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Production of Cellulase from *Myrothecium verrucaria* NCIM 903 by Solid State Fermentation Utilizing Wheat Bran as Substrate

Supriyo Basak[1*] • Meenakshi Kumari[2] • Amrita Kumari[2] • Prasanna Jyothi D[2]

[1] Indian Institute of Technology Guwahati-781039, Assam, India
[2] Vinayaka Missions Kirupananda Variyar Engineering College, Vinayaka Missions University, Periya Seeragapadi, Salem-636 308, Tamil Nadu, India
Corresponding author: * **supriyo.basak@yahoo.in**

ABSTRACT

In the present work, an attempt has been made to produce fungal cellulase enzyme from *Myrothecium verrucaria* NCIM 903 by solid substrate fermentation. Potato dextrose agar was taken as a seed medium for maintenance of the culture. Substrate used for solid substrate fermentation was wheat bran. Total cellulase activity was analyzed by filter paper assay and the maximum cellulase activity of 0.23 U/g was found after 3 days of fermentation.

Keywords: fungus, filter paper, hydrolysis

INTRODUCTION

The irreversible trend in urbanization, with inputs of food and outputs of agricultural, municipal, industrial cellulosic waste product in an ever-accelerating cycle, directly affects waste disposal problems in cities and indirectly creates the same problems in peripheral rural areas. Many microorganisms have the capacity to degrade these cellulosic wastes. Filamentous fungi like *Myrothecium verrucaria* have been found to produce a wide spectrum of polysaccharide-hydrolytic enzymes. Cellulases (EC 3.2.1.4) fall into the group of hydrolase enzymes. Cellulose is a linear polymer of anhydrous residues in the chain configuration held together by β-1-4 linkages (Figini *et al.* 1997). Cellulases are important enzymes for conversion of lignocellulosic biomass to bioethanol (Varga *et al*. 2002).

To date, the production of cellulases has been widely studied in submerged fermentation, but the high cost of enzyme production has hindered the industrial application of cellulose bioconversion. Solid state fermentation is a potential technology for production of cellulases utilizing agro-industrial residues as solid substrate due to low capital investment, low energy requirement, eco-friendly operation, and higher yield compared to submerged fermentation and lower chance of contamination due to low moisture level (Pandey 2003; Bhargav *et al*. 2008).

M. verrucaria is a plant pathogen (Murakami *et al* 2004). It is common throughout the world, often found on materials such as paper, textiles, canvas and cotton. This fungus has been used as a biocontrol agent (Clarke *et al*. 2007) and in the production of bilirubin oxidase (Kataoka *et al* 2005), laccase, and cellulases (Saunders *et al* 1948). There is no report on the cellulase produced by this microorganism by solid state fermentation. In this work, cellulase produced by *M. verrucaria* has been evaluated in solid state fermentation.

MATERIALS AND METHODS

Microorganism

M. verrucaria NCIM 903 was obtained from the National Chemical Laboratory, Pune. Spores of *M. verrucaria* NCIM 903 were cultivated on 2% potato dextrose agar (PDA) and incubated at 30°C for 7 days. The isolates formed white colonies containing sporodochia with a flattened or convex spore mass and one-celled conidia on cylindrical, bundled phialides.

Solid substrate

Wheat bran was obtained from the local market of Salem, Tamil Nadu and used throughout for the study.

Cellulase production

M. verrucaria was initially grown on the surface of PDA slants at 30°C for 7 days. The fermentation process was carried out in 250-ml conical flasks. Each flask was filled with 5 g of solid substrate followed by the addition of 3 ml of water. Then the flasks were plugged with cotton and autoclaved at 121°C for 15 min at 15 psi. Under aseptic conditions fungal spores were transferred from culture slants to the solid substrate and mixed thoroughly. Then the flasks were incubated in incubator maintained at 30°C. The extracellular enzyme was extracted by soaking the fermented solid material with 50 ml of sterile water overnight at 4°C and filtering through muslin cloth. The filtrate was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was used to measure the amount of cellulase produced.

Optimization of the incubation period

The protocol adopted for optimizing the process parameters influencing cellulase yield was to optimize the incubation period. An experiment with different incubation periods was carried out in 250-ml conical flasks at 30°C. All experiments were carried out in triplicate and the mean values are reported.

Analysis method

Filter paper activity for total cellulase present in the culture supernatant was determined according to the method recommended by Ghose (Ghose 1987). Appropriately diluted culture supernatant was added to 4 ml sodium acetate buffer (pH 4) containing 100 mg of Whatman No. 1 filter paper (Sigma–Aldrich, St. Louis, MO) strips. After incubation for 1 h the reducing sugar released was estimated by the dinitro salicylic acid method (Miller 1959). 1 unit

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Research Note

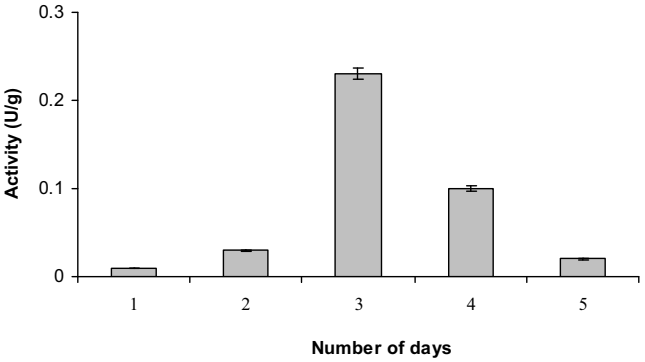

Fig. 1 Optimization of incubation period for production of cellulase from *Myrothecium verrucaria* NCIM 903. Values represent mean ± standard error (SE).

(U) of filter paper activity was defined as the amount of enzyme releasing 1 µmol of reducing sugar from the filter paper//min/g dry solid.

Statistical analysis

All experiments were carried out in triplicate and results were reported as means ± standard deviations of triplicate measurements using Microsoft Office 2003 Excel.

RESULTS AND DISCUSSION

The appropriate incubation period is one of the important parameters for cellulase production in solid state fermentation and is governed by the microorganism. The result shows optimal incubation period for production of cellulase (**Fig. 1**). The maximum cellulase activity after 3 days of fermentation was 0.23 U/g.

The growth of the microorganism as well as the level of the produced enzymes was dependent on many factors including: the time of incubation. The plant pathogen M. *verrucaria* NCIM 903 was able to utilize the cellulose present in wheat bran for the production of cellulase. Enzyme activity was increased when the incubation period increased to 3 days. Similarly, *Trichurus cylindricus* and *T. viride* produced cellulases when grown on wheat bran supplemented medium (Neudoerffer and Smith 1970) and *Trichoderma reesei* produced cellulose-free xylanase in xylan containing enriched medium (Kar *et al.* 2006). The decrease in cellulase activity after 3 days of incubation may be due to denaturation of the enzyme.

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