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ISOLATION OF CELLULOSE DEGRADING FUNGI FROM SOIL AND OPTIMIZATION FOR CELLULASE PRODUCTION USING CARBOXY METHYL CELLULOSE

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ABSTRACT

Cellulose is a linear polysaccharide composed up of glucose residues. Cellulose can be broken down by microorganisms which mainly utilize sugars for their growth. The main objective of the present study was to isolate a potential fungal strain and to optimize the culture conditions to get maximum cellulase. Potential fungus was isolated, screened and identified for cellulase production and the culture conditions were optimized. Study revealed the best condition for cellulase production for fungi was found to be pH 6 with temperature 30°C±2 and ammonium chloride as nitrogen source supported the maximum cellulase production of 0.117U/ml.

Keywords: **Cellulose, fungi, optimization and cellulase activity**

INTRODUCTION

Lignocellulosic biomass is a complex structure mainly composed up of cellulose, hemicellulose and lignin. Agricultural residue contains 40-50% cellulose [1]. Hemicelluloses are heterogeneous polymers made up of five carbon sugars and form 20-30% of plant biomass. Cellulose can be converted to fermentable sugars (saccharification) by many bacteria and fungi. Microorganisms use the released sugars for their growth by producing extracellular enzyme cellulase [2], that catalyze the process of hydrolysis. Fungi such as *Aspergillus sp*., *Fusarium sp*., *Penicillium sp*., *Trichoderma sp*., *Chaetomium sp*., have been reported to be efficient cellulase producers [3]. Cellulose is a linear polymer composed of D-glucose subunits linked by β-1, 4 glycosidic bonds forming the dimer cellobiose. These form long chains linked together by hydrogen bonds and van der Waals forces. Cellulose present in crystalline form and a small amount of non organized cellulose chains forms amorphous cellulose. In the latter conformation, cellulose is more susceptible to enzymatic degradation [4]. Cellulose appears in nature to be associated with other plant compounds and this association may affect its biodegradation.

Cellulases are a group of enzymes composed of three major components: endo-glucanases, exo-glucanases and β-glucosidase [5]. They are used in the detergents, and other industries for production of biofuels. Various bacteria and fungi present in nature are producers of complex enzyme cellulases and they grow on inexpensive cellulosic biomass [6]. Screening of such cellulose degrading microorganisms has industrial and environmental implications[7]. Various physical and chemical parameters such as temperature, pH and media components such as carbon and nitrogen sources play an important role in enzyme production. Interactions of these components is critical, hence the optimization is an essential process in the production and commercial application of such enzymes. Considering the commercial importance of cellulases, an attempt was made to optimize the growth parameters for efficient cellulase production.

MATERIALS AND METHODS

Isolation and screening of cellulase producing fungi

The soil samples were collected from agricultural fields Bangalore, India and serially diluted with sterile distilled water. Isolation of the fungal strain was carried by performing serial dilution method and spread plate technique on PDA plates. The fungal isolates was grown in agar plates containing 1% CMC agar media and cellulase activity was checked for seven days. Plates were flooded with aqueous solution of 1% Congo red for 15 min at room temperature and the plates were thoroughly washed with 1N NaCl for counter staining the Petri-plates [8].

Identification of cellulose degrading fungi

The fungal isolate demonstrating highest zone of cellulose break down was selected and identified. The

morphological characteristics of the fungi such as surface, color, border and spores were carefully observed and recorded. Further, the strain was sent to Agharkar research institute, Pune, India for species level identification.

Cellulase production and assay

Cellulase production was carried out in Erlenmeyer flasks (250 ml) with100ml of autoclaved cellulosic basal medium (CBM), supplemented with 1% carboxy-methyl cellulose (CMC). The media also contained ammonium tartrate (5g/L), KH_2PO_4 (1gL/), $MgSO_4.7H_2O$ (0.5g/L), yeast extract (0.1g)/L, $CaCl_2.2H_2O$ (0.001g/L) maintained at pH 6. Flasks were inoculated with *Paecilomyces variotii* (2×10^6 spores/ml) and incubated at room temperature for 7 days. The samples were withdrawn at regular intervals to determine the enzyme activity. Cellulase activity was determined using filter paper assay [9]. One unit of enzyme activity is defined as amount of enzyme necessary to release 1µmol of glucose per ml per minute. The reducing sugar was measured by the dinitrosalicylic acid method [10].

Optimization of cellulase production

Optimization of growth media was carried out based on one factor analysis by varying the growth parameters like carbon source, nitrogen source, inoculum size, pH and temperature. All the experiments were carried out in triplicates.CMC concentration ranging from 0.5 - 3% with 0.5% difference were added to cellulosic basal medium and incubated at standard condition for maximal cellulase production. Similarly 1% of various nitrogen sources such as peptone, tryptone, yeast extract, beef extract, casein, ammonium chloride and sodium nitrate were selected and optimized. Different inoculum sizes such as 0.5 - 3% (v/v) were tested for their ability to induce cellulase production in the production medium. Various physical parameters were also optimized by varying the pH (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) and temperature (20, 25, 30, 35 and 40°C) for maximal cellulase production.

RESULTS AND DISCUSSION

Figure 1
Fungi grown in CMC media

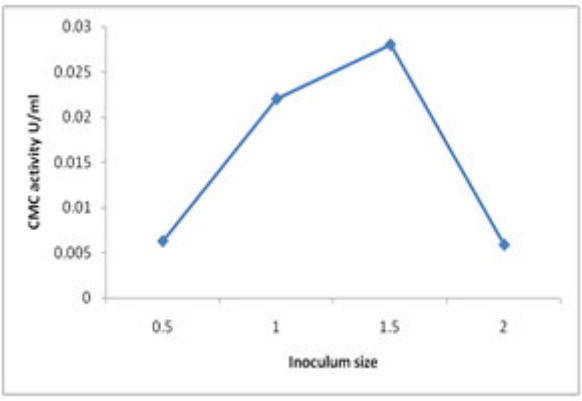

Figure 2
Effect of inoculum size on cellulase activity

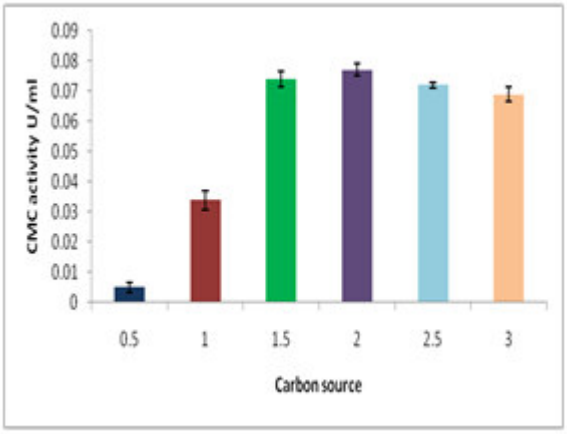

Figure 3
Effect of concentration of carbon sources on cellulase activity

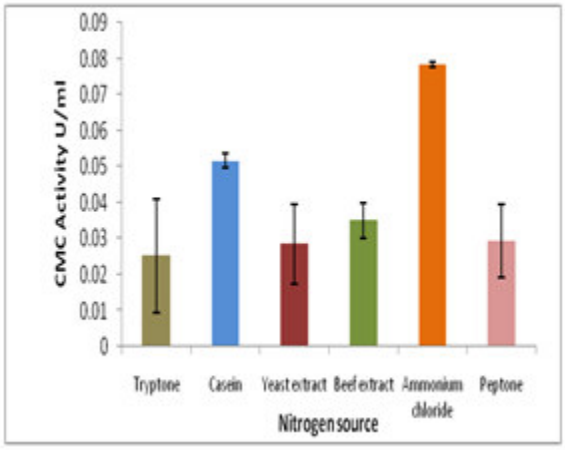

Figure 4
Effect of organic and inorganic nitrogen sources on cellulase activity

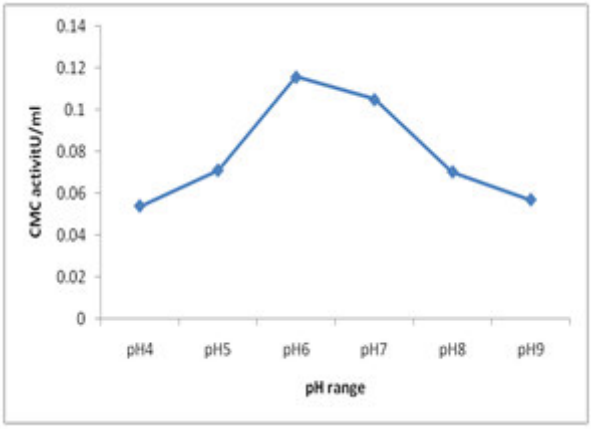

Figure 5
Effect of pH on cellulase activity

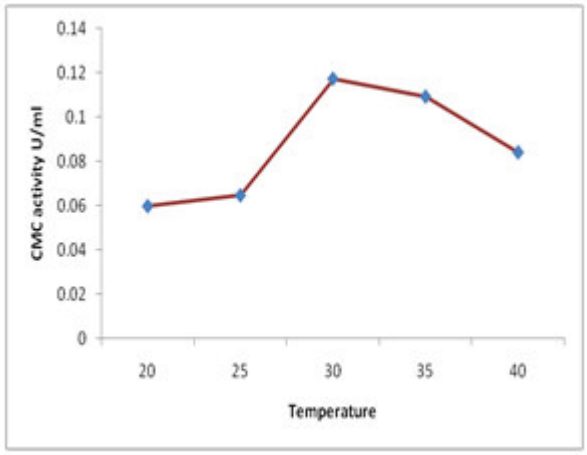

Figure 6
Effect of temperature (°C) on cellulase activity

The observed morphological and biochemical properties of the hyperactive strain revealed the characteristics criteria of *Paecilomyces variotii* NFCCI 3343. Hydrolysis capacity (HC) ratio of the isolate was calculated by dividing the zone of clearance. The diameter for the organism was found to be 1.33cm which was higher than the other isolates. *Paecilomyces variotii* has fast growing capability and is a common wood degrader. Hence, cellulase production which is beneficial for faster cellulase production work carried out by Millala et al., [11] and Abo et al.,[12] also reported that the other fungal isolates like *Aspergillus* and *Penicillium* are good cellulase producers. Inoculum density of 1.5% gave maximum cellulase activity of 0.03U/ml (Figure 2). Any further increase in the Inoculum density did not had much effect on the enzyme activity rather the activity decreased the possible reason could be because of the nutrients depletion in the medium Similar work done by Lugani, [13] also reported that increase of spores in the media decreases the cellulase activity because of depletion of nutrients into the medium. Cellulase activity at different concentrations of CMC as carbon source showed maximum CMCase activity at 2% concentration about 0.077U/ml (Figure 3). The results were in conformity with the work carried out by Ahmed et al., [14] who reported that CMC as the best substrate for cellulase production due to its soluble nature compared to other substrates. Nitrogen is an essential component for microbial growth, maximum cellulase activity about 0.078 U/ml was observed in media supplemented with ammonium chloride was shown in Figure 4. Higher cellulase yield can be obtained with ammonium compound used as nitrogen source in the growth media. The addition of organic nitrogen sources like casein and peptone also resulted in higher cellulase production but they are not an effective replacement for nitrogen sources because of higher cost [15]. Effect of pH on the cellulase production was shown in figure 5. Results of the present study revealed that pH 6.0 was best suited for higher cellulase yield. Similarly, effect of temperature on cellulase production showed highest activity of 0.117U/ml at 30±2 °C (Figure 6) at increased temperature the activity was reduced gradually is due to the instability at higher temperature. Previous studies have reported that different pH and temperatures for maximum cellulase production using different fungal strains, which suggests that the optimal physico chemical parameters depends on strain variation. Effect of pH on cellulase production by present strain supports the study conducted by Gautam et al., [16] who reported the best pH suited for the fungi strain is pH 6.

CONCLUSION

The combined effect of all the growth factors was observed that there was a trend of increase in the cellulase activity at every step of the optimization process which confirms the optimization of the process parameters. The isolated fungal strain will be a promising tool for cellulase production using lignocellulosic residues. Further study would focus on enhancing the cellulolytic ability and extraction of cellulase enzyme for its commercial application.

REFERENCES

1. Yang B, Wyman CE. Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioproducts and Biorefining. 2008 Jan 1;2(1):26-40.
2. Immanuel G, Dhanusha R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. International Journal of Environmental Science & Technology. 2006 Dec 1;3(1):25-34.

3. Gupta P, Samant K, Sahu A. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. International journal of microbiology. 2012 Jan 18;2012.
4. Pérez J, Munoz-Dorado J, de la Rubia TD, Martinez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. International Microbiology. 2002 Jun 1;5(2):53-63.
5. Anwar Z, Gulfraz M, Irshad M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. Journal of radiation research and applied sciences. 2014 Apr 30;7(2):163-73.
6. Singh A, Kuhad RC, Ward OP. Industrial application of microbial cellulases. Lignocellulose Biotechnology: Future Prospects. 2007:345-58.
7. Esa F, Tasirin SM, Rahman NA. Overview of bacterial cellulose production and application. Agriculture and Agricultural Science Procedia. 2014 Dec 31;2:(1)3-9.
8. Carder JH. Detection and quantitation of cellulase by Congo red staining of substrates in a cup-plate diffusion assay. Analytical biochemistry. 1986 Feb 15;153(1):75-9.
9. Das A, Bhattacharya S, Roopa KS, Yashoda SS. Microbial utilization of agronomic wastes for cellulase production by Aspergillus niger and Trichoderma viride using solid state fermentation. Dyn Biochem Process Biotech Mol Biol. 2011;5:18-22.
10. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959 Mar;31(3):426-8.
11. Milala MA, Shugaba A, Gidado A, Ene AC, Wafar JA. Studies on the use of agricultural wastes for cellulase enzyme production by Aspergillus niger. Res. J. Agric. Biol. Sci. 2005;1(4):325-8
12. Abo-State MAM, Hammad AE, Selin M, Gannam RB. Enhanced production of cellulases by Aspergillus sp on agricultural wastes by solid state fermentation. Am Eurasian J Agric Environ Sci. 2010; 402 – 410.
13. Lugani Y, Singla R, Sooch BS. Optimization of Cellulase Production from Newly Isolated Bacillus sp. Y3. Journal of Bioprocessing & Biotechniques. 2015 Nov 28;2015
14. Ahmed S, Aslam N, Naeem S, Jamil A. Induction of xylanase and cellulase genes from Trichoderma harzianum with different carbon sources. Pakistan Journal of Biological Sciences (Pakistan). 2003
15. Sun T, Liu BH, Li ZH, Liu DM. Effects of air pressure amplitude on cellulase productivity by Trichoderma viride SL-1 in periodic pressure solid state fermenter. Process Biochemistry,1999, 34: 2529
16. Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S. Optimization of the medium for the production of cellulase by the Trichoderma viride using submerged fermentation. International journal of environmental sciences. 2010 Oct 1;1(4):656.