brazilian journal of microbiology xxx (2018) xxx–xxx

BRAZILIAN JOURNAL OF MICROBIOLOGY

http://www.bjmicrobiol.com.br/

Short communication

Multi-enzyme complex of white rot fungi in saccharification of lignocellulosic material

Wilton Soares Cardoso[a,*], Paula Viana Queiroz[b], Gabriella Peterlini Tavares[c], Fernando Almeida Santos[d], Filippe Elias de Freitas Soares[c], Maria Catarina Megumi Kasuya[e], José Humberto de Queiroz[c]

[a] Instituto Federal do Espírito Santo, Venda Nova do Imigrante, ES, Brazil
[b] Universidade Federal de Viçosa, Departamento de Química, Viçosa, MG, Brazil
[c] Universidade Federal de Viçosa, Departamento de Bioquímica, Viçosa, MG, Brazil
[d] Universidade Estadual do Rio Grande do Sul, Departamento de Bioenergia, Porto Alegre, RS, Brazil
[e] Universidade Federal de Viçosa, Departamento de Microbiologia, Viçosa, MG, Brazil

ARTICLE INFO

Article history:
Received 8 February 2017
Accepted 18 May 2018
Available online xxx
Associate Editor: Solange I. Mussatto

Keywords:
Enzymes
Cellulases
Fungus
Hydrolysis

ABSTRACT

The multi-enzyme complex (crude extract) of white rot fungi *Pleurotus ostreatus*, *Pleurotus eryngii*, *Trametes versicolor*, *Pycnosporus sanguineus* and *Phanerochaete chrysosporium* were characterized, evaluated in the hydrolysis of pretreated pulps of sorghum straw and compared efficiency with commercial enzyme. Most fungi complexes had better hydrolysis rates compared with purified commercial enzyme.

© 2018 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Enzymes extracts from different fungi have been a strategy used in saccharification of lignocellulosic biomass, blending (2 or more extracts of different fungi) or only extract. The crude enzyme extracts offers low cost, no activities are lost in concentration/purification processes, a wide spectrum of enzyme activities is maintained and synergy among enzymes.[1–6]

This study produced, by solid-state fermentation (SSF) of forage sorghum straw, the crude extracts (called multi-enzyme complex) of 5 different fungi. The complexes obtained of each of the fungi were characterized to the apparent activities of cellulases and were evaluated for enzymatic saccharification of own *in natura* sorghum straw (not pretreated) and

* *Corresponding author.*
E-mail: wilton.cardoso@ifes.edu.br (W.S. Cardoso).
https://doi.org/10.1016/j.bjm.2018.05.006
1517-8382/© 2018 Sociedade Brasileira de Microbiologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

sorghum pretreated straw pulps, and the results compared to the hydrolysis carried out by a commercial cellulase complex.

Were evaluated the potential of crude enzymatic extracts of white rot fungi: *Pleurotus ostreatus* PLO06, *Pleurotus eryngii* PLE04, *Trametes versicolor* TRAM01, *Pycnosporus sanguineus* PYC02 and *Phanerochaete chrysosporium* PC and, obtained by SSF on straw forage sorghum. Fungi are from the collection of the Department of Microbiology, Federal University of Viçosa, Viçosa, Minas Gerais – Brazil.

To SSF were used straw forage sorghum BRS 655 (stem and leaves without the panicle with the grain) cultivar developed by EMBRAPA Maize and Sorghum and cultivated in the city of Sete Lagoas. Sorghum was cut with 120 days of planting and sun dried, ground and stored in a dry place away from light and moisture.

The sorghum straw was moistened for final humidity of 70%. 100 g of the prepared substrate were placed in polypropylene filter bags and sealed with adhesive tape for subsequent autoclaving at 121 °C for 60 min. After cooled to room temperature, each bag with substrate received two discs of mycelia, with a diameter of 2 inches, of each fungus previously cultured in Petri dish of BDA for 7 days at 30 °C. The bags were inoculated in a laminar flow cabinet and incubated in a BOD at 28 °C until complete colonization of the substrate. Monitoring of the mycelial growth was carried out visually. After the total substrate colonization by fungi, 20 days after inoculation were obtained the crude extracts (or multi-enzyme complex).

To obtain the enzyme extract, 5 g samples of substrate were placed in 250 mL Erlenmeyer flasks containing 50 mL of sodium citrate buffer (50 mM pH 4.8) and shaken at 150 rpm for 2 h at 5 °C. Then filtered through a sieve and placed in 2 mL Eppendorf tubes following centrifugation at 12,000 × *g* at 5 °C. The supernatant was transferred to another Eppendorf tube, and subsequently identified with the fungus and incubation time, sealed and stored at −18 °C.

The reagents used in this study were purchased from Sigma Chemicals companies or Vetec chemistry with analytical grade.

Total cellulase activity or Filter paper activity (FPase) was determined essentially according to the IUPAC[7] instructions, and the liberated reducing sugars were estimated by the DNS method.[8] FPase activity corresponds to 1 μM of reducing sugars as glucose equivalents liberated per min under the assay conditions.

Endoglucanase activity (carboxymethylcellulase, EC 3.2.1.4) or carboxymethycellulase (CMCase) activity was estimated by adding 250 μL of the enzyme complex in 1 mL of 1% solution of carboxymethylcellulose in 0.05 M citrate buffer, pH 4.8 and incubated at 50 °C for 30 min. One CMCase unit is the amount of enzyme necessary to produce 1 μM reducing sugar as glucose equivalents per min under the standard assay conditions.

Exoglucanase activity (EC 3.2.1.91) or AVICELase consisted of adding 250 μL of crude enzyme complex in 1 mL of 1% solution of microcrystalline cellulose (Avicel) in 0.05 M citrate buffer, pH 4.8 and incubated at 50 °C for 30 min. Periodically, the enzyme-substrate system was stirred in order to maintain the pulp in suspension. One AVICELase unit is the amount of enzyme necessary to produce 1 μM reducing sugar as glucose equivalents per min under the standard assay conditions.

Xylanase activity (endo-1,4-β-xylanase, EC 3.2.1.8) was determined in the mixture of 1 mL of the enzyme complex, 1 mL of xylan solution (1% xylan birchhood – SIGMA) in citrate buffer 0, 05 M, pH 4.8 and incubated at 50 °C for 30 min. Periodically, the enzyme-substrate system was stirred in order to maintain xylan suspension. One Xylanase unit is the amount of enzyme necessary to produce 1 μM reducing sugar as glucose equivalents per min under the standard assay conditions. The liberated reducing sugars were estimated by the DNS method.[7]

The β-glucosidase (EC 3.2.1.21) activity was determined by incubating 1 mL of p-nitrophenyl-β-D-glucopyranoside (PNPG) substrate 0.005 M, 0.05 M citrate buffer, pH 4.8 with 100 μL of the enzyme complex (crude extract), for 15 min at 50 °C. The reaction was stopped by adding 2.0 mL of 1.0 M sodium bicarbonate and absorbance was measured at 410 nm. The unit of β-glucosidase activity was defined as the amount of enzyme capable of releasing 1 μmol of p-nitrophenol per minute under the test conditions.

Laccase activity was determined by the oxidation of 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; SIGMA, St. Louis, USA) at 37 °C according to Buswell et al.[9] The reaction mixture (1 mL) contained 600 μL enzyme extract, 300 μL sodium acetate buffer pH 5.0 (0.1 M) and 100 μL ABTS solution (1 mM). Oxidation was followed via the increase in absorbance at 420 nm (γ 420 = 36.000 M^{-1} cm^{-1}). One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 μmol of ABTS per minute.

Quantification of protein used the method of Bradford.[10]

The apparent activity of enzymes FPase (Total Cellulase) AVICELase, CMAase and β-glucosidase were characterized as to pH and temperature, as well as the thermostability of maximal enzyme activity in the temperature through tests to determine activity of those enzyme with varying pH of the buffer or the reaction temperature.

The pH ranged from 3 to 8. The buffer systems used were 50 mM citrate buffer (pH 3.0–6.0) and 50 mM phosphate buffer (pH 6.0–8.0). The incubation temperature for the measurement of enzyme activity ranged from 30 to 80 °C. To estimate the thermostability, the enzymatic complex (samples) was stored in the apparent optimum temperature (results of temperature characterization) of each enzyme in the extract crude, and then made to measure the residual activity of the enzyme 12 in 12 h for 48 h.

The saccharification experiments were conducted in Erlemeyers 125 mL in shaker (Tecnal – TE-421) stirred at 120 rpm at 50 °C. 0.5 mL samples were collected every 6 h to 24 h, and after 12 12-h to 72 h, and each sample was heated at 100 °C for 5 min to inactivate the enzymes, centrifuged and subsequently was determined the concentration of reducing sugars and glucose. Saccharification was performed *in natura* sorghum straw (not pre-treated) and pulps of the forage sorghum obtained pretreatments as Cardoso et al.[11] For comparison with the commercial enzyme (enzymatic complex Genencor Multifect GC) were conducted saccharification of the pulp obtained pretreatment acid/delignified of the forage sorghum as Cardoso et al.[11]

LAP 008 protocol used as enzyme saccharification method by NREL,[12] suggests an enzyme load in the order of 25 FPU g^{-1} (25 FPase per gram of cellulose). However in this work, because

Please cite this article in press as: Cardoso WS, et al. Multi-enzyme complex of white rot fungi in saccharification of lignocellulosic material. *Braz J Microbiol.* (2018), https://doi.org/10.1016/j.bjm.2018.05.006

Download English Version:

https://daneshyari.com/en/article/11013175

Download Persian Version:

https://daneshyari.com/article/11013175

Daneshyari.com