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Research Article
Enzyme Activity of Cellulolytic Bacteria from Biological Education and Research Forest Floor Andalas University

Fuji Astuti Febria, Chairul, Anthoni Agustien, Riri Kurnia Ilahi and Elsa Rohmah

Department of Biology, Faculty of Mathematics and Natural Science Andalas University, Padang, West Sumatra, Indonesia

Abstract

Background and Objective: The composition of the waste consists mostly of plant biomass. Cellulose is the largest component of plant biomass and cellulolytic bacteria are needed to degrade it. This study aimed to determine enzyme activity possessed by bacterial isolates from Biological Education and Research Forest floor Andalas University. **Materials and Methods:** The isolation stage was carried out with NA (Nutrient agar) medium, Screening with CMC (Carboxymethyl Cellulose) medium with congo red dye and enzyme activity testing was carried out using the Nelson-Somogyi method. **Results:** We found 16 bacterial isolates obtained from Biological Education and Research Forest Floor Andalas University, 10 of them were positive for cellulolytic bacteria with the highest cellulolytic index value of 2.59 on FFB 2 isolates. **Conclusion:** The bacterial isolate with the best enzyme activity value was FFB 2 isolate 0.166 U mL^{-1} for 72 hrs.

Key words: Agricultural waste, cellulases, cellulose, clear zone, CMC, degradation, endoglucanase, lignocellulose

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Corresponding Author: Fuji Astuti Febria, Department of Biology, Faculty of Mathematics and Natural Science Andalas University, Padang, West Sumatra, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Piles of garbage can cause various environmental problems. The composition of the waste is mostly dominated by organic waste. Lignocellulose is a component in plant cell walls that accounts for 60% of the total biomass on earth. Different based on variations in the type of source, leaves and stems of plants consist of lignocellulose consisting of 35-50% cellulose, 20-35% hemicellulose, 10-25% lignin and small amounts of other components[1]. Cellulose, which is the largest component of lignocellulose, is hampered by its utilization process because of its complex structure. Cellulase enzymes are the key to biologically effective utilization of cellulose[2], with the help of cellulolytic systems, cellulose can be converted into glucose which is a multifunctional product, in a process that is much cheaper and biologically beneficial[3].

Biodegradation and biological decomposition of cellulose require synergistic work between 3 components of cellulase enzymes, namely endoglucanase, exoglucanase and glucosidase that works synergistically[4]. Many bacteria and fungi can utilize cellulose as a carbon source by synthesizing cellulases enzyme that is responsible for the degradation of cellulose into simple sugars consisting mostly of glucose[5]. Cellulolytic bacteria live freely in the environment and hydrolyze cellulose in the environment such as in piles of dead plants on the forest floor and agricultural waste or living in the digestive tract of animals that help break down food intake in the form of cellulose. Cellulolytic bacteria are proving to be ecologically significant due to their important role in global carbon recycling. Cellulolytic bacteria are also of industrial importance because they can serve as excellent resources for use in the paper, food and bioenergy industries[6,7].

Biological Education and Research Forest have an area of ±150 ha that is located on (0'55'S, 100'28'E) the western edge of Barisan Hill, West Sumatra and includes lowland tropical rain forest[8]. This forest is one of the locations that can be used to obtain cellulolytic bacteria isolates that have good enzyme activity values so that these isolates can be used further later. This study aimed to determine enzyme activity possessed by bacterial isolates from Biological Education and Research Forest floor Andalas University.

MATERIALS AND METHODS

Study area: The research was conducted from February-June, 2021 at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences and Sumatran Biota Biotechnology Laboratory andalas University, Padang. Sampling was carried out in Biological Education and Research Forest Andalas University, Padang

Screening of cellulolytic bacteria: Screening with CMC (Carboxymethyl Cellulose) medium and incubated for 96 hrs then dripped with 1% congo red for 15 min, after that dropped again with 1 M NaCl. The clear zone would form around colonies if the isolates Cellulolytic positive bacteria. After that, the cellulolytic index value was calculated[9].

Cellulolytic bacteria growth profile: To see the growth profile of cellulolytic bacteria, cellulolytic bacteria were cultured in a liquid culture of basal medium with CMC added. Observations were made once every 24 hrs with a spectrophotometer (Genesys 150 UV-Visible Spectrophotometers, Madison, USA).

Enzyme activity test: Enzyme activity test was carried out using the Nelson-Somogyi Method[10]. About 0.5 mL of 1% CMC with acetate solvent with a pH of 5.6 (PH/EC TEMP 983, Guangdong, China) was placed in a 40°C water bath for 5 min. A total of 0.5 mL of enzyme extract was added and incubated for 30 min. After that, 0.5 mL of Nelson's reagent was added and heated for 20 min. after 20 min it was cooled to 25°C and added again with 0.5 arsenomolybdate solutions then vortexed and added 3 mL of distilled water, waited 30 min then centrifuged (MPW 150R, 04-347 Warszawa Poland) and the absorbance of the supernatant was measured by a spectrophotometer (Genesys 150 UV-Visible Spectrophotometers, Madison, USA).

RESULTS AND DISCUSSION

Selection of cellulolytic bacteria: After obtaining bacterial isolates from the isolation stage, a selection process was carried out to determine whether the bacterial isolates obtained were cellulolytic bacteria that could produce cellulase enzymes that could hydrolyze cellulose. The results obtained from the selection process can be seen in Fig. 1 and Table 1.

The data of Fig. 1 shows that during the selection process for cellulolytic bacteria using NA medium added with CMC, a clear zone is formed around the bacterial colony which indicates that the bacteria produce cellulase enzymes that can hydrolyze cellulose in the CMC medium. This clear zone can be formed because the cellulose around the bacterial colonies has been hydrolyzed by bacterial enzymes which break the glycosidic bonds between monosaccharides in the cellulose in the medium used. According to Ladeira research

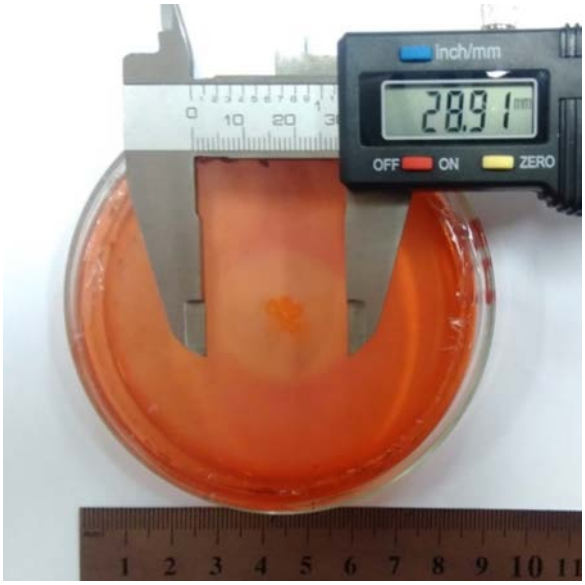

Fig. 1: Cellulolytic bacteria selection

Table 1: Cellulolytic index values

Isolate codes	Cellulolytic index
FFB 1	-
FFB 2	2.59
FFB 3	0.28
FFB 4	0.24
FFB 5	-
FFB 6	0.07
FFB 7	0.30
FFB 8	0.14
FFB 9	-
FFB 10	0.13
FFB 11	-
FFB 12	0.71
FFB 13	-
FFB 14	0.42
FFB 15	-
FFB 16	1.53

FFB: Forest floor bacteria

Carboxymethylcellulose is one of the endoglucanase groups. Cellulase can be produced by fungi, bacteria and plants. Various studies have been carried out on the degradation of cellulose using fungi because of their ability to produce large amounts of cellulolytic enzymes. Nonetheless, recent research has focused more on bacteria due to several clear advantages over fungal enzymes. Bacteria are considered strong and functional producers of enzymes because of their high growth rate and therefore a higher rate of enzyme production. It has stability under harsh conditions and the availability of multi-enzyme complexes[11].

Based on Table 1, it is known that from the 16 bacterial isolates, 10 of them were cellulolytic bacteria. The different cellulolytic index values in each isolate showed that the ability of each isolate to produce cellulase enzymes to hydrolyze cellulose was also different. There is an isolate that has the potential to produce cellulase enzymes where the cellulolytic index value is more than 2. In this study, there were isolates with a cellulolytic index value greater than 2, namely, isolate FFB 2 with an index value of 2.59. To improve the ability of the potential bacterial isolates, we can optimize the bacterial isolates so that the ability of the bacteria can be further improved. optimization that can be done such as optimization of pH, temperature, carbon source and other things that can affect the performance of microbial enzymes. According to Liang's research, who have conducted a selection of cellulolytic bacteria in nature reserves in the subtropics of China, 22 isolates showed hydrolysis zones in the medium containing CMC-Na after congo red staining[12]. The size of the clear zone formed around the colony can indicate potential cellulolytic activity and can calculation with cellulolytic index formula[13].

Cellulolytic bacteria growth profile: Based on the research that has been done, the growth curves of the ten isolates that were positive for cellulase enzymes were obtained based on the results of the previous selection process. The growth profiles of the ten isolates can be seen in Fig. 2.

The result of Fig. 2 shows the lag phase (adaptation), the log phase (exponential), the stationary phase and the death phase. Through this growth curve, we can find out the right time for the utilization of bacteria, in our research we can find out that cellulase enzyme activity is likely to be high in the 72-120 hour range because from the picture above the stationary phase takes place in that period. In this phase usually, the increase in the number of cells and the rate of cell death is relatively balanced and in this phase also metabolites are produced.

In addition, Fig. 2 also shows that 9 bacterial isolates (FFB2, FFB3, FFB4, FFB7, FFB8, FFB10, FFB12, FFB14 and FFB16) have a fast lag phase (adaptation) which is less than 24, but there is 1 bacterial isolate that has a lag phase range (adaptation) of 48 hrs, namely FFB 6. This may have happened because the FFB 6 isolate was not able to utilize the cellulose media used so that the adaptation process took a long time. About 9 bacterial isolates have a log phase (exponential) 24-48 hrs, namely, FFB 2, FFB 3, FFB 4, FFB 7, FFB 8, FFB 10, FFB 12, FFB 14 and FFB 16 and the stationary phase is in the range of 48-120 hrs, while 1 isolate, namely FFB 6 had an exponential phase at 48-120 hrs without having a stationary phase, this may have happened because the ability of FFB 6 isolate

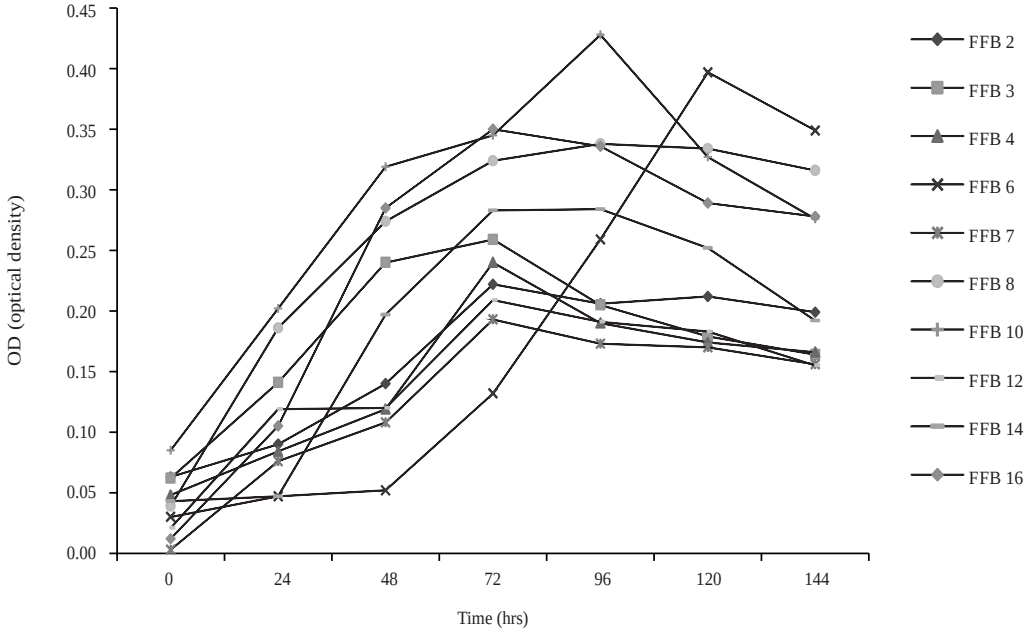

Fig. 2: Growth curve profile of 10 cellulolytic bacteria isolates

Table 2: Enzyme activity value 10 cellulolytic bacterial isolates

Isolate codes	Enzyme activity value (U mL^{-1})	Best enzyme activity at the (hrs)
FFB 2	0.166	72
FFB 3	0.109	96
FFB 4	0.091	72
FFB 6	0.120	96
FFB 7	0.124	72
FFB 8	0.157	96
FFB 10	0.107	96
FFB 12	0.104	72
FFB 14	0.146	96
FFB 16	0.099	72

bacteria to utilize carbon sources in the form of cellulose was quite low. After all, cellulose was included in a complex polysaccharide. Bacteria more easily utilize simple carbon sources than complex carbon sources. Meanwhile, at 120 hrs on average, all bacterial isolates had entered the death phase. The different growth times in the ten isolates were found because each isolate had different physiological abilities to grow in a new environment.

Microorganisms have varying growth times. Each microorganism has a growth curve consisting of several phases, namely first, the lag phase which is the adjustment phase of cells to the environment for the formation of enzymes to break down substrates. The lag phase is the earliest stage of the bacterial growth cycle. The lag phase can also be assumed as the adaptation phase needed by bacterial cells to start exploiting their new environmental conditions[14].

The second phase is the exponential phase, wherein in this phase, there is an increase in the number of cells in which cell activity is greatly increased. The third phase is the stationary phase, wherein in this phase, the increasing number of cells and the number of dead cells are relatively balanced where secondary metabolites can be harvested. Next is the death phase, wherein in this phase the number of dead cells is more than the number of living cells, resulting in a decrease in the number of cells. Moreover, these growth phases namely, lag phase (adaptation), log phase (exponential), stationary phase and death phase represent a very complex state regulated by various environmental and physiological cues[15].

Enzyme activity test: Based on the calculation of the enzyme activity value that has been carried out, it is obtained an enzyme activity curve that shows the ability of the enzyme to hydrolyze cellulose substrate into glucose. The enzyme activity curve can be seen in Fig. 3 and Table 2.

From Fig. 3 and Table 2, we can see that the optimum enzyme activity value for each isolate was shown at different times. 5 isolates showed the optimum value of enzyme activity at 72 hrs, namely isolates FFB 2, FFB 4, FFB 7, FFB 12 and FFB 16. While the other 5 isolates showed the optimum value of enzyme activity at 96 hrs, namely isolates FFB 3, FFB 6, FFB 8, FFB 10 and FFB 14. In addition, based on Table 2, it can be seen that the isolate that showed the best enzyme activity was isolated FFB 2 where the optimum enzyme activity value was 0.166 U mL^{-1} and had reached the optimum value at 72 hrs. From Fig. 2, we can also see that each isolate reached the optimum value of enzyme activity at different times, some within 72 hrs and some within 96 hrs.

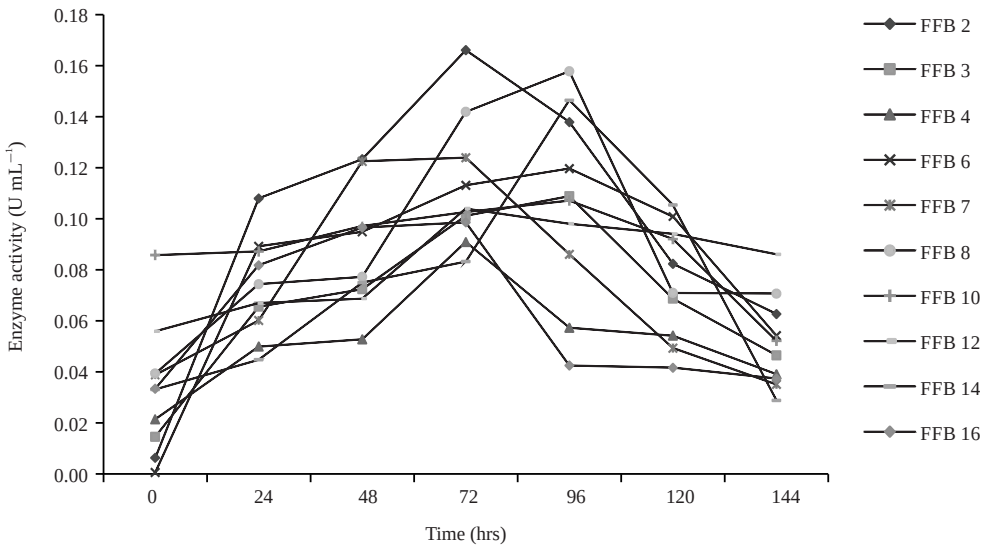

Fig. 3: Enzyme activity of 10 cellulolytic bacteria isolates

The different enzyme activity values in each isolate and the different optimum times for each isolate was due to the different ability of each isolate to produce enzymes, where to hydrolyze cellulose requires synergistic performance between multi-enzyme components, not from Only one enzyme, the synergism will affect the ability of microbial enzymes to hydrolyze cellulose[16]. Bacteria will try to take advantage of the various resources around them in various ways[17]. In general, enzymes are monomeric globular proteins whose catalytic activity depends on the native configuration of their active sites. Cellulase enzymes break down cellulose into smaller polysaccharides or completely into-glucose units which can be further fermented into bioethanol[18]. Although the chemical composition of cellulose is quite simple, it has several crystalline and amorphous topologies. This heterogeneity of the structure of cellulose makes cellulose a difficult substrate for hydrolysis. Microorganisms that can hydrolyze cellulose usually have a multienzyme system. These enzymes work synergistically to carry out effective hydrolysis[19]. By doing this research, it was found that bacterial isolates have the potential to produce cellulase enzymes which can later be used to overcome environmental problems, especially the accumulation of organic waste made from plant biomass and it is hoped that these cellulolytic bacteria can be further developed and molecular identification is carried out to determine the type.

CONCLUSION

The conclusion from the research that has been done is that of the 16 bacterial isolates obtained from Biological Education and Research Forest Floor Andalas University, 10 of them were positive for cellulolytic bacteria with the highest cellulolytic index value of 2.59 on FFB 2 isolates and the bacterial isolate with the best enzyme activity value was FFB 2 isolate 0,166 U mL^{-1} for 72 hrs.

SIGNIFICANCE STATEMENT

This study found cellulolytic bacteria and their activity with the potential to degrade cellulose as one of the main components of plant-based organic waste. These isolates are expected to be developed in the processing of organic waste that is environmentally friendly in the future.

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REFERENCES

1. Mood, S.H., A.H. Golfeshan, M. Tabatabaei, G.S. Jouzani, G.H. Najafi, M. Gholami and M. Ardjmand, 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew. Sust. Energy Rev., 27: 77-93.

2. Li, F., Y. Xie, X. Gao, M. Shan, C. Sun, Y.D. Niu and A. Shan, 2020. Screening of cellulose degradation bacteria from Min pigs and optimization of its cellulase production. Electron. J. Biotechnol., 48: 29-35.
3. Gupta, P., K. Samant and A. Sahu, 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int. J. Microbiol., Vol. 2012. 10.1155/2012/578925.
4. Kostylev, M. and D. Wilson, 2012. Synergistic interactions in cellulose hydrolysis. Biofuels, 3: 61-70.
5. Lee, S.M. and Y. Koo, 2001. Pilot-scale production of cellulase using *Trichoderma reesei* Rut C-30 fed-batch mode. J. Microbiol. Biotechnol., 11: 229-233.
6. Zhang, S., D. Shan, X. Liu and M. Sun, 2018. Cellulose-degrading strains: Their screening and application to corn straw in low-temperature environments. Pol. J. Environ. Stud., 27: 2349-2355.
7. Nkohla, A., K. Okaiyeto, U.U. Nwodo, L.V. Mabinya and A.I. Okoh, 2017. Endoglucanase and xylanase production by *Chryseobacterium* species isolated from decaying biomass. Pol. J. Environ. Stud., 26: 2651-2660.
8. Chairul and T. Yoneda, 2006. Leaf longevity of tropical shrub species in an open forest in Sumatra. Tropics, 15: 201-207.
9. Cappuccino, J.G. and C. Welsh, 2017. Microbiology: A Laboratory Manual. 11 Edn., Pearson Education, Edinburgh. England.
10. Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.
11. Ladeira, S.A., E. Cruz, A.B. Delatorre, J.B. Barbosa and M.L.L. Martins, 2015. Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility. Elect. J. Biotechnol., 18: 110-115.
12. Liang, Y.L., Z. Zhang, M. Wu, Y. Wu and J.X. Feng, 2014. Isolation, screening and identification of cellulolytic bacteria from natural reserves in the subtropical region of china and optimization of cellulase production by *Paenibacillus terrae* ME27-1. Biomed. Res. Int., Vol. 2014. 10.1155/2014/512497.
13. Ferbiyanto, A., I. Rusmana and R. Raffiudin, 2015. Characterization and Identification of cellulolytic bacteria from gut of worker *Macrotermes gilvus*. HAYATI J. Biosci., 22: 197-200.
14. Rolfe, M.D., C.J. Rice, S. Lucchini, C. Pin and A. Thompson *et al*., 2012. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. J. Bacteriol., 194: 686-701.
15. Llorens, J.M.N., A. Tormo and E. Martinez-Garcia, 2010. Stationary phase in gram-negative bacteria. FEMS Microbiol. Rev., 34: 476-495.
16. Ilahi, R.K. and F.A. Febria, 2021. Screening of cellulolytic bacteria from biological education and research forest floor Andalas university, Indonesia. Pak. J. Biol. Sci., 24: 612-617.
17. Febria, F.A., I.J. Zakaria, L. Syukriani, S.P. Rahayu and M.A. Fajri, 2016. The highest mercury resistant bacteria as a mercury remediator from gold mining soil in West Sumatera. Indonesia. J. Chem. Pharm. Res., 8: 394-397.
18. Subhedar, P.B. and P.R. Gogate, 2014. Enhancing the activity of cellulase enzyme using ultrasonic irradiations. J. Mol. Catal. B: Enzymatic, 101: 108-114.
19. Schwarz, W.H., 2001. The cellulosome and cellulose degradation by anaerobic bacteria. Appl. Microbiol. Biotechnol., 56: 634-649.