Vol.46, n. 3 : pp. 333-337, June 2003
ISSN 1516-8913 Printed in Brazil

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY
AN INTERNATIONAL JOURNAL

Cellulase Activity of a *Lentinula edodes* (Berk.) Pegl. Strain Grown in Media Containing Carboximetilcellulose or Microcrystalline Cellulose

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ABSTRACT

Endoglucanase and exocellobiohydrolase produced b Lentinula edodes (Berk.) Pegl. strain thatt was cultivated in carboxymetilcellulose (CMC) or microcrystalline cellulose (Avicel) liquid media. The concentration and type of cellulose influenced the enzyme activity and production. Extra-cellular cellobiase activity was not detected in CMC or Avicel media. This enzyme was detected in mycelial extracts only. With 1.7% Avicel liquid medium, the strain did not produce exocellobiohydrolase, but 74 μmol RBBR/mg protein/min was detected with 0.5% Avicel. The substitution of Avicel by 0.5% CMC reduced this activity. Endoglucanase also had maximum activity in 0.5% Avicel medium (approximately 820 UI/mg protein) after 96 h incubation. In supernatants from 0.5% CMC, the maximum activity attained was 200 UI/mg protein only.

Key words: *Lentinula edodes*, cellulase, liquid medium cultivation

INTRODUCTION

During the period between 1986 to 1991, the world production of Shiitake mushroom [*Lentinula edodes* (Berk.) Pegl.] increased 64.4% (Miles and Chang, 1997). The greatest Shiitake producers of Brazil are located in the Southeast region, mainly in São Paulo State. Although this region has large forest reserves, mainly of *Eucalyptus* species, the use of lignocellulosic residues can be a more economically-viable process for the large scale cultivation due to rapid bioconversion (Buswell et al., 1996). Towards this, various strategies have been developed in many parts of Asia to utilize part of the vast quantities of waste lignocellulose generated through the agricultural, forestry and food processing industries (Chang and Miles, 1991).

The utilisation of lignocellulosic substrates by mushrooms depends on the production of a pool of hydrolytic and oxidative enzymes able to convert lignocellulosic compounds, of high molecular weight, into low molecular molecules that can be assimilated and converted into biological energy (Buswell and Chang, 1993). The aim of this workwas to study the profile of the cellulolytic enzymes exocellobiohydrolase (EC. 3.2.1.91), endoglucanase (EC. 3.2.1.4) and cellobiase (EC. 3.2.1.21) produced in cellulolytic media by *L. edodes*.

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MATERIALS AND METHODS

Organism and culture conditions

Lentinula edodes (Berk.)Pegl., strain CC17 was obtained from the Laboratório de Cogumelos Comestíveis, Centro Nacional de Recursos Genéticos e Biotecnologia - CENARGEN-EMBRAPA, and maintained at 5°C on Malt-Extract-Agar (MEA) slants with periodic transfer. To determine cellulolytic enzyme production by *L. edodes*, the fungi was cultivated on MEA Petri dishes and five mm diameter culture edge plugs were cut off and transferred (in three sets) 250ml Erlenmeyer flasks containing 50ml defined medium modified from Soundar and Chandra (1998) (SC medium), containing (g/l): Avicel or CMC 5g, Peptone 1g, $Ca(NO_3)_2.4H_2O$ 5.5g, KH_2PO_4 1.3g, $MgSO_4.7H_2O$ 0.5g, plus 1ml nutrient solution. A culture was also carried out in a Jurasek and Paice (1988) medium (JP medium), containing Avicel (17g) and peptone (20g). The medium components were dissolved in deionized water and sterilized at 121°C for 30 min. The flasks were incubated on rotatory shaker (FANEN®) for 10 days at 150 rpm and 28.0 ± 1.0°C. Aliquots were collected each two days, centrifuged and supernatants used for enzyme assays. Chemicals were obtained from SIGMA/MERCK.

Enzyme assays

Exocellobiohydrolase, endoglucanase and cellobiase (EC. 3.2.1.21) activities were determined according to Wood and Bhat (1988) procedure. In Avicelase assays, 1ml supernatant was pipetted to 10ml test tubes and 1.5ml RBBR-Avicel suspension containing 100mg/3ml was added. The mixture was incubated for 30 min. at 50°C, after which the tubes were placed in a boiling water bath to stop reaction. The hot suspension was centrifuged and the supernatant was read in spectrophotometer at 595nm. The activity was expressed in μmol of RBBR, calculated from its molar extinction coefficient (5901.1 $mol^{-1}.cm^{-1}$). The endoglucanase activity was determined by incubating 0.5ml of supernatant conveniently diluted with citrate buffer (pH 4.8) in a 25ml test tube for 30 min at 50°C. After incubation, 3ml DNSA reagent was added and the solution was boiled for 5min. The absorbance was measured at 540nm and the glucose content was obtained by comparing to a glucose standard graph prepared by the same procedure. The enzyme activity was expressed in IU/mg protein. Cellobiase was measured by using p-Nitrophenil-β-D-glucoside method. The supernatant protein content was determined by Bradford (1976) methodology. One millilitre of p-Nitrophenyl-β-D-glucoside substrate was pipetted into a test tube along with 1.8ml of acetate buffer pH 4.8 at 50°C. Diluted enzyme (200μl) was added and the mixture incubated at 50°C for 30 min. Glycine buffer, pH 10.8 was added to stop reaction and the liberate p-nitrophenol was measured at 430nm against a standard curve of p-nitrophenol. The unit of activity was expressed in μmol p-nitrophenol/min.

RESULTS AND DISCUSSION

Lentinula edodes CC-17 showed endoglucanase activity in supernatants of all culture media examined (JP and SC media). However, exocellobiohydrolase showed only activity in SC medium. Celobiase activity was not detected in either JP or SC media. This enzyme was detected in mycelial extracts only.

Buswell et al. (1996) and Leatham (1985) *L. edodes* as reported as a weak hydrolytic enzyme producer when cultivated on microcrystaline cellulose media. Many reports pointed the lack of detection of cellulases of this fungi. Reducing sugar was not found in all supernatant samples collected. This behaviour suggest as a inherited characteristic of the strain.

The concentration and type of cellulose influenced the exocellobiohydrolase activity. At 1.7% Avicel (JP medium), no exocellobiohydrolase was detected. However, at 0.5% Avicel (Fig.1), two peaks were obtained at each 4 days of incubation, with the highest peak around 74 μmol RBBR/mg protein/min. When Avicel was replaced by CMC, the activity decreased to less than the half of activity reached with Avicel.

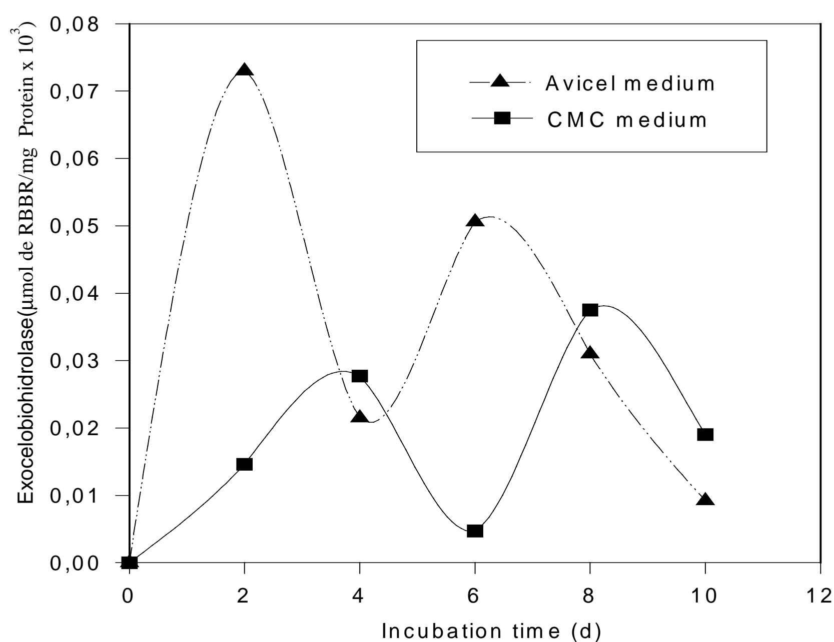

Figure 1 - Exocelobiohydrolase activity of *Lentinula edodes* (Berk.) Pegl., strain CC-17, on Avicel and CMC (SC medium).

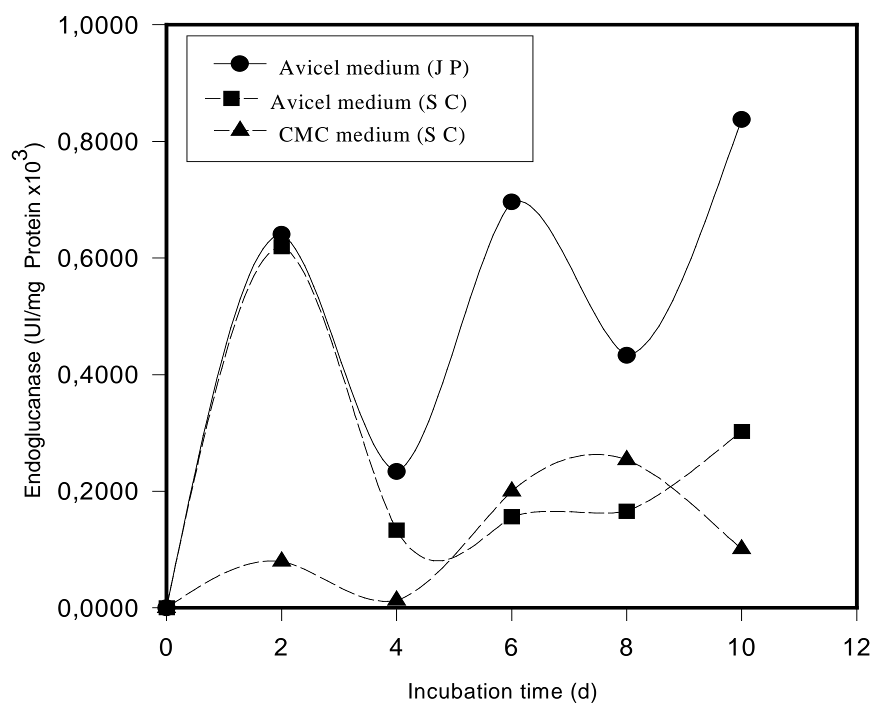

Figure 2 - Endoglucanase activity of *Lentinula edodes* (Berk.) Pegl., strain CC-17, on Avicel (JP media and SC media) and CMC (SC medium).

In both substrates (Avicel and CMC), the cultures showed two activity peaks followed by a activity decrease. This fact can be explained by the following: (a) substrate heterogeneity, such as the substrate portions more easily degraded are firstly hydrolysed; (b) end product inhibition and (c) thermal inativation and irreversible adsortion of part of enzyme by the substrate (Mandels and Reese, 1960).
Endoglucanase (EC 3.2.1.4) activity was distinct at different cellulose concentrations and composition (Fig.2). In supernatants obtained from 1.7% Avicel (JP medium) was 820 UI/mg protein. At 0.5% Avicel medium (SC medium), this enzyme showed a single peak (620 UI/mg protein) at 48 h incubation. In CMC medium, endoglucanase showed the lowest activity (maximum 200 UI/mg protein).
The absence of detectable glucose in supernatants of both culture media suggested a effect of substrate on the synthesis and cellulase excretion, without a end product repression, since the glucose inhibition level 0.5%, and restored when 90% of glucose was consumed (Mandels and Reese, 1960). Non cellobiase activity was detected in all supernatants collected.

ACKNOWLEDGEMENTS

This work was supported by a grant from Banco do Nordeste do Brasil - BNB/FUNDECI and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES. We thank to Departamento de Morfologia e Fisiologia - DMFA of UFRP for providing the laboratories were this research was done.

RESUMO

Neste trabalho foram estudadas as atividades de endoglucanase, exocelobiohidrolase e celobiase em uma linhagem de *Lentinula edodes* (Berk.) Pegl. cultivada em meio líquido contendo carboximetilcelulose (CMC) ou celulose microcristalina (Avicel). Foram detectadas as atividades de endoglucanase e exocelobiohidrolase no sobrenadante das culturas crescidas tanto em meios contendo CMC como nos meios contendo Avicel, sendo observada a influência da concentração e do tipo de celulose. Não foi detectada atividade de celobiase nos sobrenadantes, sendo a mesma detectada somente no extrato micelial. Com uma concentração de 1,7% de Avicel, a linhagem estudada não demonstrou atividade de exocelobiohidrolase. Porém, à concentração de 0,5% obteve-se uma atividade de 74 μmol de RBBR/mg de proteína/min. Com a substituição de Avicel por CMC a 0,5%, a atividade de exocelobiohidrolase foi reduzida a menos de 50%. A máxima atividade de endoglucanase em sobrenadantes obtidos em meio com Avicel a 0,5% foi em torno de 800 UI/mg de proteína, após 96 horas de cultivo. Em sobrenadantes obtidos de meio com CMC, a atividade desta enzima foi de apenas 200 UI/mg de proteína.

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Received: April 05, 2001;
Revised: October 30, 2001;
Accepted: October 31, 2002.