sterile petri plates with media were taken for inoculation. 1 ml of the suspension was inoculated into each petri plate. These inoculated plates were incubated at room temperature for 9 days.

4. UV treatment:
The UV treatment was given after about 9 days of inoculation into petri plates, when proper growth of culture (*Chaetomiun globosum)* was seen. The UV treatment was given to six out of seven petri plates with culture, where one was kept as control. UV treatment was given to the culture by using technique proposed by *David B. Fankhauser* (2001). Initially UV chamber was cleaned up and sterile by alcohol before using it. One hour before the experiment UV lamp was switched on to sterile the chamber. The petri plates with culture were exposed to UV light without lead one by one at different time interval i.e. 10min; 20min; 30min; 40min; 50min; 60min; which named as C*10*, C*20*, C*30*, C*40*, C*50*, C*60* respectively and one Petri plate was not expose to UV light kept as control which named as C*0.* After UV treatment all treated and one control Petri plates kept at room temperature for one day.

5. Extraction of Enzyme:
Next day after UV treatment and control cultures were used for enzyme extraction. The experiment was based on the methods described by Mandels*et.al.* (1976). Culture from Petri plates i.e. C*0,* C*10*, C*20*, C*30*, C*40*, C*50*, and C60 were scrap lightly by sterile nichrome loop. In each Petri plate added 15 ml sterile distilled water were added. Water with culture was transferred separately into 7 sterile test tubes which were further well shaken using a vortex mixer to obtained uniform spore suspension. Inoculums were further used for enzyme assay. Reese liquid media with cellulose was used as broth. One ml from seven inoculums were further inoculated separately inseven 250 ml conical flasks with 100 ml Reese liquid media with cellulose. These inoculated conical flasks were incubated at room temperature on a rotary shaker at 180 rpm. for 8 days. After 8 days broths were filter through glass wool. These filtrates were stored in freezer which used as enzyme source for determining

cellulase activity. Seven enzymes were extracted from 6 UV treated i.e. 10min; 20min; 30min; 40min; 50min and 60 min. and one control culture of *Chaetomiun globosum* named as E*10*, E*20*, E*30*, E*40*, E*50*, E*60* and E*0* respectively.

7. Cx and C1 enzyme activity:
The seven filtrates (enzymes) were extracted from6 UV treated and one control culture used to determined Cx and C1 enzyme activity. The Cx and C1 enzyme activities of the filtrates were determined by estimating the reducing sugars formed, using DNSA reagent determined by Mandels*et.al* ,1976cited (Gosavi., 2008).

8. Protein estimation:
It was done for enzyme extracted from 6 UV treated and one control culture based on method proposed by Lowry *et al;* (1951).

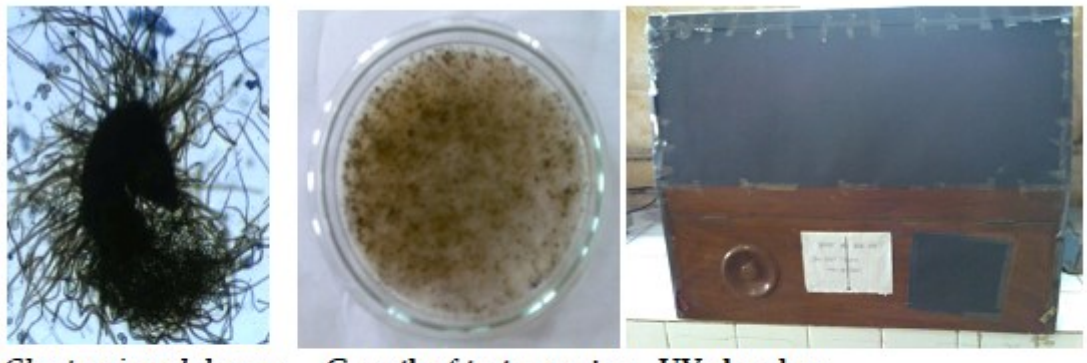
9. Reducing sugar estimation:
Enzymes extracted from UV treated culture and control was further proceeding for estimation of reducing sugar by DNSA method. Estimation of reducing sugar was done by DNSA method determined by Miller (1972).

RESULTS & DISCUSSION

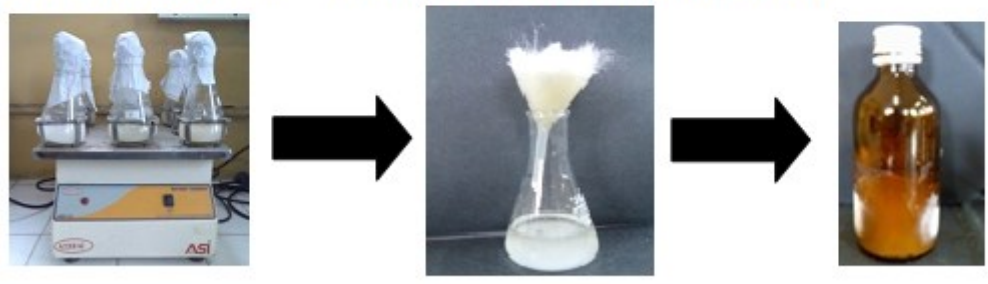
1. The Cx and C1 enzyme activity
Enzyme activity increases from enzyme E*10* to E*20* and again decline from E*30* to E*60.* The enzyme E20 show maximum enzyme activity than other enzyme extracted from treated culture and control culture *Chaetomiunglobosum*. Similar results were seen in *Aspergillusfumigatus* after treatment of UV (Prabakaran*et al;* 2009).

2. Protein estimation:
The mg of protein content per ml of enzyme increased from enzyme E*10* to E*20* and again decline from enzyme E*30 to* E*60.* The maximum protein content was observed in *enzyme E20 than other enzyme extracted from UV treated culture and control culture Chaetomiun globosum*. Similar results of protein estimation were obtained to that Aspergillus *fumigatus, Penicillium chrysogenum and Verticillium terrestre* after treatment of UV(Prabakaran *et al;* 2009).

3. Reducing sugar estimation:
The mg of reducing sugar content per mg of protein in enzyme increased from enzyme E*10* to E*20* and again decline from enzyme E*30 to* E*60.* The maximum mg of



Chaetomiumglobosum Growth of test organism UV chamber



Extraction of Enzyme

TABLE NO.1: Cx enzyme activity

Enzyme	mg of sugar/mg of protein
E0 *(control)*	35
E*10*	20
E*20*	36.36
E*30*	30
E*40*	26.67
E*50*	20
E*60*	20

TABLE NO.2: C_1 enzyme activity

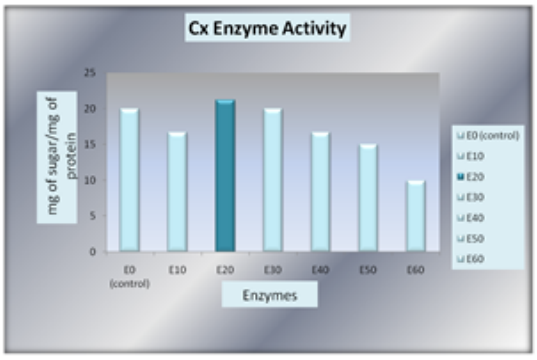
Enzyme	mg of sugar/mg of protein
E0 *(control)*	35
E*10*	20
E*20*	36.36
E*30*	30
E*40*	26.67
E*50*	20
E*60*	20

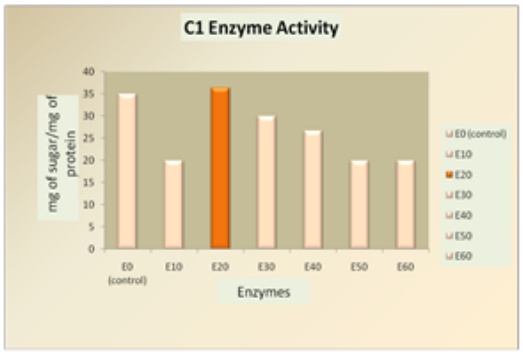
TABLE NO.3: Estimation of Proteins

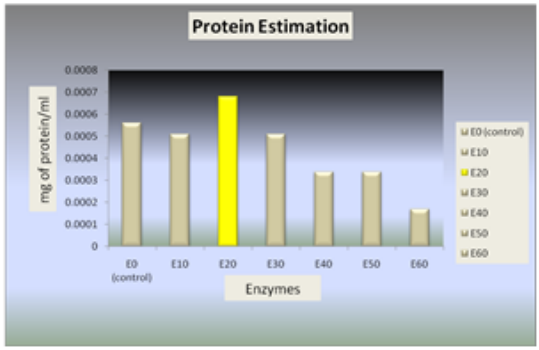
E0 *(control)*	0.000561
E*10*	0.00051
E*20*	0.00068
E*30*	0.00051
E*40*	0.00034
E*50*	0.00034
E*60*	0.00017

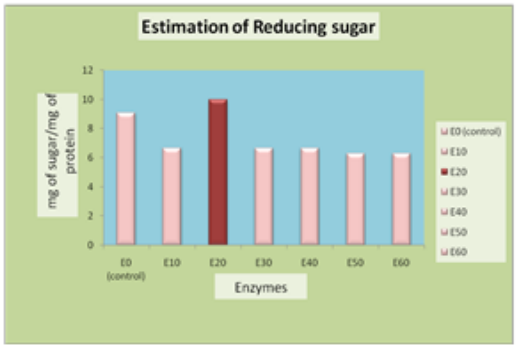
TABLE NO.4: Estimation of Reducing sugars

E0 *(control)*	9.09
E*10*	6.66
E*20*	10
E*30*	6.66
E*40*	6.66
E*50*	6.29
E*60*	6.29









reducing sugar content per mg of protein in enzyme seen in enzyme E*20* than other UV treated and control culture of *Chaetomiun globosum.* This experiment is supported by isolation and partial purification of extracellular enzyme (1,3)-3-D Glucanase from *Trichoderma ressei* (Saravanan*et al*;2007).

In many cases, mutations by UV are harmful, but occasionally it may lead to a better adapted organism to its environment with improved biocatalytic 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 *(Prabakaranet al., 2009).*

The above data support the view that cellulase production seen in culture of *Chaetomiun globosum* which exposed to UV light for 20 min. than other culture which exposed to UV light for 10min; 30min; 40min; 50min and 60 min. and control culture of *Chaetomiun globosum.*

Therefore we concluded that Chaetomiun*globosum*get mutated after exposed to UV light, the result of that enzyme cellulase production of *Chaetomiun globosum* after expose of UV light increased till certain time i.e. 20 min. and more expose to UV light enzyme cellulase production of *Chaetomiun globosum* started to decline.

Conflicts of interest: The authors stated that no conflicts of interest.

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