JURNAL BIOLOGI

El-Hayah

Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

Original research article

Isolation and Identification of Cellulose Degrading Bacteria from Banana Peel Compost

Yendania Grevitara P.[1], Badriyatur Rahma F.[1], Hellen Septirangga P.[1], Irma Dahlia Y. [1], Endang Suarsini[1]

[1]*Post Graduate, Biology Education, Universitas Negeri Malang*

[*]*Corresponding author*
Email: yendaniagrevitara3931@gmail.com
DOI: 10.18860/elha.v7i1.7241

Article Info

Article history:
Received 02 September 2018
Received in revised form
14 October 2018
Accepted 20 October 2018

Keywords:
Isolation,
Identification,
Cellulolytic bacteria,
Banana peel compost

Abstract

Cellulolytic bacteria are bacteria that have the ability to hydrolyze cellulose complexes into smaller oligosaccharides and eventually become glucose. Glucose is used as a carbon and energy source for bacterial growth. This study was conducted to isolate the cellulose degrading bacteria from banana peel compost that produce cellulose enzymes based on the clear zone that visible around the colony. The cellulolytic activity was determined by the ability of bacteria to hydrolyze the Carboxymethyl Cellulose (CMC) substrate. Determination of cellulolytic activity is known based on cellulolytic index calculation, the diameter total minus the diameter of the colony and divided by the diameter of the colony. The result of five bacterial isolates was found but only one bacterium had the potential to be a cellulose degradation. Based on the Microbact Gram-Negative Identification System, the bacterium is Burkholderia cepacia. These bacteria have an important role in nature as decomposers of various complex compounds, such as cellulose, hemicellulose, lignin, and pectin.

1. INTRODUCTION

Indonesia as an agricultural country has the potential to develop various agricultural commodities such as food crops, plantations and forestry. Indonesia's agrarian waste can be used as compost that has economic value. The compounds contained in compost waste are mostly lignocellulose which consists of three polymers, namely cellulose, hemicellulose and lignin (Perez *et al*, 2002).

Cellulose is the main constituent compound in agricultural waste and in nature, these compounds can be destroyed by microorganisms that grow on cellulose. Cellulose is a glucose polymer in the form of a linear chain and is linked by a β-1.4 glucosidic

bond. Glucoside bonds in cellulose fibers can be broken down into glucose monomers by cellulase enzyme complexes. The enzyme complex consists of three main enzyme types (endoglucanase, exoglucanase, and β-glucosidase) which degrade cellulose and release reducing sugar (cellobiose and glucose) as the end product (Schwarz, 2001).

The use of cellulase enzymes in waste degradation is more beneficial than using chemicals because they can avoid corrosion and environmental pollution problems and form a little by-product. This cellulose solution can be done with cellulose. Cellulosic is a biological process that is controlled and carried out by the presence of cellulase enzymes (Gupta, 2011). In plants, cellulose is coated with polymers consisting mostly of xylan and lignin. Xylan can be degraded by xylanase, but lignin is very difficult to degrade. If xylan and lignin are removed, cellulose can be degraded by cellulase from bacteria or cellulolytic mold to produce cellobiose and glucose.

This research was conducted to utilize cellulase-producing bacteria in cellulose degradation. Further benefits can be used as activators in making banana peel compost, moreover, banana peel compost containing high cellulose. Cellulase bacterial activity will accelerate cellulose degradation in agricultural wastes so that compost can be obtained effectively and efficiently.

2. MATERIALS AND METHODS

Study Location

The study was conducted from October to December 2017. Research activities included composting, compost sampling, laboratory analysis, and the ability to test bacterial isolates. Laboratory analysis activities were carried out at the Microbiology Laboratory of FMIPA State University of Malang.

Tools and materials

The tools used in this study included composting tools, test tubes, staining rack, petri dishes, Erlenmeyer flasks, graduated cylinders, beaker glass, glass rod, volume pipettes, lab burners, loop, micropipettes and tips, rulers, shakers, incubators, autoclaves, laminar air flow (LAF), microscopes, vortex, glass objects, wash bottle, and cameras.

The ingredients used are banana skin, husk, water, every 100 mL of solid CMC media containing 0.2 g yeast extract, 0.4 g of beef extract, 0.51 g of peptone, 0.1 g of KH_2PO_4, $MgSO_4.7H_2O$ 0.02 g , $CaCl_2$ 0.3 g, $FeCl_3$ 0.028 g, Na_2HPO_4 0.1 g, CMC 0.5 g and bacto to 2.5 g (Baharuddin dkk., 2010). Alcohol, sterile distilled water, cotton, gauze, matches, label paper, and gram bacterial coloring reagents.

Procedure

Composting

Composting is started with (1) collecting banana peel waste. Furthermore, the waste of banana skin is chopped until it is destroyed; (2) coat the basket with cardboard on all four sides so that no insects or flies enter and lay eggs in a box; (3) inserting chaff pads in a box that aims to absorb excess water from organic waste or steam produced from composting; (4) put small pieces of banana peel waste which has been chopped; (5) adding some of water to the banana skin waste; (6) cover with a paddle again to anticipate the steam produced from the composting process; (closing the box with a porous black cloth, the aim is to avoid insects or flies from entering the box. The composting process is carried out for approximately 3 weeks.

Isolation of Cellulose Degradation Bacteria

Compost samples were collected from compost made from banana peel. Samples are stored at room temperature. A total of 25 grams of sample were included in a 250 mL physiological saline solution and then vortexed to obtain a 10^{-1} dilution. The suspension of 10^{-1} dilution was taken by 1 ml and then put into 9 ml of physiological saline solution or 85% NaCl to obtain a 10^{-2} dilution. Next, serial dilution was carried out until 10^{-7} dilution was obtained. Then pour plate and spread plate technique on Carboxymethyl Cellulose (CMC) agar medium.

Prepare 10 mL of Carboxymethyl Cellulose (CMC) agar medium in Petri dishes. Next 1 mL sample from 10^{-3} dilution, 10^{-4},10^{-5},10^{-6} and 10^{-7} are included in the Petri dish (Lamid *et al.*, 2011).

The medium in the petri dish that had contained the sample was incubated at 37◦C for 1x24 hours. After bacterial isolates grow, inoculate a single colony on the CMC agar medium in a test tube to get pure isolates. Furthermore, inoculation was carried out on the CMC medium in the petri dish again. To see the hydrolysis activity, soaking the medium in 0.1% neutral red then incubated at 37◦C for 1x24 hours and rinsed with 1M NaCl. Measured the clear zone formation and bacterial colonies. The measurements to determine the activity of bacterial enzyme production. The largest ratio is assumed to indicate the highest activity (Howard et al, 2003).

Identification of Isolates

Identification of bacterial isolates was carried out by looking at colony morphology and some biochemical characters. The investigation was carried out based on microbact: a Gram-negative identification system by looking at changes in pH on several substrates and substrate utilization tests. The bacteria were tested based on two different substrate strips, 12A and 12B. Each strip has 12 different biochemical tests. At 12A was used for identification of negative oxidase, nitrate-positive glucose fermenter, and was used continuously with 12B for the identification of positive oxidase, nitrate-negative glucose nonfermenter.

Parameters include characteristics of bacterial colonies, shape, size, gram staining, oxidase, motility, nitrate, lysine, ornithine, H2S, glucose, mannitol, xylose, ONPG, indole, urease, Voges-Proskauer (VP), citrate, TDA, gelatin, malonate, inositol, sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin and arginine tests. The reaction is then converted into the octal code and inputted in the Microbact computerized identification package, which will show bacterial identification (Ohara, 2005).

3. RESULTS

Isolate Cellulolytic Bacteria From Banana Peel Waste

The cellulolytic index of bacterial isolates found in each plate is described in **Table 1** as follows.

Table 1. Cellulolytic Index

Dilution	Cellulolytic Index
10^{-3}	0,75
10^{-4}	1,36
10^{-5}	0,5
10^{-6}	1,19
10^{-7}	1,2

Source: Personal observation

The appearance of colonies of bacterial isolates that produce the largest cellulolytic index is 10^{-4} at the dilution stage presented in **Figure 1** as follows

Figure 1. Bacterial colonies in medium CMC 10^{-4} dilution plates

The appearance of colonies of bacterial isolates that produce the largest cellulolytic index in oblique CMC medium is presented in **Figure 2** as follows.

Figure 2. Pure culture of bacteria has the potential to degrade cellulose

Determination of bacterial species that have the potential to degrade cellulose can be done by testing Gram purified bacteria if the results show that bacterial colonies are Gram negative and form bacilli then identification can be continued. Morphological observations were made before Gram testing, based on observations of bacterial colonies having concentrated character, round shape, slippery edges, flat elevation, yellow color, gloomy, 0.375 mm in diameter. Furthermore, the results of Gram testing showed that bacteria with 10^{-4} dilution were Gram negative bacteria and were bacillary so that specific species identification could be continued. The appearance of Gram bacterial colonies that have the potential to degrade cellulose is presented in **Figure 3.**

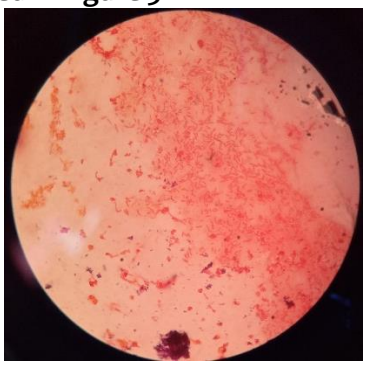

Figure 3. The appearance of gram of bacterial colonies with 10-4 dilution microscopically which has the potential to degrade cellulose

Identification of bacterial isolates

Based on identification results (Table 2) using Microbact: Gram Negative Identification System shows that bacterial isolates obtained at 10-4 dilution have the characteristics shown in Table 2 as follows.

Table 2. Results of bacterial identification

Parameter	10^{-4}
Oxidase	+
Motility	+
Nitrase	+
Lysine	-
Ornithine	-
H_2S	-
Glucose	+
Mannitol	-
Xylose	+
ONPG	+
Indole	-
Urease	+
V-P	-
Citrate	+
TDA	-
Gelatin	+
Malonate	+
Inositol	-
Sorbitol	-
Rhamnose	-
Sucrose	-
Lactose	-
Arabinose	+
Adonitol	-
Raffinose	-
Salicin	-
Arginine	-

The identification results show that the bacteria are Gram-negative bacteria with bacillary form. After being tested using the Microbact Gram-Negative Identification System, the bacteria were determined as *Burkholderia cepacia*. *Burkholderia cepacia* bacteria are a group of catalase and lactose-nonfermented producing bacteria. These bacteria have an important role in nature as decomposers of complex compounds such as cellulose and lignin (Akita, 2017).

4. DISCUSSION

Burkholderia cepacia is a biological control agent (Van, 2002). Understanding biological agents according to FAO (2004) are microorganisms, such as bacteria, fungi, viruses and protozoa, as well as genetically modified microorganisms that are used to control organisms that disturb plants (OPT). Besides that *Burkholderia cepacia* functions as a biodegradation agent in contaminated water and soil such as phenol, diesel, camphor, fertilizer, therefore *Burkholderia cepacia* can be found naturally in soil, water and rhizosphere of plant roots (Sausa, 2011). But *Burkholderia cepacia* is pathogenic in humans. Discussed by a panel of experts (Scientific Advisory Panel; SAP) on July 20-23, 1999 in Arlington Virginia (USA) (SAP Report 1999) *Burkholderia cepacia* can cause harm to humans which are characterized by drastically reduced health symptoms in someone who has been infected the bacteria because bacteria can enter the blood vessel system

5. CONCLUSION

The bacterial isolates obtained are able to produce cellulase enzymes which can be seen from the presence of clear zones around the colony. Of the 5 cellulolytic bacterial isolates derived from composting banana skin waste which has the highest cellulolytic index value of 10^{-4} isolates with a value of 1.36. Compost bacterial isolate of banana peel is able to decompose banana skin, which is indicated by the activity during the decomposition process of banana peel. The results of the identification of bacterial isolates are *Burkholderia cepacia*. *Burkholderia cepacia* is a group of catalase and lactose-nonfermented producing bacteria. These bacteria have an important role as decomposers of various complex compounds such as cellulose and lignin.

6. REFERENCES

Akita, Hironaga., Kimura, Zen., Yusoff Mohd., Nakashimma, Nobutaka dan Hoshino, Tamotsu. 2017. Identification and Characterization of Burkholderia multivorans CCA53, BMC Research Notes NCBI. (Online) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5501517/).

Baharuddin, A.S., Razak, M.N.A., Hock, L.S., Ahmad M.N., Aziz, S.A., Rahman, N.A.A dan Shah, U.K.M. 2010. Isolation and Characterization of Thermophilic Cellulase-Producing Bacteria from Empty Fruit Bunches-Palm Oil Mill Effluent Compost, *American Journal of Applied Sciences, 7:* 56-62.

FAO. 2004. Fertilizer use by crop in Brazil. Land and Plant Nutrition Management Service. Land and Water Development Division. *Food and Agriculture Organization of The United Nations*. Rome. http://ftp.fao.org/agl/agll/docs/fertusebrazil.pdf 64 pp.

Gupta, Pratima, Samant, Kalpana, Sahu, Avinash. 2011. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. *International Journal of Microbiology.* Volume 2012.

Howard, R.L., Masoko, P. and Abotsi, E,. 2003. Enzyme activity of a Phanerochaete chryso-sporium cellobiohydrolase (CBHI. 1) expressed as a heterologous protein from Escherichia coli. *African Journal of Biotechnology, 2(9).* 296-300. Sept 2003.

Lamid, M., Nugroho, T.P., Chusniati, S. dan Rochiman, K. 2011. Eksplorasi bakteri selulolitik asal cairan rumen sapi potong sebagai bahan inokulum limbah pertanian. *Jurnal Ilmiah Kedokteran Hewan* 4(1): 37-42.

Ohara, Caroline M., 2005. Manual and Automated Instrumentation for Identification of *Enterobacteriaceae* and Other Aerobic Gram-Negative Bacilli. *Clinical Microbiology Reviews, Jan 2005, p. 147-162.*

Perez, J., J.M. Dorado, T. Rubia, J. Martinez. 2002. Biodegradation and Biological treatments of Cellulose, Hemicellulose

and Lignin: An Overview. *Int. Microbiol*. 5, 53-63.

Sausa, Silvia., Ramos, G.C & Leitau, H. J. 2011. Burkolderia cepacia Complex: Emerging Multihost Pathogens Equipped with a Wide Range of Virulence Factors and Determiants. *International Journal of Microbiology* DOI: 10. 1155-2011-607575.

Schwarz, W. H., 2001. The Cellulosome and cellulose degradation by anaerobic bacteria. *Appl Microbiol Biotechnol* 56:634–649.

Van Antwerpen, T., R.S. Rutherford, and J.L. Vogel. 2002. Assessment of sugarcane endophytic bacteria and rhizospheric burkholderia species as antifungal agents. *Proceeding of South African Sugar Technology Association* 76:301-304.