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Research Article

Screening of Cellulolytic Bacteria from Biological Education and Research Forest Floor Andalas University, Indonesia

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Abstract

Background and Objective: Organic waste dump is a problem that needs to be solved, one of which is by using microbe. Cellulolytic bacteria's ability to produce cellulase enzymes that can hydrolyze cellulose. Cellulose is the major component of the plant cell walls that are difficult to endure degradation naturally. This study aimed to find cellulolytic bacterial isolates on Biological Education and Research Forest floor Andalas University and characterize the cellulolytic bacteria found. **Material and Methods:** CMC (Carboxymethyl Cellulose) medium is used as a screening for bacteria isolate and this study used the survey method and also conducted catalase, glucose and lactose test for characterizations. **Results:** We found 16 bacterial isolates on Biological Education and Research Forest floor where 9 isolates were in a shaded area and 7 isolates were in an unshaded area. There were 12 isolates from 16 isolates that have the positive cellulolytic ability with a variety of clear zone sizes, where there were 8 isolates with the large clear zone and 4 isolates producing very small clear zones. Characteristics of bacterial isolates with the large clear zone obtained were 2 gram-positive coccus isolates with positive catalase test, 3 gram-negative coccus isolates with positive glucose test and 3 gram-negative bacilli isolates with negative fructose test. **Conclusion:** We identified 2 potential isolates with a cellulolytic index value greater than 2, isolate UCB 4 with a cellulolytic index value of 3.5 and UCB 6 with a cellulolytic index value of 2.2.

Key words: Cellulases, cellulose, clear zone, CMC, degradation, enzymes, microbe

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

INTRODUCTION

The waste dump is a problem that needs to be carefully handled because it can adversely affect the quality of the environment and human health. This pile of garbage can cause various environmental problems because the accumulated garbage can pollute community water sources and cause various diseases caused by pathogenic bacteria carried by polluted water. The composition of this waste is mostly dominated by organic waste. Organic waste consisting of food scraps and plant residues, among all the composition of organic waste, plant residue is the largest composition.

The biodegradation process of plant biomass takes a bit longer period in nature^{1,2}. Plant biomass consists of a lot of cellulose³. Plant cell walls have a complex structure consisting of polysaccharides, proteins and lignin. Among the polysaccharides that make up the plants cell walls, cellulose and hemicellulose are the main components⁴. Cellulose hydrolysis is a stage of biodegradation¹. Enzymatic hydrolysis of cellulose is necessary for microorganisms. However, there are so many microorganisms, only a small portion of which can degrade cellulose, because the cell walls are difficult to degrade. Cellulolytic organisms are very diverse because their natural substrate depends on plant cell wall structure. Cellulase is different from most other enzymes, because of its ability to degrade insoluble substrates⁵.

Biological Education and Research Forest are located on the western edge of Barisan Hill as a part of Kamalau Hill, Limau Manis, in Padang, West Sumatra (0°55'S, 100°28'E). This forest is an artificial open forest that represents a secondary forest. The forest includes lowland tropical rain forest comprising an area of ± 150 hectares and is located at an altitude of 250-450 m above sea level. Biological Education and Research forest had a high diversity of animals and plants including several of Sumatera's endemic species. Based on previous studies, species treasures in Biological Education and Research Forest were estimated at around 42 species in the open forest, such as *Aleurites moluccana*, *Archidendron jiringa* and *Hevea brasiliensis*. The number of species observed in the closed forest was 20, including some species also observed in the open forest⁶.

According to the number of plant species in the Biological Education and Research forest, certain organisms can decompose the fallen leaves and dead trees on the forest floor which are common mentioned as leaf litter. Isolation of cellulolytic biodiversity in the Biological Education and Research forest floor can provide a basis for further studies on the use of cellulolytic bacteria as a decomposition agent of plant organic waste that has cellulose composition. The

findings elucidate an effective way of degradation waste dump by using cellulolytic bacteria.

MATERIALS AND METHODS

Study area: The research was conducted for 5 months from February-June, 2020 at the Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, Indonesia. Sampling was carried out in Biological Education and Research Forest Andalas University, Padang, Indonesia.

Bacteria isolation: The sample of litter was diluted to a concentration of 10^{-6} and then diluted with 10^{-4} - 10^{-6} concentration inoculated 1 mL on NA medium with pour plate technique. Samples that have been inoculated are then incubated for 24 hrs⁷.

Screening of cellulolytic bacteria: Screening of cellulolytic bacterial was done by inoculating bacterial isolates that have been purified on the NA modification with CMC medium and incubated for 96 hrs then the bacterial colonies were dripped with 1% congo red for 15 min, after which it dropped again with 1 M NaCl. Cellulolytic positive bacteria would form clear zones around bacterial colonies⁸.

Cellulolytic index: The measurement of cellulolytic index could be done by measuring the clear zone formed around the bacterial colony by using the formula⁸:

$$\text{Cellulolytic index} = \frac{\text{Diameter of zone} - \text{Diameter of bacterial colony}}{\text{Diameter of bacterial colony}}$$

Macroscopic observation: Macroscopic observations were made by directly observing the forms, elevation and margins of bacterial colonies⁷.

Microscopic observations: Microscopic observation was carried out with gram staining to determine whether the bacterial isolate was gram-positive or gram-negative and to determine the shape of the bacterial cell⁷.

Biochemical tests: Biochemical tests were being carried out based on the results of gram staining and cell shape. If the bacterial isolate obtained was gram-positive and in the form of bacilli, a spore staining would be carried out, while those in the form of coccus the catalase would be tested. If the bacterial isolate obtained was gram-negative and

coccus-shaped, a glucose test would be carried out, while those in bacilli form would be evaluated for lactose test⁷.

RESULTS AND DISCUSSION

Cellulolytic bacterial screening is a selection effort on the ability of isolates found. This research was producing cellulase enzymes. Screening results showed that out of the 16 isolates found there were 12 isolates of cellulolytic positive bacteria producing cellulase enzymes that were analyzed qualitatively as indicated by the formation of clear zones around the colony. The results of cellulase bacterial selection can be seen in Fig. 1a, b.

This research revealed the potential activity of cellulolytic bacteria that were isolated from the Biological Education and Research Forest floor. As shown in Fig. 1a,b shows the formation of a clear zone around the bacterial colony indicating that the bacterium was cellulolytic. This happens because the bacterial isolate was found capable of producing cellulase enzymes that could hydrolyze cellulose on the CMC medium. The clear zone was formed caused by the degradation of CMC (carboxymethyl cellulose) around the bacterial colony⁹. Most microorganisms can degrade cellulose but only a few microorganisms can produce cellulase enzymes in large quantities so that they can fully hydrolyze cellulose. Endoglucanase activity (β 1-4 endoglucanase-EC 3.2.1.4) can be tested by measuring the amount of reduced sugar from cellulose by cellulase enzymes. Cellulase is an enzyme complex that can hydrolyze cellulose into glucose so that it can be useful in the utilization of plant biomass¹⁰.

Screening results from Table 1 and 2 showed that out of the 16 isolates found there were 12 isolates of cellulolytic

positive bacteria produced of cellulase enzymes that were analyzed qualitatively as indicated by the formation of clear zones around the colony. In this research we also found the cellulolytic index varied in each isolate tested due to the different ability of bacterial isolates to hydrolyze cellulase in CMC substrate. Figure 2a showed the highest cellulolytic index value in shade area was isolate SCB 7 which was 1.60 (Table 1) and Fig. 2b showed the highest cellulolytic index value in unshaded area was isolated UCB 4 which was 3.50. Figure 2b also shows 2 isolates were obtained with a cellulolytic index value greater than 2 from biological education and research

Table 1: Bacteria screening results from shade area of biological education and research forest floor

Isolate code	Produce cellulase enzymes	Cellulolytic index
SCB 1	+	0.14
SCB 2	+	0.21
SCB 3	+	0.11
SCB 4	+	0.04
SCB 5	-	-
SCB 6	+	0.25
SCB 7	+	1.60
SCB 8	-	-
SCB 9	-	-

SCB: Shade cellulolytic bacteria, +: Positive, -: Negative

Table 2: Bacteria screening results from unshaded area of biological education and research forest floor

Isolate code	Produce cellulase enzymes	Cellulolytic index
UCB 1	-	-
UCB 2	+	0.09
UCB 3	+	0.01
UCB 4	+	3.50
UCB 5	+	1.20
UCB 6	+	2.20
UCB 7	+	0.71

UCB: Unshaded cellulolytic bacteria, +: Positive, -: Negative

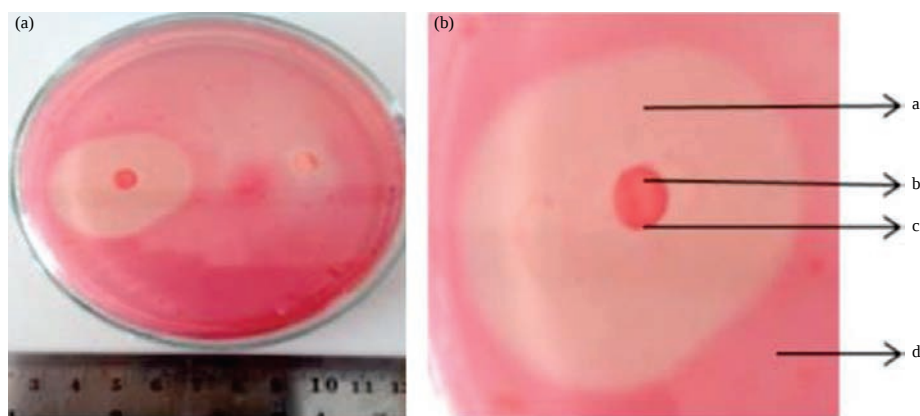


Fig. 1(a-b): Screening of cellulolytic bacteria with CMC medium and congo red

(a) Screening of cellulolytic bacteria (UCB 4) and (b) Zoom of screening cellulolytic bacteria (a: Clear zone, b: Paper dish, c: Bacterial colony, d: The modified NA medium is bind to congo red)

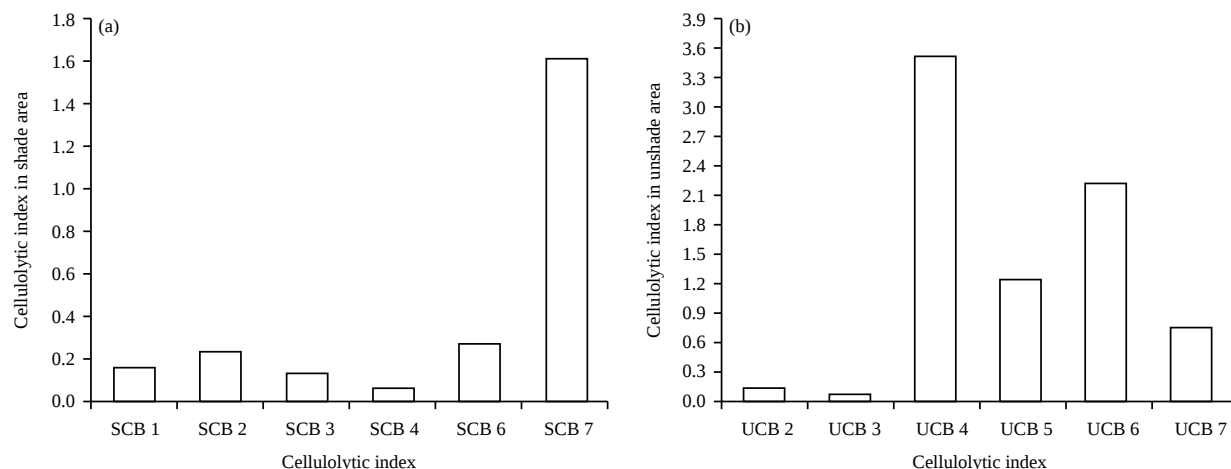


Fig. 2(a-b): Cellulolytic Index in different areas

(a) Bacteria cellulolytic index value of bacteria isolated from shade and (b) Unshaded area of biological education and research forest (HPPB) floor

Table 3: Macroscopic, microscopic and biochemical tests of cellulolytic bacterial isolates from the biological education and research forest floor

	Numbers							
	1	2	3	4	5	6	7	8
Isolate code	SCB 1	SCB 2	SCB 6	SCB 7	UCB 4	UCB 5	UCB 6	UCB 7
Macroscopic observation								
Form	Irregular	Circular	Irregular	Circular	Circular	Irregular	Circular	Rhizoid
Margin	Undulate	Entire	Lobate	Undulate	Entire	Filamentous	Undulate	Filamentous
Elevation	Umbonate	Raised	Umbonate	Convex	Convex	Raised	Umbonate	Raised
Microscopic observation								
Gram	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Negative
Shape of cell	Coccus	Coccus	Basil	Coccus	Basil	Coccus	Basil	Coccus
Biochemical tests								
Catalase test	Positive	-	-	-	-	Positive	-	-
Spore	-	-	-	-	-	-	-	-
Staining	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-
Test	-	Positive	-	Positive	-	-	-	Positive
Lactose	-	-	-	-	-	-	-	-
Test	-	-	Negative	-	Negative	-	Negative	-

:- Not tested

forest floor, which were isolate UCB 4 with a cellulolytic index value of 3.50 and UCB 6 with a cellulolytic index value of 2.20. This indicated that the two isolates namely UCB 4 and UCB 6 were potential bacterial isolates in producing cellulase enzymes (Table 2).

The bacterial cellulolytic activity can be distinguished based on the size of the clear zone formed around the colony and the calculation of cellulolytic index values. The cellulolytic index value can be calculated by measuring the diameter of the clear zone minus the diameter of the bacterial colony and then divided by the diameter of the bacterial colony⁸. With cellulolytic index greater than or equal to 2 is more likely to produce cellulase enzymes.

In other isolation of cellulolytic bacteria from the gut of Worker *Macrotermes gilvus*, the research found 4 isolates of cellulolytic bacteria. Those were RU1, RU3, RU4, RA2 with the value of the cellulolytic index respectively 0.87, 0.75, 0.81 and 2.50⁸. From this research, we know Biological Education and Research Forest floor is one of the potential places to isolate cellulolytic bacteria with a high cellulolytic index. Characteristics of cellulolytic bacteria from biological education and research forest floor are shown in Fig. 3a-b that showed gram staining of cellulolytic bacteria and Table 3 shows macroscopic, microscopic and biochemical tests of cellulolytic bacterial.

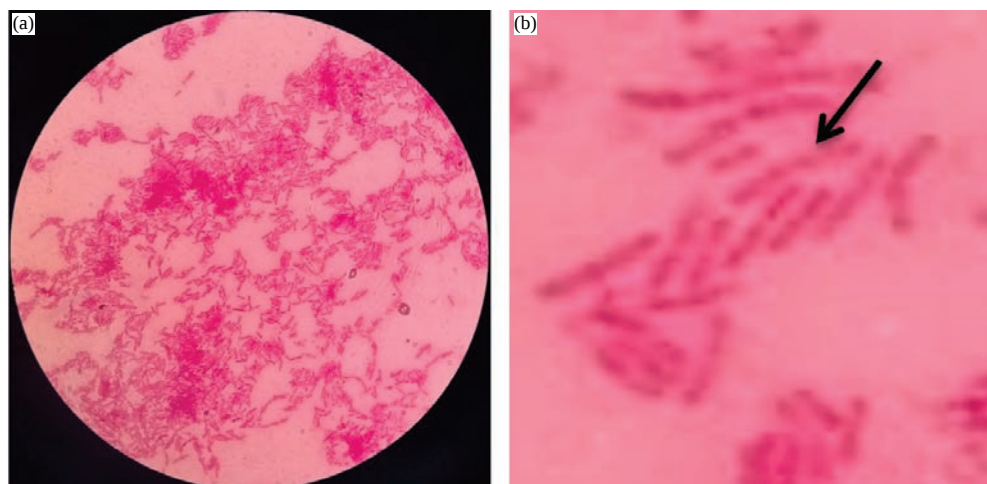


Fig. 3(a-b): Gram staining of cellulolytic bacteria

(a) Microscopic observation of UCB 6 cellulolytic bacteria isolate classified as gram-negative and (b) Shape of stem cells

Cellulolytic bacteria from Biological Education and Research Forest floor have various characterizations. Table 3 showed characteristics of bacterial isolates obtained, 2 gram-positive coccus isolates with positive catalase (SCB 1 and UCB 5), 3 gram-negative coccus isolates with a positive glucose test (SCB 2, SCB 7 and UCB 7) and 3 gram-negative bacilli isolate with negative fructose test (SCB 6, UCB 4 and UCB 6).

For gram-positive bacteria in the form of coccus, further testing was done by the catalase test. The catalase test is positive when air bubbles are produced during the test. The catalase test that has been carried out also obtained positive results, then based on the bacterial identification scheme by Cappuccino and Welsh⁷ the bacteria obtained were thought to belong to the genus *Micrococcus* spp. and *Staphylococcus* spp.

For gram-negative bacteria in the form of coccus, the next test was the glucose test. The glucose test is a part of the carbohydrate fermentation test. The test will be positive if the colour of the medium turns yellow due to the process of fermentation of carbohydrates to become acidic⁷. In the glucose test, the colour of the medium changed to yellow, so the bacteria obtained were thought to belong to the genus *Neisseria* spp. According to Cappuccino and Welsh⁷ gram-negative bacteria in the form of bacilli, the next test is the lactose test. The lactose test is a part of the carbohydrate fermentation test. The test will be positive if the colour of the medium turns yellow due to the process of fermentation of carbohydrates to become acidic. In the lactose test, there was no change in the colour of the medium, so the bacteria obtained were thought to belong to the genus *Proteus* spp. and *Pseudomonas* spp.

The characterizations carried out in this research were not able to confirm the type of bacteria found but, even though this is only an assumption, it will require molecular identification by analyzing 16 s rRNA gene sequences.

CONCLUSION

From this study 16 bacterial isolates were found on the Biological Education and Research Forest floor with 12 isolates had the positive cellulolytic ability and 2 potential isolates a cellulolytic index greater than 2, isolate UBS 4 with a cellulolytic index value of 3.5 and UBS 6 with a cellulolytic index value of 2.2. The 2 potential cellulolytic bacterial isolates can be further developed to be used as agents for degrading cellulose-based waste.

SIGNIFICANCE STATEMENT

This study found cellulolytic bacteria 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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