



Labscale Production and Purification of Cellulase Enzyme from *Aspergillusniger*

Patil Kalyani C., Patil Ashwini M., Wagh Mohini C. and Chandratre Sangita J.

Department of Microbiology, Moolji Jaitha College, North Maharashtra University, Jalgaon, 425001, INDIA

Available online at: www.isca.in, www.isca.me

Received 30th July 2015, revised 8th October 2015, accepted 23th October 2015

Abstract

The aim of this paper is to determine the production of cellulase enzyme from fungal strain *Aspergillusniger* on labscale. Cellulase enzyme and various cellulosic product obtained from microorganisms have shown to have immense industrial potential by using to chemical cellulase as being traditionally used in industries such as a, food industry, textile industry, paper and pulp industry, biofuel production, brewing and agricultural industry etc. cellulase being broadly used for industrial purpose and the enzyme cellulase being an extremely complex system, research oriented work for its production has importance periodically. The availability of substrate for the production of this enzyme was abundant. The most abundant carbohydrate polymer in biosphere is cellulose, having an annual production that estimated to be over 7.5×10^{10} tones. Cellulase is the inducible enzyme produced by various microorganisms like bacteria, fungi while there growth on carbon containing sources that is cellulose used as a substrate. As compare to bacteria; fungi was most suitable microbes for the enzyme production. Filamentous fungi that is slender thread like structure of fungi is mostly preferred for cellulase enzyme production and it has good capacity to produced extracellular enzyme. The analysis of production of cellulase enzyme was done by Di-nitrosalicylic acid (DNS) method. The present study aimed to screen fungi *Aspergillus niger* for the production of cellulase enzyme and further enzyme purification.

Keywords: Cellulase, cellulosic materials, *Aspergillusniger*, Partial purification.

Introduction

The cellulose is abundantly found in plant biomass. From all the different carbon sources for the production of cellulase enzyme carboxy methyl cellulose is the most efficient carbon sources which can be used for the abundant production of enzyme. Naturally it is only present in plant cell, while some bacterial species also tend to produce it. Cellulose rich plant biomass is a reliable and sustainable source of fuel, as animal feed stock and feed stock for chemical synthesis.

Degradation of cellulose, containing biomass has a major role as cellulose has enormous potential as a renewable source of energy, is an important part in the carbon cycle within the biosphere. It also plays an important part in reducing the environmental pollution, by converting the cellulosic biomass to fermentable sugars through biocatalyst cellulases obtained from cellulolytic organisms. Thus considering the bioconversion, feasibility and efficiency it can be considered of vast industrial significance. There are many applications of the Cellulase in industry that used for many years in textile production, food processing, waste-water treatment, detergent for mulationfeed and other areas. Other applications of cellulase are for beer production for production of wine, and for production of fruit juice and also for production of biofuels and ethanol. In this study, protocol for measuring enzyme activity of cellulase is taken from International Union of Pure and Applied Chemistry that is (IUPAC). According to IUPAC guide line a cellulase

enzyme contains three important components that are, i. β -glucosidase (EC-3.2.1.21), ii. exo- β -glucanase (EC-3.2.1.21), iii. endo- β -glucanase (EC-3.2.1.4).

As the accumulation of biomass persistently increases in the biosphere so does the production of cellulosic components. Bioconversion of these cellulosic materials provides tremendous opportunities for achieving higher benefits and economically feasible products. Thus with the growing need and demand, approach towards the synthesis of biomass has to be diversified in such a way that maximum yield is obtained. Various forms of degradation processes that are faster must be accepted for which use of microorganisms is far easier than use of chemicals. Decomposition of organic matter is generally carried out by microorganisms.

Scientific classification of fungi:

Domain – Eukaryota
Kingdom – Fungi
Phylum – Ascomycota
Subphylum – Pezizomycotina
Class – Eurotiomycetes
Order – Eurotiales
Family – Trichocomaceae
Genus – *Aspergillus*
Species – *A. niger*

Why fungi are used- Microorganisms like bacteria, fungi and

actinomycetes produced cellulase enzyme by utilizing carbon sources. In all microorganism fungi has main producer microorganism for cellulase enzyme. Most of the times, filamentous fungi are preferred because it gives more yield of cellulase enzyme than that of the other fungi. *Aspergillus niger* is filamentous fungi therefore indirectly it gives more production of β -glucosidase, endo- β -glucanase and exo- β -glucanase and crude enzyme produced by the fungal cultures having wide variety of application especially in agricultural use.

Material and Methods

Phenotypic Characters: Phenotypic characters were studied by the slide culture technique. In which size shapes and other morphological characters etc. were studied by using lacto phenol cotton blue stain on the slide. Fungi are the spore bearing, non chlorophyllous organism which, and whose usually filamentous structures are surrounded by cell walls containing chitin or fungal cellulose or both. Culture of *Aspergillus niger* continuously subculturing by using potato dextrose agar slant and store at 4°C for further use. *Aspergillus niger* shows growth on Czapek-Dox agar medium containing 1% carboxy methyl cellulose and has ability to use cellulose as a carbon source.

Qualitative Method for Screening of Cellulytic Activity of Fungi¹: For screening of cellulolytic activity of fungi is done in Czapek-dox agar medium with following composition (g/l): sucrose – 30, KCl – 0.5, FeSO₄– 0.01, NaNO₃ – 2, Agar agar – 20, carboxy- methyl cellulose – 1%, MgSO₄ – 0.05, K₂HPO₄– 1, pH–5.

These all chemicals were used in preparation of media. Inoculate spore suspension density 2×10^6 . 0.1 ml spore suspension of known density was inoculated in 6mm cavity made in Czapek Dox agar medium, plates were incubated for three days that is 72 hours at room temperature (28°C), when fungal growth appeared on media After incubation, staining with 10ml of 1% Congo - Red staining solution was perform for detection of cellulolytic activity.

Production of Cellulase: From fungal isolates, fungal culture, *Aspergillus* sp. was used to know their potential for cellulases production. For the production; 100 ml of Czapek-Dox broth medium amended with 1% of carboxymethyl cellulose was distributed into separate 250 ml Erlenmeyer conical flasks and adjusted pH to 5. Spore suspension of fungal culture was inoculated after autoclaving the production media. Incubate at temperature 32°C on shaking condition 120rpm for a week/ 7 days. Growth of organism shows in the form of beads. Then the contents of flasks were centrifuge to separate mycelial beads from culture broth. Then the supernatant as crude enzyme was used for estimation of total protein content and total activity of cellulase by filter paper activity.

Enzyme Purification: By Salt Precipitation Method²⁻³: Take 250 ml Czapek-dox broth medium containing 1% carboxy methyl cellulose. Inoculate a aliquot of 0.1 ml suspension of given organism that is *Aspergillus niger*. Incubate flask for 7 days in 120 rpm. After incubation centrifuge the medium in sterile centrifugation tubes at 5000 rpm for 10 min. Remove the supernatant in separate clean flask and discard the precipitate. Measure the supernatant in measuring cylinder and purify the cellulase enzyme by ammonium sulfate precipitation, dialysis technique. After dialysis partially purified enzyme was obtained from crude sample.

Quantitative Method for Purification of Cellulase Enzyme⁴: In this method, enzyme activity of crude as well as partially purified enzyme was determined. The enzyme activity was calculated as per procedure describes by International Union of Pure and Applied Chemistry (IUPAC) guide line By using Whatman filter paper No.1 as substrate. Enzyme activity was calculated by following formula in FPU/ml.

Formula= Filter Paper Activity = $(0.37 / [\text{enzyme}]) \times \text{releasing } 2.0$ (mg glucose) units/ml.
Derivation used for the FPU⁵.

Protein estimation by Folin Lowry method: In it, protein was estimated by the protocol of Folin Lowry method. For that we had taken stock concentration 1000 μ g/ml, reagent C and reagent FC was also used. Then OD was taken at 660nm in spectrophotometer.

Results and Discussion

Qualitative Method by Screening of Cellulolytic Fungi: The fungal culture produced zone of hydrolysis (fig.2) in 1% carboxy methyl cellulase containing Czapek- Dox agar plate after flooding with 1% Congoed solution after three days. The cellulase activity of *Aspergillus niger* was confirmed by Congo red decolouration. The results obtain from these study shows much more similarity to that of earlier reports which used for reference purpose.

Discussion: This study should be demonstrated that the cellulase enzyme was partially purified on lab scale. The screening of Cellulase enzyme was takes place and which was detected by the clear zone of hydrolysis around the growth and the results were match with earlier reports of Muhammad Sohail, Aqeel Ahmed and Shakeel Ahmed Khan, 2014.

The cellulase enzyme was partially purified by Ammonium salt precipitation with dialysis method this reports is matches with Sri Lakshmi S. and Narasimha G. 2012. By which reports enzyme activity and specific activity of partially purified enzyme were increased than that of the crude enzyme.

Our future aspect is Purification of enzyme and use of agricultural waste material and lignocellulosic material as a

substrate for the production of enzyme and biofuels. In the area of Jalgaon district there is abundant amount of agricultural waste so we again elaborate our work on the base of agricultural waste as a substrate.



Figure-1
Phenotypic characters



Figure-2
The fungal culture produced zone of hydrolysis

Table -1

Quantitative Method for Total Protein and Enzyme Activity

Test	Protein Conc.	Enzyme activity FPU/ML	Specific activity
Crude Enzyme	496.6µg/ml	2units/ml	4.08units/mg
Partially Purified Enzyme	414.4µg/ml	2.3units/ml	5.60units/mg

Conclusion

From this study, we concluded that the fungi culture, that is *Aspergillusniger* possess cellulolytic activity. Cellulase enzyme was produced by *Aspergillusniger* fungi on lab scale and then partial purification was carried out by using ammonium sulphate precipitation technique followed by dialysis method. filter paper activity was increased of dialysed sample that is 2.3units/ml as compare to crude enzyme that is 2units/ml and specific activity of crude and partially purified enzyme was 4.08units/mg and 5.60units/mg determined respectively.

Acknowledgement

I am thankful to Moolaji Jaitha College, Jalgaon for providing the infrastructure and facilities for project work.

References

1. Sri Lakshmi S. and Narasimha G., Production of cellulases by fungal cultures isolated from forest litter soil, Ann For. Res., **55(1)**, 85-92, (2012)
2. Alzahrani Z., Salting in , Salting out and Dialysis of Protein., BCH, **233**, (2010)
3. Devi C.M, Kumar S.M., Production, optimization and partial purification of cellulose by *A. niger* fermented with paper and timber sawmill industrial wastes. J. Microbiol, Biotech. Res., **2(1)**, 120-128, (2012)
4. Adney B. and Baker J., Measurement of Cellulase Activities, NREL/TP, **5(1)**,426-428, (1996)
5. Ghosh T.K., Measurement of cellulase Activities. Pure and Appl Chem, **59(2)**, 257-268, (1987)
6. Elad Y., Biological control of foliar pathogens by means of *Trichodermaharzianum* and potential modes of action. Crop Prot., **19**, 709-714,(2000)
7. Beguin P. and Aubert J.P., The biological degradation of cellulose. FEMS Microbiol. Rev., **13**, 25-28, (1994)
8. Bhat M.K., Cellulases and related enzymes in biotechnology, Biotechnol. Adv. **18**, 355-383, (2000)
9. Miller G.L., Use of Dinitrosalicylic Acid Reagent for Determination of Reducing sugar, Anal. Chem., **3(1)**, 426-428 (1959)
10. Sohail M., Ahmad A., Khan S.A., Comparative study of production of cellulase from three strain of *A. niger*. Dipartment of microbiology, Karachi, **46(5)**, 1911-1914, (2014)
11. Upadhyay A. *et.al.*, Biophysical chemistry, Principles and Techniques, 301-343, (1993)