Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

ISOLATION OF CELLULOLYTIC BACTERIA FROM TERMITES WITH CELLULOSE OF CORN COBS AS A CARBON SOURCE

NYI MEKAR SAPTARINI, WIWIEK INDRIYATI

Faculty of Pharmacy, Padjadjaran University Jl Raya Bandung Sumedang Km 21 Jatinangor West Java Indonesia 45363

Email: nyi.mekar@unpad.ac.id

Received: 03 Feb 2014 Revised and Accepted: 19 Feb 2014

ABSTRACT

Objective: The aims of this study is to isolate the cellulolytic bacterial from termites, then produce crude cellulase and determined its cellulolytic activity.

Methods: The steps of this study consisted of cellulose isolation, cellulolytic bacteria isolation, production of crude cellulase, and determination of cellulolytic activity.

Results: Cellulose content of corn cobs were ranged from 23.13 to 27.90%. Bacterial isolates found to be positive on screening media producing clear zone. After incubation of bacterial isolates in broth medium, the media turned into yellowish clear solution. Endoglucanase and total cellulase activity of isolated bacteria was 0.021 U and 0.022 U, respectively.

Conclusion: Isolated cellulose from corn cobs can used as sole carbon source in isolation of celulolytic bacterial from termites.

Keywords: Corn cobs, Termites, Endoglucanase activity, Total cellulase activity

INTRODUCTION

Cellulolytic is a biological process which controlled and processed with cellulase system. Cellulase system consists of three classes of soluble extracellular enzymes, i.e 1,4-β-endoglucanases, 1,4-β-exoglucanases, and β-glucosidases (β-D-glucoside glucohydrolases or cellobiases) [1]. These enzymes hydrolyze cellulose to glucose [2]. One of the best source for cellulolytic system is symbiotic microorganism in intestinal tract of organism with cellulose as source of metabolizable sugar (glucose). Termites had symbiotic microorganism in their intestinal tract which digest the cellulolytic food [3,4]. Cellulase used in various industrial processes, including biofuels such as bioethanol [5], triphase biomethanation [6], plants and agriculture waste processing [7,8], chiral separation and ligand binding studies [9]. In 2008, Indonesian corn production reached 15.86 million tons of dry corn kernels, which corn cobs estimated at 6.8 million tons [10]. It is necessary to use corn cobs to increase the added value and prevent the environmental pollution. In this study, corn cobs used as cellulose sources to cellulase production of isolated bacteria from termites.

MATERIALS AND METHODS

Corn cobs and termites were collected from Jatinangor West Java Indonesia. All chemical reagent are analytical grade (Merck).

Cellulose Content Analysis and Cellulose Isolation from Corn Cobs

Corn cobs were washed, chopped, dried, milled, and sieved to 20 mesh then 40 mesh. Cellulose content analysis was conducted using Chesson method as described by Mudyantini [11]. Cellulose isolation was conducted by modifying method as described by Norashikin and Ibrahim [12].

Screening and Isolation of Cellulolytic Bacteria

Termites were collected from dead trees in Jatinangor area. Termites were crushed in 0.9% saline solution under sterile condition. The 0.1 mL extract was inoculated in basal salt media ($NaNO_3$ 2.5 g; KH_2PO_4 2 g, $MgSO_4$ 0.2 g, NaCl 0.2 g, $CaCl_2.6H_2O$ 0.1 g, and distilled water 1 L) containing 2.5 g of isolated cellulose from corn cobs. These cultures were incubated for 7 days in a shaker incubator at 37 °C and 100 rpm. Bacterial colony was isolated on an agar media composed of KH_2PO_4 0.5 g; $MgSO_4$ 0.25 g, 2 g of isolated cellulose from corn cobs, agar 15 g, gelatin 2 g, and distilled water 1 L, pH adjusted to 6.8-7.2 [13].

Confirmation of the cellulolytic ability of bacterial isolates was performed by streaking on the cellulose Congo-Red agar media composed of KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, 2 g of isolated cellulose from corn cobs, agar 15 g, Congo-Red 0.2 g, gelatin 2 g, and distilled water 1 L, pH adjusted to 6.8-7.2. Colonies which discoloration of Congo-Red were said as positive cellulolytic bacteria [8]. Cellulolytic potential of positive isolates were qualitatively estimated by calculating hydrolysis capacity (HC), i.e. the ratio of diameter of clearing zone and colony [14].

Cellulase Production

The selected isolat were cultured at 37 °C and 150 rpm in production media composed of KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, gelatin 2 g, 2.5 g of isolated cellulose from corn cobs, and distilled water 1 L, pH adjusted to 6.8-7.2. Broth culture were incubated for 3 days, then centrifuged at 5000 rpm and 4 °C for 15 min. Supernatant was collected and stored as crude enzyme preparation at 4 °C. Pellet was subjected to gravimetric analysis to determine the residual cellulose [15].

Cellulase Assay

Endoglucanase activity was determined by incubating 0.5 mL supernatant with 0.5 mL of 2% cellulose in 0.05 M sodium citrate buffer (pH 4.8) at 50 °C for 30 min. Total cellulase activity was determined by incubating 0.5 mL supernatant with 1 mL of 0.05 M sodium citrate buffer (pH 4.8) containing 50 mg of cellulose. After incubation for 1 hour at 50 °C, the reaction was terminated by adding 3 mL 3,5-dinitrosalicylic acid (DNS) reagent to 1 mL of reaction mixture. Reducing sugar were estimated spectrophotometrically using glucose as standards [16]. The enzymatic activity were defined in international units (IU). One unit of enzymatic activity defined as the amount of enzyme that releases 1 µmol reducing sugar (measured as glucose) per mL per minute.

RESULTS AND DISCUSSION

Cellulose Content Analysis and Cellulose Isolation From Corn Cobs

The sieving process used to increase the surface area contact and improve the performance, because of uniformity of particle size. Table 1 showed that the smaller particle size of simplicia had smaller cellulose content. This is because cellulose is polymer, so reducing of particle size can break the glycosidic bond between monomers thus damage the cellulose structure.

The yield of cellulase isolation from corn cobs was 1.25 ± 0.48 g. The steps of cellulose isolation consisted of delignification, hemicellulose hydrolysis, and bleaching. The yield of isolated cellulose (12.5%) smaller than cellulose content (23.13 to 27.90%). This is caused by cellulose lost during the process. So, we need shorter isolation steps, but isolated cellulose meets the requirements.

Screening and Isolation of Cellulolytic Bacteria

Isolation of bacteria carried on termites, because these insects can digest wood components. Bacterial isolates found to be positive on screening media (isolated cellulose Congo-Red agar) producing clear zone beneath and around the colonies (Fig. 1). The colonies which formed clear zone was chosen to determine the hydrolysis capacity (HC) from bacterial isolate.

Table 1: Cellulose Content Analysis

Mesh	Cellulose content (%)
40	23.13 + 0.43
20	27.90 + 0.38

Table 2: Hydrolytic Capacity Measurement of Bacterial Isolates

Colony	Colony diameter (mm)	Clear zone diameter (mm)	Hydrolytic capacity
1	5.1	26.7	5.24
2	4.7	23.6	5.02
3	4.9	25.3	5.16
Average		25.2 + 1.56	5.14 + 0.09

The hydrolytic capacity value (Table 2) obtained is similar to range reported by Gupta et al (2011), i.e. ranged from 4.32 to 5.49. This is indicates that the bacterial isolate had ability to degradate the cellulose, so termites can digest cellulose to produce glucose for carbon sources.

Fig. 1: Clearing zone on isolated cellulose Congo-Red agar for bacterial isolate after 48 hours of incubation

Congo red is used as an indicator of the presence of cellulolytic enzymes which secreted into the media. This is because congo red showed strong interaction with polysaccharides containing β-(1→4)-D-glucopiranocyl bond and β-(1→3)-D-glucan bond, and perhaps with some galactoglucomannan hemicellulose [17]. The advantage of this method is able to calculate and characterize cellulolytic microorganisms based on the color intensity of congo red-glucan complex.

Cellulase Production

Isolated cellulose from corn cobs is used as the sole carbon source for cellulolytic bacteria which cultured in broth media. Cellulase which produced by bacterial isolates are extracellular enzyme [1]. This has been confirmed from the results of isolation of cellulose-degrading bacteria that produce clear zone beneath and around the colonies.

After 72 hours of incubation of bacterial isolates in broth medium, the media turned into yellowish clear solution. Centrifugation conducted to separate the crude enzyme in the supernatant from the sediment. The sediments containing residual cellulose which not hydrolyzed. Gravimetric analysis showed that the residual cellulose was 0.0053 mg (0.011%).

Cellulase Assay

Cellulase system consist of 1,4-β-endoglucanase, 1,4-β-exoglucanase, and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase) [1]. The synergy of third enzymes do complete hydrolysis of cellulose to glucose [2]. In this study, glucose used as standard and DNS reagent used to stop the enzymatic reaction, so the reaction product can be measured. The reaction between glucose and the DNS reagent gave maximum absorption at 450 nm.

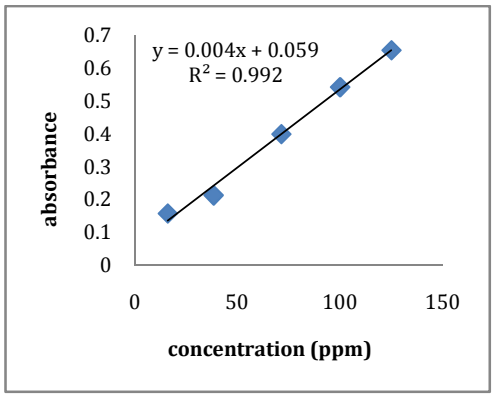

Fig. 2: Glucose calibration curve

Incubation of cellulase and isolated cellulose from corn cobs gave absorbance of 0.505 for endoglucanase activity and 0.547 for total cellulase activity. Hence, we found that the glucose concentration were 111.5 ppm and 122.0 ppm. So, bacterial isolates had endoglucanase and total cellulase activity at 0,021 U and 0,022 U, respectively.

CONCLUSION

Cellulose content of corn cobs were ranged from 23.13 to 27.90%. Isolated cellulose from corn cobs can used as sole carbon source in

isolation of celulolytic bacterial from termites. Bacterial isolates which isolated from termite had endoglucanase and cellulase total activity, i.e. 0.021 U and 0.022 U, respectively.

ACKNOWLEDGEMENTS

We thank Ghina Rahimah Az-Zahra and Prita Dwi Anggraeni for their technical support.

REFERENCES

1. Shewale, J.G. Glucosidase: its role in cellulase synthesis and hydrolysis of cellulose, International Journal of Biochemistry, 1982, 14 (6): 435-443.
2. Ryu, D.D.Y. & Mandels, M. Cellulases: biosynthesis and applications, Enzyme and Microbial Technology, 1980, 2 (2): 91-102.
3. Saxena, S., Bahadur, J. & Varma, A. Cellulose and hemicellulose degrading bacteria from termite gut and mould soils of India, The Indian Journal of Microbiology, 1993, 33: 55-60.
4. Dillon, R.J. & Dillon, V.M. The gut bacteria of insects nonpathogenic interaction, Annual Review of Entomology, 2004, 49: 71-92.
5. Vaithanomsat, P., Chuichulcherm, S. & Apiwatanapiwat, W. Bioethanol production from enzymatically saccharified sunflower stalks using steam explosion as pretreatment, Proceedings of World Academy of Science, Engineering and Technology, 2009, 37: 140-143.
6. Chakraborty, N., Sarkar, G.M. & Lahiri, S.C. Cellulose degrading capabilities of cellulolytic bacteria isolated from the intestinal fluids of the silver cricket, Environmentalist, 2000, 20 (1): 9-11.
7. Mswaka, A.Y. & Magan, N. Wood degradation, and cellulase and ligninase production, by *Trametes* and other wood-inhabiting basidiomycetes from indigenous forests of Zimbabwe, Mycological Study, 1998, 102 (11): 1399–1404.
8. Lu, W.J., Wang, H.T., & Nie, Y.F. Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process, Journal of Environmental Science and Health, 2004, Part B, 39 (5-6): 871–887.
9. Nutt, A., Sild, V. Prtterson, G. & Johansson, G. Progress curve as a means for functional classification of cellulases, Europian Journal of Biochemistry, 1998, 258: 200.
10. http://pse.litbang.deptan.go.id
11. Mudyantini, W. Pertumbuhan, Kandungan Selulosa, dan Lignin pada Rami (*Boehmeria nivea* L. Gaudich) dengan Pemberian Asam Giberelat (GA3). Biodiversitas, 2008, 9 (4): 269-274.
12. Norashikin, M. Z. and M. Z. Ibrahim. Fabrication and Characterization of Sawdust Composite Biodegradable Film. World Academy of Science, Engineering and Technology, 2010, 65: 864-867.
13. Gupta, P., Samant, K., and Sahu, A. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. Intl. J of Microbiology, 2011, 2012: 1-5.
14. Hendricks, C.W., Doyle, J.D. & Hugley, B. A new solid medium for enumerating cellulose-utilizing bacteria in soil, Applied and Environmental Microbiology, 1995, 61 (5): 2016–2019.
15. Tailliez, P., Girard, H., Millet, J. & Beguin, P. Enhanced cellulose fermentation by an asprogenous and ethanol tolerant mutant of *Clostridium thermocellum*, Applied Environmental Microbiology, 1989, 55: 207-211.
16. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analytical Chemistry, 1959, 31 (3): 426–428,.
17. Wood, P. J. Specificity in the interaction of direct dyes with polysaccharides. Carbohydr. Res. 1980, 85:271-287.