AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Characterization of Cellobiohydrolase I (CBHI) from recombinant *Aspergillus niger*

Norashikin Othman and Jamaliah Md. Jahim

Department of Chemical and Process Engineering, Faculty of Engineering & Built Environment, National University of Malaysia, Malaysia.

ARTICLE INFO

Article history:
Received 15 April 2014
Received in revised form 22 May 2014
Accepted 25 October 2014
Available online 10 November 2014

Keywords:
Cellobiohydrolase (CBH), Endoglucanase (EG), β-Glucosidase, Enzyme Assay, Aspergillus niger

ABSTRACT

This study investigated the development of assay method for the characterization of the inducible cellobiohydrolase enzyme (CBHI) by recombinant *A. niger*. Analysis on substrate specificity show highest enzyme activity was towards pNPC at 33.2 U/ml. Enzyme activities between CBHI towards CMC and pNPG substrate recorded the value of 9.25U/ml and 3.92 U/ml respectively. Analysis for overall celulase enzyme activity using filter paper as substrate recorded the value at 0.34FPU, showing the capability of CBHI to hydrolyze cellulose even with low concentration of endoglucanase and β-glucosidase. The optimum working conditions for CBHI were at 65°C using acetate buffer at pH 3. CBHI shows high stability and able to retain more than 98% of it activity from 20°C to 65°C. CBHI was also stable in low pH and able to retain its enzyme stability from pH 3 to pH 5.

To Cite This Article: Norashikin Othman and Jamaliah Md. Jahim., Characterization of Cellobiohydrolase I (CBHI) from recombinant *Aspergillus niger*. ***Aust. J. Basic & Appl. Sci.,*** *8(19): 227-229, 2014*

INTRODUCTION

Cellulase is a complex enzyme mixture comprised of three different enzymes with different roles. The hydrolysis of cellulose by cellulase enzyme was initiated by cellobiohydrolase (CBH) acting on the reducing and non-reducing end of cellulose, to produce cellobiose. On the other hand, Endoglucanase (EG) helps to speed up reaction by breaking up the internal cellulose chain and amorphous region, to produce new chain in CBH reaction. The hydrolysis of cellulose was completed by β-glucosidase that reacts on cellobiose, producing simple glucose sugar (Haan, R. Den, 2013).

Cellulase enzyme play an important role in production of fermentable sugar originated from lignocellulosic material. Pretreatment methods are required to completely break down all the cellulose, lignin and hemicellulose chain in the lignocellulosic structure. Enzyme hydrolysis by cellulase is one of the method widely useas pretreatment. However, due to the complex mechanisms of cellulase, complete studies on the kinetic and cellulase reactions still has not been achieved (Dashtban, M., 2010). The problems arise due to the insolubility of cellulose material, variety of end product from cellulase reactions and inhibition of enzyme by cellobiose (Ahamed, A., P. Vermette, 2008). New insight in functions of CBH shows that although slow, complete solubilization of cellulose can be achieved even without the presence of EG (Teeri, T.T., 1996). Most engineered CBHs also show high thermostability and stable in low pH (Heinzelman, P., 2010).

Experimental Procedure:

Static Surface Liquid Culture (SSFC) technique was used in the fermentation (Noh, A., 2012). The medium used for the fermentation was Minimal Medium J (MMJ) containing mixture of glucose and maltose at the ratio of 1:1. 10% (v/v) spore suspension *A. niger* was cultivated with 200ml MMJ (150g/l) in a glass petri dish with a diameter of 150mm. The petri dish was incubated inside a fermentation box and placed in an incubation room at 30°C for 10 days. The enzyme was filtered using filter paper to separate the mycelium and medium. The filtered medium was then partially purified by protein precipitation method using ammonium sulphate at 60% saturation (Wenk, M., 2007). The CBHI produced was tested against different substrate, filter paper (FPU), p-nitrophenol-cellobioside (pNPC), carboxymethyl cellulose (CMC) and p-nitrophenol glucopyranoside (pNPG) to determine its substrate specificity and to identify the presence of other enzyme. Optimum temperature was measured by incubating the enzyme with substrates from temperature 20°C to 90°C for 30 minutes. Optimum buffer and pH for the purified CBH was determined by incubating the enzyme diluted in 0.1M acetate buffer, citrate buffer and phosphate buffer ranges from pH 3 to pH 8 respectively. Thermal stability was investigated by incubating the enzyme in water bath without substrates for 15 minutes at different range of temperature from 20°C to 90°C. For pH stability, the enzyme was diluted in 0.1M acetate buffer at various pH ranges from pH 3 to pH 8. Inactive

Corresponding Author: Norashikin Othman, Department of Chemical and Process EngineeringFaculty of Engineering & Built Environment, National University of Malaysia, Malaysia,
Ph: +60173529279, E-mail: norashikin.nbo@gmail.com

Australian Journal of Basic and Applied Sciences, 8(19) Special 2014, Pages: 227-229

enzyme was used as control enzyme by incubating the enzyme at 100°C for 10 minutes. All assays were triplicate and the data represents as mean value of the triplicate experiments.

RESULTS AND DISCUSSION

Effect of CBHI with different Substrates:

Each enzyme in cellulase group have different roles in order to hydrolyze the complex structure of cellulose. Different substrates are required to identified another protein apart of CBHI. CMC is used as specific substrate to characterize EG activity while pNPC and pNPG reacts with CBHI and β-glucosidase Analysis using FPU was used to determine the capability of CBHI to complete the hydrolysis of cellulose without the presence of EG and β-glucosidase. Table 1 shows the activities of CBH1 towards different cellulosic substrates when incubated at pH 3.0 and 65°C for 30 minutes.

Table 1: Enzyme Activities of CBHI with Different Substrates.

Substrate	Activities (U/ml)
pNPC	33.2
CMC	9.25
pNPG	3.92
Filter Paper (FPU)	0.34

The data shows that the highest activity on CBHI was when using PNPC at 33.2 U/ml and CMC gave lowest activity towards at 9.25 U/ml. The use of PNPG when react with β-glucosidase, was at 3.92 U/ml. CBHs can be divided into two types, CBHI that reacts with the reducing end and CBHII that reacts with non-reducing end of cellobiose (Kleman, K.M., 1996). P-nitrophenol (pNP), the products formed from the reaction of p-NPC with CBHI connects with the reducing ends of cellobiose thus making pNPC a suitable substrate for CBHI. Low enzyme activity between CBHs and CMC can be supported by Kleman (1996) as CMC was specifically engineered to react with EG only, creatingamorphous site for CBH reaction. A low activity for CMC shows that there still EG present in the enzyme solution. PNPG was also the key component used in many studies regarding β-glucosidase. The reaction data has resulted in high production of CBHI and low productivity of EG and β-glucosidase in the fermentation medium.

Analysis using FPU is the main method used to determine total cellulase activity. The value for CBHI enzyme activity when analyzed using FPU method shows a result at 0.34 FPU. Analysis by single cellulase enzyme using only CBHs enzyme form a noncomplex cellulse system (Kanokratana, P., 2008). It required a reaction between exo to ezo type of enzymes , or CBHI and CBHII that react on the reducing and non-reducing end of microcrystalline cellulose (Teeri, T.T., 1996). Complete solubilization of cellulose was possible using only CBHI. This reaction occur at slower rate (Kim, Y.H., 2010).

Characterization of CBHI:

The analysis to quantify CBHI was conducted to study the effect of thermal and pH stability for the best working conditions for CBHI. Figure 1 shows the result for analysis on incubation temperature, pH, thermal and pH stability on relative enzyme activity.

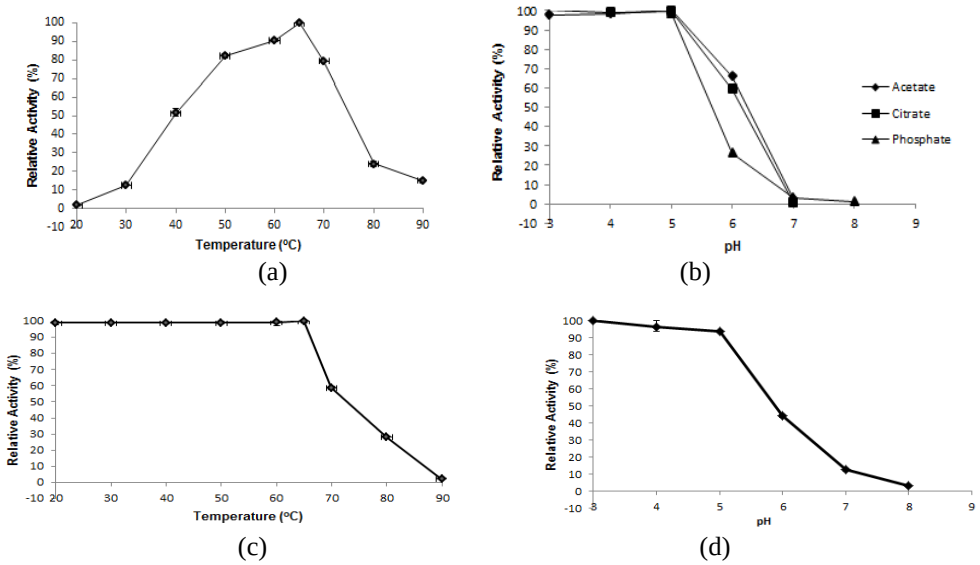

Fig. 1: Effect of (a) incubation temperature and; (b) pH on relative enzyme activity of CBHI; (c) Thermostability of enzyme CBHI at different temperature and; (d) pH stability on enzyme CBHI at different pH.

Australian Journal of Basic and Applied Sciences, 8(19) Special 2014, Pages: 227-229

The optimum incubation temperature for CBHI was found at 65^{o}C. Theactivity of 80% was retained at temperature over 50^{o}C but starting to decline when temperature reaching 70^{o}C. CBHI was thermal stable enzyme and can retain more than 98% of its maximum activity when incubated at wide range of temperature from 20^{o}C to 65^{o}C. Temperature more than 70^{o}C result in the loss of active site in protein structure caused by protein denaturation. More than 96% of maximum activities were retained at lower pH for all buffers, with highest value by acetate buffer at pH 3 with 99% activity. From the analysis of pH stability, the graph show that the enzyme retain it stability from pH 3.0 to pH 5.0 before decreasing to less than 50% activity at pH 6.0. Most CBH reported had the highest enzyme activity at acidic pH range of 3.0 to 6.0.

Conclusion:

The enzymatic activity of CBHI against different substrates and at various pH and temperature were observed for development of the enzyme assay method. PNPC, which reacts with CBHI enzymes, gave highest enzyme activity,in specific substrate analysis, but CBHI can be used for complete hydrolysis of lignocellulosic material to simpler sugar. The best working condition of CBHI was at 65^{o}C in acetate buffer pH3. The stability of enzyme in extreme temperature and acidic environment can help to increase the pretreatment of lignocellulosic material prior to enzymatic hydrolysis of the materials.

ACKNOWLEDGEMENT

The author would like to thank Universiti Kebangsaan Malaysia for financial support (research grant ERGS/1/2011/TK/UKM/02/23).

REFERENCES

Ahamed, A., P. Vermette, 2008. Enhanced enzyme production from mixed cultures of Trichoderma reesei RUT-C30 and Aspergillus niger LMA grown as fed batch in a stirred tank bioreactor. Biochemical Engineering Journal, 42(1): 41-46.

Dashtban, M., M. Maki, K.T. Leung, C. Mao, W. Qin, 2010. Cellulase activities in biomass conversion: measurement methods and comparison. Critical reviews in biotechnology, 30(4): 302-9.

Haan, R. Den, H. Kroukamp, Zyl, J.D. Van, Zyl, W.H. Van, 2013. Cellobiohydrolase secretion by yeast : Current state and prospects for improvement. Process Biochemistry, 48(1): 1-12.

Heinzelman, P., R. Komor, A. Kanaan, P. Romero, X. Yu, S. Mohler, C. Snow, *et al*., 2010. Efficient screening of fungal cellobiohydrolase class I enzymes for thermostabilizing sequence blocks by SCHEMA structure-guided recombination. Protein engineering, design & selection : PEDS, 23(11): 871-80.

Kanokratana, P., D. Chantasingh, V. Champreda, S. Tanapongpipat, K. Pootanakit, L. Eurwilaichitr, 2008. Identification and expression of cellobiohydrolase (CBHI) gene from an endophytic fungus, Fusicoccum sp. (BCC4124) in Pichia pastoris. Protein Expression and Purification, 58(1): 148-153.

Kim, Y.H., M. Jeya, J. Lee, Y. Kim, 2010. Purification and Characterization of a Thermostable Cellobiohydrolase from Fomitopsis pinicola. Journal of Micbiology and Biotechnology, 20: 1681-1688.

Kleman, K.M., M. Siika Aho, T.T. Teeri, T.K. Kirk, 1996. The Cellulases Endoglucanase I and Cellobiohydrolase II of Trichoderma reesei Act Synergistically To Solubilize Native Cotton Cellulose but Not To Decrease Its Molecular Size. Applied and environmental microbiology, 62(8): 2883-7.

Lynd, L.R., P.J. Weimer, Zyl, W.H. Van, I.S. Pretorius, 2002. Microbial Cellulose Utilization : Fundamentals and Biotechnology, 66(3): 506-577.

Noh, A., J. Jahim, A. Munir, A. Murad, F. Diba, A. Bakar, 2012. Effect of Various Cultivation Methods on Cellobiohydrolase Production from Aspergillus niger. International Journal on Advanced Science Engineering and Information Technology, 2(4).

Teeri, T.T., 1996. Crystalline cellulose degradation : new insight into the function of cellobiohydrolases G3 7799: 141-148.

Wenk, M., 2007. Protein Purification. A manual for Biochemistry Protocols, 1-11.