ENHANCING OF β-GALACTOSIDASE PRODUCTION FROM ASPERGILLUS SPECIES BY INDUCED MUTAGENESIS UNDER SOLID-STATE FERMENTATION

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Abstract

The first antibiotic was discovered in 1896 by Ernest Duchesne and "rediscovered" by Alexander Flamming in 1928 from the filamentous fungus *Penicilium notatu*m, Antibiotics can be selectively toxic by targeting such features as the bacterial cell wall. In present study the *Penicillum* species was isolated from soil sample through serial dilution and plating method and the genus was identified by wet-mount technique after that the fungi .The *Penicillum*sp was grown in the growth medium , after mat formation it was transferred in to production medium and the production was conformed by estimating the enzyme by DNS method. The same way the *Penicillium* sp was subjected to mutation by UV irradiation and Ethidioum Bromide and it was compared with wiled type. In that compere with wiled type I got the better production in UV mutation, in Ethidium Bromide mutation , the production was reduced so the to conclude my work the UV mutant strain was produced better result in production of Penicillinase enzyme. In future i will go for mass production of the enzyme and reduce the cost of the Antibiotic.

Key: Beta galactosidase, mutation, DNS, Ethidium Bromide

INTRODUCTION

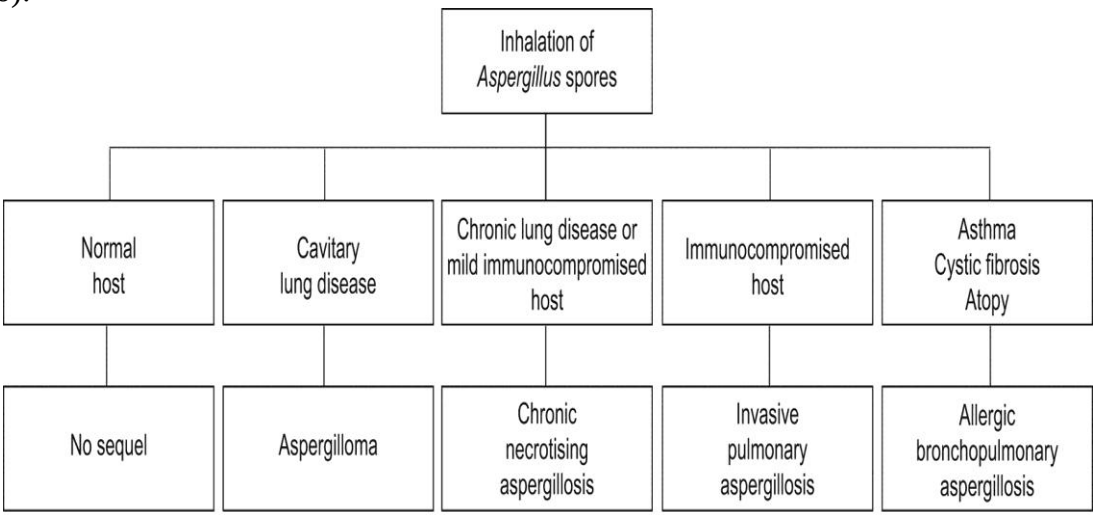
Aspergillus is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of *Stachybotrys* (species of which have also been called "black mould") Flannigan B, Miller JD (2001). *Aspergillus oryzae* is an asexual, ascomycetous fungus used for hundreds of years in the production of soy sauce, miso and sake without recorded incidents. There are conflicting opinions about whether *A.oryzae* can be isolated in nature. Although the details of the genetic relationship between *A.oryzae* and *A. flavus* remain unclear, the two species are so closely related that all strains of *A.oryzae* are regarded by some as natural variants of *A.flavus* modified through years of selection for fermenting of foods. *A.oryzae* is regarded as not being pathogenic for plants or animals, though there are a handful of reports of isolation of *A.oryzae* from patients. There are also several reports of products of *A.oryzae*fermentations, e.g. α-amylase, that seem to be associated with allergic responses in certain occupations with high exposure to those materials. *A. oryzae* can produce a variety of mycotoxins when fermentation is extended beyond the usual time needed for production of these foods. While wild *A.flavus* isolates readily produce aflatoxins and other mycotoxins, *A.oryzae* has not been shown to be capable of aflatoxinproducts M. Ichinohe and (Y. Yanagihara. 1987).

Generally identification of the *Aspergillus* species is based on the morphological characteristics of the colony and microscopic examinations.1 Although molecular methods

continue to improve and become more rapidly available, microscopy and culture remain commonly used and essential tools for identification of *Aspergillus* spp. Cultureing of *Aspergillus*spp often described as slow, perhaps creating misconception about its value for the detection of *Aspergilli*. *A. fumigatus* is a rapid gr. J MdMycol 2005.The biology, pathogenesis, molecular biology, and virulence factors of *A. fumigatus* have been exhaustively reviewed . This brief article focuses on how *A. fumigatus* is equipped with the features necessary for a ubiquitous pathogen.(Kwon-Chung et al., (2013).Fungi are major plant and insect pathogens, but they are not nearly as important as agents of disease in **(** M. R. Bragulat et al., 1994.Specifically, scientific findings in the following areas will be presented: classification and phylogenetic analyses of *A. flavus*, population biology, ecology and pathogenicity in agricultural environments, classical genetics including linkage group and mutant analyses, gene clusters, regulation of aflatoxin biosynthesis, and genomics.(K. A. Scheidegger and G.A Payne 2003,). *Aspergillus fumigatus* causes osteomyelitis in CGD patients almost as frequently as A. nidulans and much more frequently than A. flavus. Osteomyelitis due to A. nidulans is associated with higher mortality than A. fumigatus. (2011 Blackwell Verlag GmbH)

These observations demonstrate that voriconazole could be an effective and well-tolerated therapeutic option for the management of *Aspergillus* IOE.(Amonoo-Kuofi, K., P. Tostevin, and J. R. Knight. 2005). The ascospores of *A. fumigatus* germinate after heating at 70°C for 30 min and should survive at core temperatures of the compost pile that can reach ≥70°C .(Latgé J-P (1999) *Aspergillusfumigatus* and aspergillosis. ClinMicrobiol Rev 12: 310–350.). Twenty-three of the identified hydrophobins could be classified as class I hydrophobins based on their conserved cysteine spacing patient and hydropathy pattern.(Jensen, Britt Guillaume 2010).

A review of noninvasive or semi-invasive aspergillosis is also provided, with particular emphasis on chronic necrotizing pulmonary aspergillosis, which is recognized as a transition form of simple pulmonary aspergilloma and invasive pulmonary aspergillosis, although few findings have been reported in this area.(.K. Shibuya et al.,2006).



This mutant yield a third generation.UV treated strain,UV-5 with a 117.6% increase in beta galactosidase productivity with respect to the parent strain.(Department of chemical technology,University of Bombay,India,RasouliI,Kulkarni PR,(1994),oct.).Applications of association analysis, regulator engineering and robust genetic selections to improve lovastatin production in *Aspergillusterreus* have been described elsewhere. These approaches have been combined with traditional metabolic engineering and mutation and selection methods, both to enhance existing commercial

processes and to develop competitive strains from wild-type isolates (Specialty Chemicals Magazine July/August 2005).

This enzyme is extracellular in the micro fungus and therefore suitable structural characteristics for this localization are endogenous .On the contrary,in similar conditions but with the E.coliprotein,cytosolic in origin,secretion did not surpass 2% in the culture medium(Kumar.V,*et al.*,(1992)). The extracellular beta galactosidase of *A. niger* presents, along its primary structure.a lower number of charged amino acids compared to the *K.lactis* beta galactosidase,showing the *A.niger* beta galactosidase a 50% reduction in the histidine and 43% in lysine content. Enzymes activities were measured by determining the reducing sugars liberated by using the di nitro salicylic method (Summer.J.B.,andG.F.Somers.(1949)). In agreement with our previous findings for D-xylose induction, D-maltose induction leads to recruitment of proteins involved in proteasome-mediated degradation.(Schaap PJ, de Graaff LH (2011).

MATERIALS AND MEDHOD

ISOLATION OF ASPERGILLUS SPECIES:

We isolated a fungal species from Soil with a sterile inoculation loop, Fungal conidial spores were touched and immediately transferred to a test tube containing PDB (Potato Dextrose Broth), and then spores were uniformly homogenized by vortex. This spore suspension was inoculated on to PDA plate and incubated at 28^{o}C for 7 days. After spore germination, it was maintained on PDA (Potato Dextrose Agar).

ISOLATON OF FUNGAI FROM SOIL BY SERIAL DILUTION

The sample was collected, from that 1ml of sample was taken and it was transfered in to the test tube containing 10 ml of distilled water , it was considered as the dilution factor 10^{-1} .

Seven test tubes were taken and each test tubes were filled with 9ml of distilled water and marked as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, $10^{-7.}$ One ml of sample was taken from 10^{-1} and it was subjected to serially dilute from 10^{-3}. Before transfering the suspension from every dilution mixing was ensured. So that there is a uniform distribution of microbes. Then prepare was nutrient agar medium and sterilized at 121◦C and pour in to the petriplates. Dilute the sample were up to 10^{-2}, 10^{-3}. Added 0.1 ml of sample from 10^{-3} on to centre of an agar medium used in sterile pipette. Then dip the glass spreader were L-rod into a beaker of ethanol. Then plates was incubated at 37◦C for 24 hours. After result were observed in the growth of microoganisms.

Sabourand dextrose agar medium was prepared and 60ml of distilled water 6 tubes was also kept for sterilization .after sterilization,1g of goat dung soil was collected from decontaminated area.This suspension was serially dilution 10-3 was taken in a sterile pipette and poured over the approximately marked agar plate aseptically. sterilized and cooled sabourand dextrose agar medium was poured into the plates and rotated well.The plates were allowed to solidify and incubation at 28C to 80C for 5 days.

INOCULATION OF SAMBLE INTO THE MEDIUM

Sabourand dextrose agar medium was prepared and it was sterilized , after that the 20 ml of sterilized medium was transfered in to petriplate and it was allowed to solidify .After solidification 0.1ml of the suspension from dilutions 10^{-3} was spreaded on the petri plates containing Sabourand dextrose agar by using L-rod .The inoculated plates were incubated at 370C for 5-7days .After incubation the spores were formed on the surface of the petriplate.

FUNGAL STAINING

SEMI PERMANENT PREPARATION:

A part of the mycelium (3-4 days cultivated) was taken and it was smeared on the glass slide cointaining lacto phenol-cotton blue solution with the aid of preparation

needle.The preparation was covered with a coverslip and carefully heated over a little flame to remove air bubbles and at the same time, the coverslip was pressed tightly by the preparation needle.The specimen was observed through 10x lens of light microscope.Conidial morphology of isolated fungal strains was analyzed through a Phase-Contrast Microscope with automated imaging software (Motic image).

PRODUCTION OF β - GALACTOSIDASE USING ASPERGILLUS SPECIES

The culture of *Aspergillus*species was inoculated to sterile petriplatecontaininsabourse dextrose agar. The plate were then incubated at room temperature for 7-10 days to obtain fungal spores .The fungal spores were inoculated aseptically into 100ml conical flask containg growth media and it was incubated at room temperature for 5 days.After incubation the fungal mat was obtained and it was washed with 0.05% tween 80.

After wash, the mat was transferred to 250ml of production medium consist and it was incubated on the incubated orbital shaking incubator (REMI-9001) at room temperature for 5 -6 days.After incubation beta galactosidase was produced and it was estimated by taking optical density at 570nm by using colorimeter (CL 157)at the regular intravel (4^{th} , 5^{th} , 6^{th} day).

RANDOM-INDUCED MUTAGENESIS

In this study, we developed mutants by both UV- and Ethidium bromide induced mutagenesis.

UV-INDUCED MUTAGENESIS:

5 ml of sterile distilled water with one drop of Tween-80 was added in to agar slant. Then the spores were scraped by using an inoculation loop and suspended in 10 ml sterile test tube.The sample was Vortexed for 3 minutes to get a uniform suspension. After that 20-25 ml of sterile distilled water was added for dilution of spores in a 50 ml sterile flask. (It will be give $4x10^6$ spores/ml).2ml of spore suspension was added onto 6 petriplates . Then the plates were kept under UV for the irradiation from 10 to 60 minutes respectively.To isolate surviving colonies, the spores were inoculated on 250ml conical flask containing 50ml of Czapekdoxbroth for wild and UV irradiated colonies. Then the flasks were incubated at 28 ºC for 3 days in an incubated shaker with 120 rpm and beta-galactosidase producing capacity was observed by measuring enzyme activity from culture supernatants by using DNS method.

ETHIDIUM BROMIDE INDUCED MUTAGENESIS:

2ml of spore suspension was added onto 6 conical flasks containing czapekdox broth, 5% Nacl and 0.5%lactose.The sample was vortexed for 3 minutes to get a uniform suspension. After that 20-25ml of sterile distilled water was added for dilution of spores in a 50ml sterile flask. To that broth 10,20,30,40,50& 60 micro liter of Ethidium bromide was added. Then the flasks were incubated at 28 ºC for 3 days in an orbital shaker with 120 rpm and β-galactosidase producing capacity was observed by measuring enzyme activity from culture supernatants using DNS method.

ENZYME ASSAY:

Enzyme assay was based on the determination of reducing sugars produced as a result of enzymatic hydrolysis of pectin by Dinitrosalicylic acid reagent (DNS) method. For this to 0.2ml of 1% pectin solution and 2.0ml of sodium citrate buffer (pH 5.0) and 1.0ml of enzyme extract was added . the reaction mixture was incubated at 35°C for 25

mints. After 25mints.0.1ml of this reaction mixture was taken and added to test tubes containing 0.5ml of sodium carbonate solution . To that 3.0ml of DNS reagent was added and the test tubes were shaken to mix the content. The test tubes were heated on the boiling water bath for 10-15min. then the reaction mixture was allowed to cool and then 20ml of distilled water was added to the contents of each tubes and the absorbance was measured at.570nm using calorimeter(CL-157) .

STARCH HYTROLYSE

40 ml of Starch Agar medium was prepared and it was sterilized by autoclaving at 121^{o} C 15lb pressure for 20 minutes. After sterilization the medium was poured in the sterile petriplate and it was allowed to solidify. After that the sample were inoculated then it was incubated at room temperature for 3 days. After incubation the iodine solution was flood on the plate to check the starch hydrolysis

RESULT AND DISCUSSION

In my present study i have isolated the *Aspergillus*sp from soil sample. It was conformed by wet-mount after observing of microscope. Then it was subjected to growth on the growth medium, in that 5 th day a mat formation was observed (Plate : 1), after that it was transferred to production medium, in that the production was observed in yellows colour (Plate :2), The production was estimated by DNS method (Table 1 & Fig : 1)

The isolated *Aspergillus*sp was mutated with UV and Ethidium bromide and it was estimated (Fig : 2) , in that a better production was observed in UV mutation at 20 minutes exposer , it was estimated by DNS method in that it was produced 1.74 OD value is the heist when compare with Ethidium bromide mutation. To compete with developed countries, the genetically modified strains would be helpful to improve the productivity and to minimize the cost of β-galactosidase from native strains while mutating to the species in which growing UV it shows the maximum mutation 20 minutes which shows 20 minutes is self sufficients for this microorganism to mutate with mutating gent vizOD radiation.

TABLE:1UV MUTATION ASPERGILLUS SPECIES ON GROWTH

SL.NO	TIME IN MIN	OD AT 570 NM	
		BLANK	TEST
1.	10	0.24	1.38
2.	20	0.24	1.46
3.	30	0.24	1.53
4.	40	0.24	1.59
5.	50	0.24	1.69
6.	60	0.24	1.74

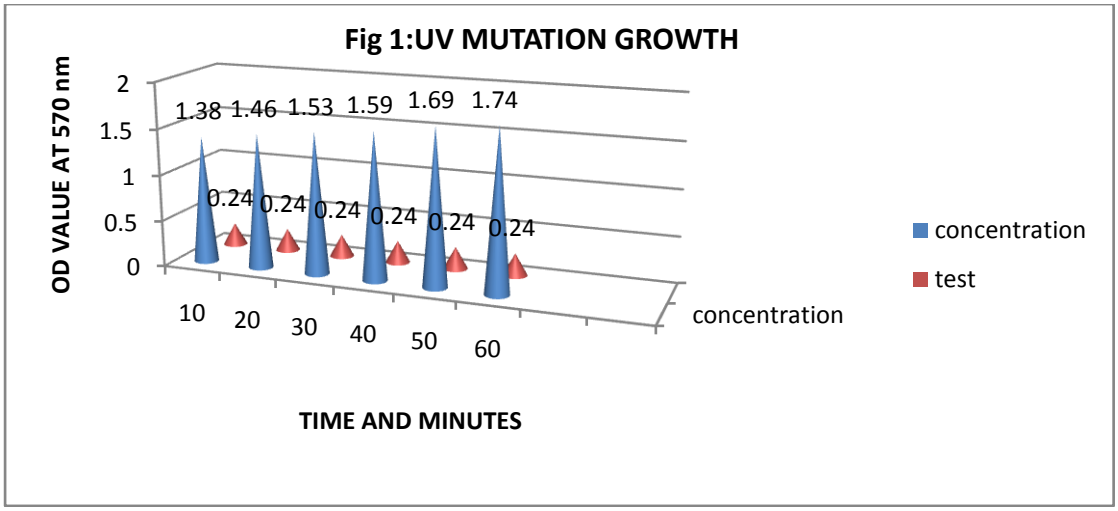
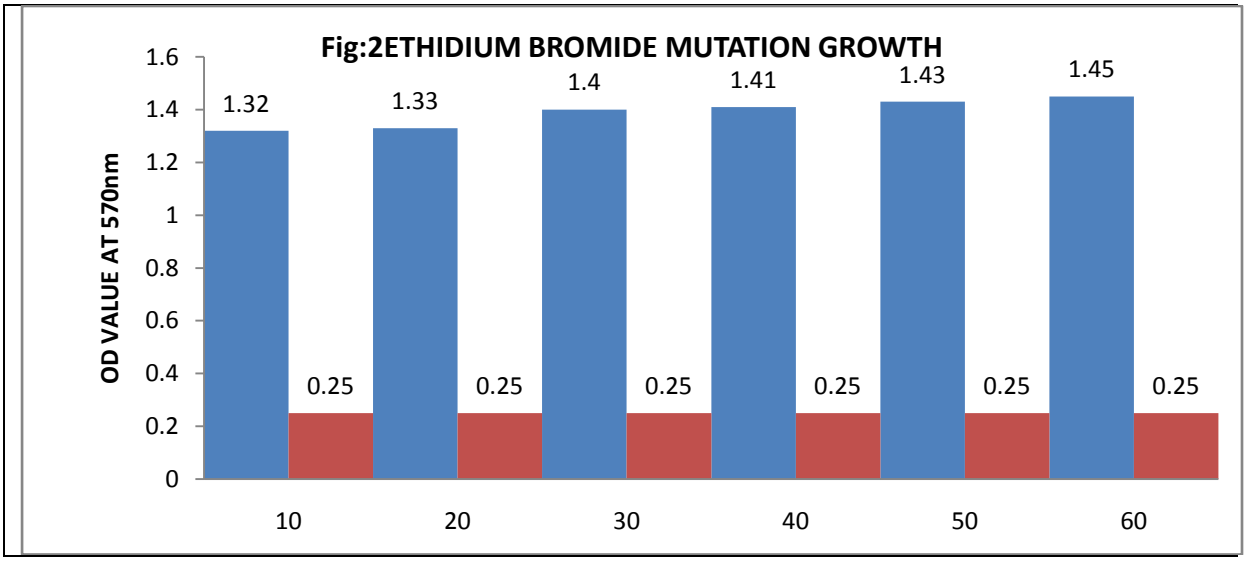


TABLE:2ETHIDIUMBROMIDE ASPERGILLUS SPECIES GROWTH

SL.NO	TIME IN MIN	OD AT 570 NM	
		BLANK	TEST
1.	10	0.25	1.32
2.	20	0.25	1.33
3.	30	0.25	1. 40
4.	40	0.25	1.41
5.	50	0.25	1.43
6.	60	0.25	1.45

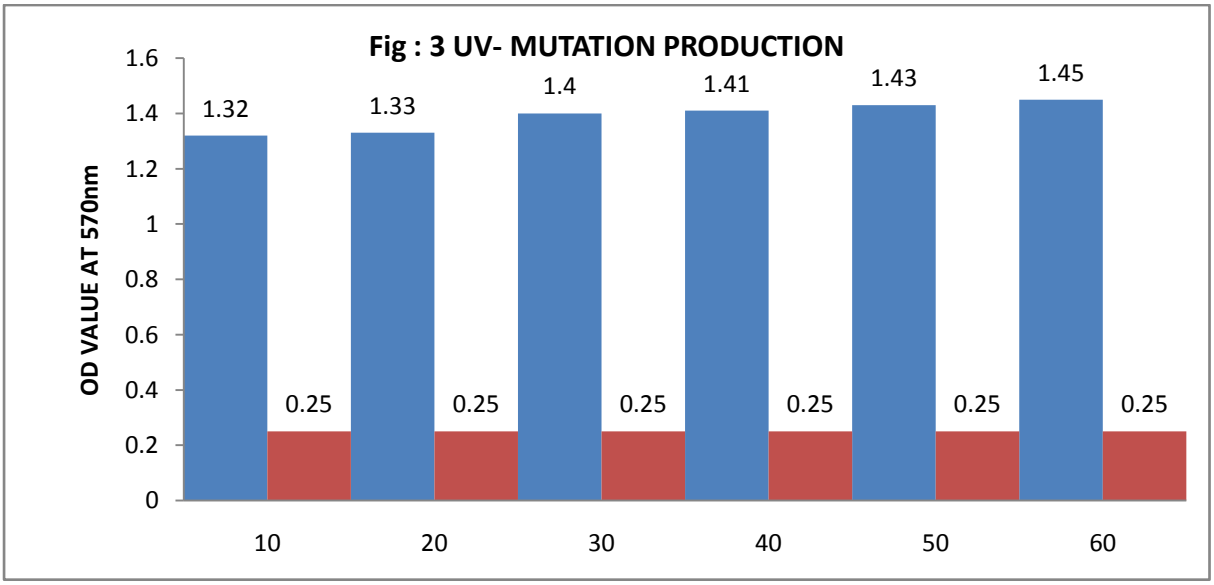


While mutating the species with EthidiumBromider in or various concentration shows a step slope which shows concentration is not a both for mutating with ethidium bromide.
While assay pectinase by DNS method it shows probable increase of activity at an absorbence of 1.45 OD at 570.

TABLE-3 UV MUTATION ASPERGILLUS SPECIES PRODUCTION

SL.NO	TIME IN MIN	OD AT 570 NM

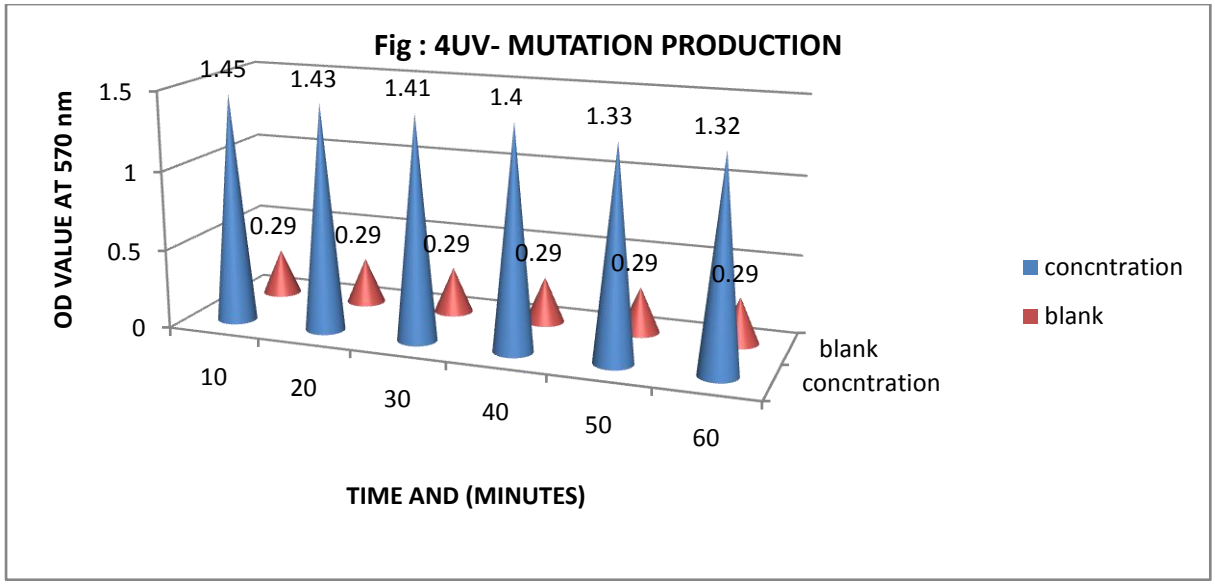
		BLANK	TEST
1.	10	0.25	1.32
2.	20	0.25	1.33
3.	30	0.25	1.40
4.	40	0.25	1.41
5.	50	0.25	1.43
6.	60	0.25	1.45



While mutating to the species in which growing TABLE-3 UV it shows the maximum mutation 20 minutes which shows 20 minutes is self sufficieents for for this microorganism to mutate with mutating vig OD radiation.

TABLE-4 ETHIDIUM BROMIDE ASPERGILLUS SPECIES PRODUCTION

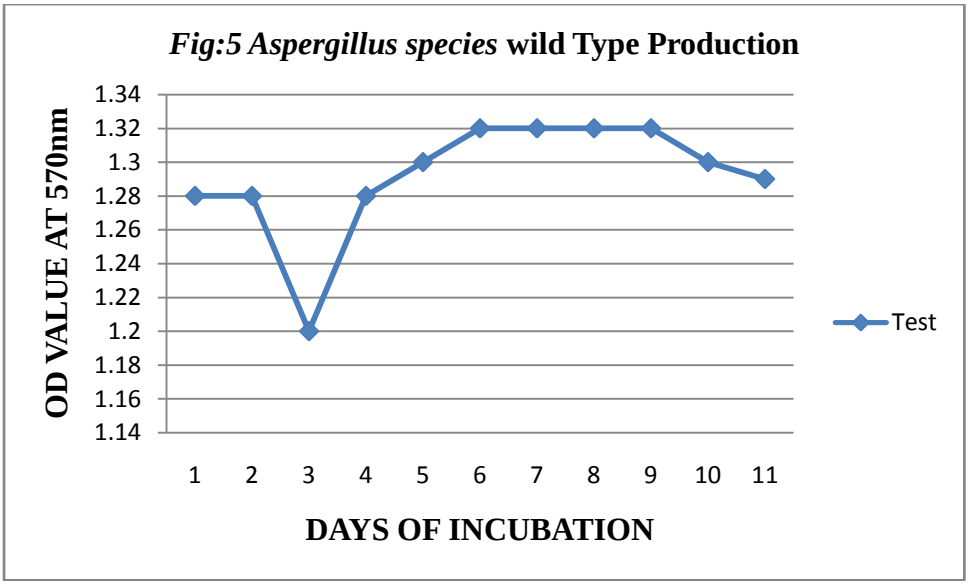
SL.NO	TIME IN MIN	OD AT 570 NM	
		BLANK	TEST
1.	10	.27	1.45
2.	20	.27	1.43
3.	30	.27	1.41
4.	40	.27	1.40
5.	50	.27	1.33
6.	60	.27	1.32



While mutating the species with EthidiumBromider in or various concentration shows a step slope which shows concentration is not a both for mutating with ethidium bromide.While assay pectinase by DNS method it shows probable increase of activity at an absorbence of 1.32 OD at 570nm.

TABLE-5 PRODUCTION OF ASPERGILLUS SP

SL.NO	DAYS	OD AT 570 NM	
		BLANK	TEST
1.	1th	0.22	1.28
2.	2th	0.22	1.28
3.	3th	0 .22	1.20
4.	4th	0 .22	1.28
5.	5^{th}	0 .22	1.30
6.	6th	0 .22	1.32
7.	7^{th}	0 .22	1.32
8.	8th	0 .22	1.32
9.	9th	0.22	1.32
10	10^{th}	0.22	1.30
11.	11^{th}	0 .22	1.29



The mat were observed in the production medium after incubation at orbital shaker (REMI-9001) at the OD value of citric acid is measured at 570nm by colorimeter(CL-157).

SL.NO	TIME IN MIN	OD AT 570 NM	
		BLANK	TEST
1.	10	0.24	1.38
2.	20	0.24	1.46
3.	30	0.24	1.53
4.	40	0.24	1.59
5.	50	0.24	1.69
6.	60	0.24	1.74

While mutating to the species in which growing TABLE-2 UV it shows the maximum mutation 20 minutes which shows 20 minutes is self sufficients for this microorganism to mutate with mutating viz OD radiation.

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