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MAXIMIZING CELLULOSE BREAKDOWN: OPTIMIZATION OF *ASPERGILLUS NIDULANS* FROM SPONTANEOUS MUTATIONS FOR ENHANCED DEGRADATION EFFICIENCY

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ABSTRACT

Cellulose, the predominant polysaccharide in nature, consists of linear chains of glucose units linked by β-1,4 glycosidic bonds. It is widely present in plant biomass and plays a crucial role in the bioconversion processes. Successful degradation of cellulose-rich materials hinges on various factors including the source of cellulose, the efficiency of cellulolytic enzymes, and the optimization of catalytic conditions. *Aspergillus nidulans*, an industrially important fungus, exhibits significant capability in breaking down plant cell wall polysaccharides. It secretes high levels of β-glucosidase (BGL) and low levels of endoglucanase (EGL). In this study, *Aspergillus nidulans* was cultivated on 1% cellulose agar plates for 12 consecutive weeks, and the hydrolytic capacity (HC) was assessed weekly. Colonies obtained in the 7th, 9th, and 11th weeks exhibited the highest HC, suggesting spontaneous mutations in *Aspergillus nidulans* that optimize its cellulose degradation efficiency. Further investigation into the genetic basis of these spontaneous mutations could provide valuable insights into the mechanisms underlying cellulose degradation optimization in *Aspergillus nidulans*. Additionally, elucidating the specific changes in enzyme expression or activity resulting from these mutations may contribute to the development of more efficient enzymatic processes for biomass conversion. This study highlights the potential of harnessing natural genetic variation in fungal species for industrial applications in biofuel production and waste management.

KEYWORDS: Cellulose, *Aspergillus nidulans*, spontaneous mutation, hydrolytic capacity, zone of clearance.

INTRODUCTION

Cellulose, the most prevalent polysaccharide in nature, consists of linear chains of glucose units linked by β-1,4 glycosidic bonds. It is abundant in plants, constituting a significant portion of their biomass, with an annual production estimated at 4 x 10^9 tons. However, its partial crystalline and insoluble nature renders it resistant to enzymatic degradation, posing a challenge for its utilization. Nonetheless, cellulose can be converted into glucose with the aid of cellulolytic systems. Microorganisms play a crucial role in converting lignocellulosic waste into valuable products such as biofuels through fermentation processes.

The successful transformation of cellulosic materials depends on various factors, including the source of cellulose, the enzymatic activity of cellulases, and the optimal conditions for enzymatic activity and production. The cellulase enzyme system consists of three classes of soluble extracellular enzymes: β-1,4 endoglucanase, β-1,4 exoglucanase, and β-glucosidase. Endoglucanase catalyzes the random cleavage of β-1,4 glycosidic bonds within cellulose chains, while exoglucanase is responsible for cleaving the non-reducing ends of cellulose chains, facilitating the breakdown of crystalline cellulose into elementary fibrils. β-glucosidase, on the other hand, hydrolyzes cellobiose and water-soluble cellodextrins into glucose.

Detritus waste, comprising discarded or spoiled plant material, is a potential source of cellulose. Fungi possessing cellulolytic degradation systems utilize three classes of hydrolytic enzymes: β-1,4 endoglucanases (EGL), exoglucanases or cellobiohydrolases (CBH), and lytic polysaccharide monoxygenase. These enzymes collectively catalyze the hydrolysis of cellulose into glucose, contributing to the degradation of detritus materials.

Because of several applications of cellulase in industries its optimization from many fungi have been studied. The filamentous plant pathogen *Trichoderma reesi* was studied for cellulose degradation by producing cellulase enzyme (Singhania et al 2013). *Aspergillus nidulans* is a industrial fungus capable of degrading plant cell wall polysaccharide efficiently. It secrete higher level of BGL and low level of EGL. Micro organisms showed potential ability to adopt changes in environment. Spontaneous mutation is that occurs naturally in micro organisms, such type of mutations are either harmful or beneficial. This property of microbes makes them ideal for strain improvement in industries by spontaneous mutations on repeated subculturing.

In this research work spontaneous mutation was studies and new strain of *Aspergillus nidulans* was obtained by repeated subculturing and cellulose degradation was optimized.

MATERIALS AND METHODS

Sample collection: sample was directly collected from vegetable waste of vegetable market in sterilized screw cap tubes randomly as 1 cm^2 blocks. Sample was scrapped/chopped with spatula /knife and brought to laboratory for the isolation of cellulose degrading microbes.

Isolation of *Aspergillus nidulans*: 1 gm of collected sample was crushed aseptically and mixed in 100 ml of sterile distill water. Serial dilutions up to 10^{-9} was prepared. A pour plate technique was used to isolate the cellulose degrading fungi. 1 ml of diluted sample was mixed with cellulose agar media composed of KH_2PO_4-0.5 gm, $MgSO_4$-0.25 gm, cellulose-2.0 gm, gelatin-2.0 gm, agar-15 gm and distill water-1L at pH 6.8 to 7.2 and 30^0C of temperature. After 48

hours of incubation selected colonies were transferred on Congo red agar media for the confirmation of cellulose degrading activity.

Composition of Congo red agar is of KH_2PO_4 .0.5 gm, $MgSO_4$-0.25 gm, cellulose-2.0 gm, gelatin-2.0 gm, agar- 15 gm, congo red -0.2 gm, distill water – 1L, pH- 6.8 to 7.2.

Congo red used in the media act as an indicator for cellulose degradation as it provides rapid and sensitive test for cellulytic microbes. Colonies showing discoloration of congo red were taken as positive cellulose degrading fungi (14). Hydrolysis capacity of positive isolates were determined by measuring diameter of clearing zone of colony (15).

Enzyme assay: Cellulose activity was assayed by using 3,5- dinitrosalisic acid (DNS) reagent and released reducing sugar from filter paper was estimated. The cellulytic activity was determined by incubating 0.5 ml of supernatant with 1.0 ml of 0.05 M sodium citrate buffer pH 4.8 containing whatman filter paper No 1 strip(1.0 X 6 cm ie 50 mg). After one hour incubation at 50^0C temperature the reaction was terminated by adding 3 ml of of 3,5- dinitrosalisilic acid (DNS) and 1 ml of reaction mixture. The amount of reducing sugar was estimated spectrophotometrically (16). One unit of enzymatic activity was defined as the amount of enzyme that releases 1µ mol reducing sugar per ml per minute.
Aspergillus nidulans was grown on malt extract agar (MEA) to produce spores. Spores were collected by using buffer containing 0.01 M acetamido aminoethane sulfonic acid (pH 6.8). α-Cellulose in a 250 ml flask was inoculated with spores of *Aspergillus nidulans* and incubated at 30^0C at 250 rpm.Agar plates were prepared containing 1% cellulose and 1% glucose. 10 µl of spore suspension was inoculated in the center of the plate and incubated at 30^0C for 48 hours.
Strain improvement: 10 µl spores suspension of *Aspergillus nidulans* was inoculated in a agar plate containing 1% α-cellulose. A repeated subculture was carried out after every 5 days on fresh agar plates with 1% α-cellulose for 12 weeks. Several colonies were purified and colony with maximum growth on cellulose containing agar plate was selected for further studies.

RESULTS AND DISCUSSION

Aspergillus nidulans positive for cellulose degradation was selected and grown on 1% α-cellulose and 1% glucose containing agar plates showed good growth figure 1 and poor growth on 1% α-cellulose containing agar plates figure 2. Spores of *Aspergillus nidulans* were harvested and used for cellulose degradation studies. Repeated sub culturing for 12 weeks was carried out on 1% α-cellulose containing agar plates. Zone of hydrolysis was measured for each plate and colony was assigned a number A01 to A12. The plates of seventh, ninth and eleventh week showed the colonies with maximum cellulose degradation which are assigned numbers A07, A09 and A11 respectively. These plates showed better growth on 1% α-cellulose containing agar plates as compare to other plates which indicate spontaneous mutation. On 1% α-cellulose and 1% glucose containing agar plates noticeable difference is not observed in these plates.

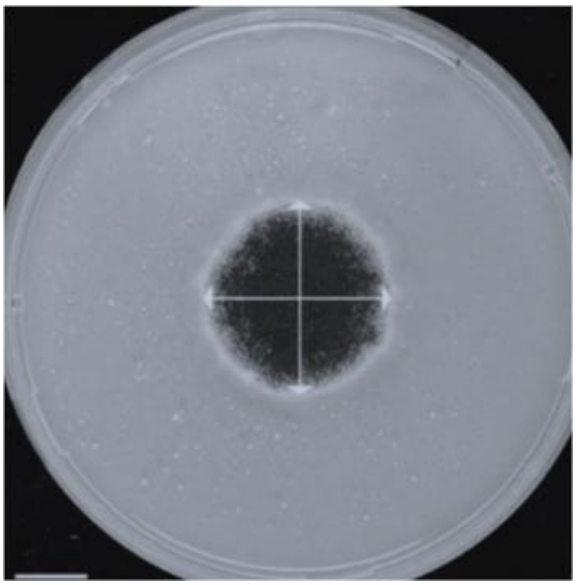


Figure 1: Growth of *Aspergillus nidulans* in 1% cellulose + 1% glucose agar plate.

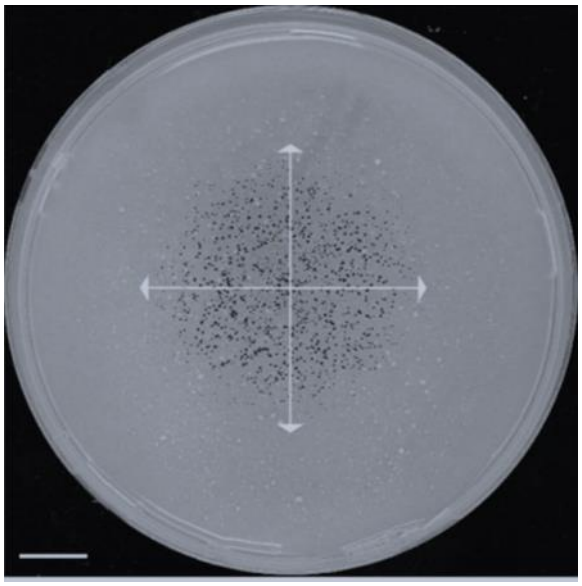
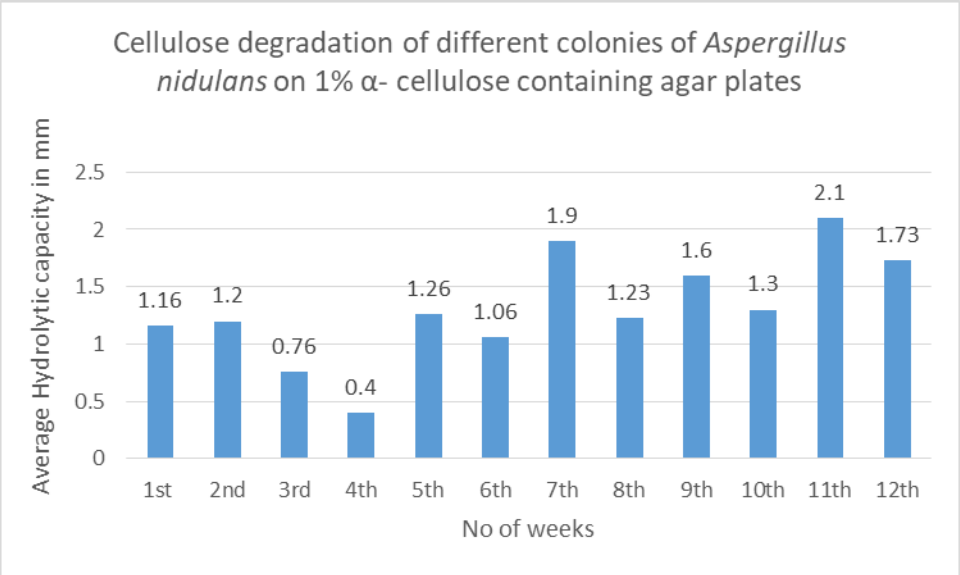


Figure 2: Growth of *Aspergillus nidulans* in 1% cellulose agar plate.

Table 1: Maximum clearing zone and Hydrolytic capacity (HC) of cellulose degrading *Aspergillus nidulans* on 1% α-cellulose + 1% glucose containing agar media for 12 weeks.

Sr. No	Weeks	Colony	Hydrolytic capacity in mm	Average Hydrolytic capacity in mm
1	1st	AA01	1.2 mm	1.16 mm
		AB01	1.1 mm	
		AC01	1.2 mm	
2	2nd	AA02	1.1 mm	1.20 mm
		AB02	1.0 mm	
		AC02	1.50 mm	
3	3rd	AA03	0.7mm	0.76 mm
		AB03	0.8 mm	
		AC03	0.8 mm	
4	4th	AA04	0.4 mm	0.40 mm
		AB04	0.5 mm	
		AC04	0.3 mm	

5	5th	AA05	1.3 mm	1.26 mm
		AB05	1.3 mm	
		AC05	1.2 mm	
6	6th	AA06	1.2 mm	1.06 mm
		AB06	1.0 mm	
		AC06	1.0 mm	
7	7th	AA07	1.9 mm	1.90 mm
		AB07	1.8 mm	
		AC07	2.0 mm	
8	8th	AA08	1.4 mm	1.23 mm
		AB08	1.1 mm	
		AC08	1.2 mm	
9	9th	AA09	1.8 mm	1.60 mm
		AB09	1.6 mm	
		AC09	1.4 mm	
10	10th	AA10	1.4 mm	1.30 mm
		AB10	1.3 mm	
		AC10	1.2 mm	
11	11th	AA11	2.0 mm	2.10 mm
		AB11	2.1 mm	
		AC11	2.2 mm	
12	12th	AA12	1.7 mm	1.73 mm
		AB12	1.8 mm	
		AC12	1.7 mm	



Graph 1: Showing Hydrolytic capacity of *Aspergillus nidulans* on plates subcultured for 12 weeks on 1% cellulose.

CONCLUSION

In this present study cellulose degradation was studied. Different types of micro organisms are able to degrade cellulose. *Aspergillus nidulans* improved its cellulose degrading capacity by spontneous mutation when subcultured on 1% α-cellulose containing agar plates. Sub culturing for 12 weeks on such media allow Aspergillus nidulans to undergo spontaneous mutation and improved its hydrolytic capacity in 7th, 9th and 11th week plates. When 1% glucose is added to the same medium confluent growth was observed while on 1% cellulose only poor growth was observrd which

indicate that these strains used cellulose as a sole source of corbon and ideal for spontaneous mutation to adopt the *Aspergillus nidulans* in provided conditions for survival.

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