Biodiversity and Enzyme Activity of Indigenous Cellulolytic and Amylolytic Bacteria in Decayed Mangrove Stem Waste Product at Waai Seashore, Ambon Island

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Abstract: Mangrove (*Sonneratia* spp.) could be found at Waai seashore, Ambon island. The remainder of the mangrove stem will be decayed and become the waste product. Some indigenous bacteria species that live in the decayed mangrove stem waste product have cellulolytic and amylolytic characters. This research was done to: (1) identify and determine the cellulolytic bacteria species; (2) identify and determine the amylolytic bacteria species; (3) determine the cellulolytic bacteria species that have the highest cellulase activity; (4) determine the amylolytic bacteria species that have the highest amylase activity. This research was conducted at the Microbiology Laboratory, Department of Biology, State University of Malang, Microbiology Laboratory, Faculty of Medicine, Brawijaya University and Chemistry Laboratory, Muhammadiyah Malang University. In the experiment, 25 g sample were grinded and diluted in 225 mL nutrient broth to get 10^{-1} suspension. Then the suspension was diluted gradually until 10^{-10} . The suspension was inoculated on nutrient agar medium with 0.1 mL each, and incubated in 37 °C during 24 h. Each bacteria colony was isolated and identified to know whether it was cellulolytic or amylolytic bacteria. Afterward, the cellulase activity as well as the amylase activity was analyzed. The research results show that: (1) there are four cellulolytic bacteria species, i.e., *Micrococcus luteus*, *Bacillus pumilus*, *Planococcus citreus* and *Bacillus cereus*; (2) there are four amylolytic bacteria species, i.e., *Bacillus firmus*, *Nitrobacter* sp., *Bacillus mycoides* and *Pseudomonas aeruginosa*; (3) *Bacillus cereus* has the highest cellulase activity; (4) *Nitrobacter* sp. has the highest amylase activity.

Key words: Biodiversity, cellulolytic bacteria, amylolytic bacteria, decayed mangrove stem waste product.

1. Introduction

Mangroves have a function as the habitat for the sea animals, i.e., crab, fish, shrimp, mollusc, etc.. The mangrove wood has a economically social value, i.e., for building, furniture and handy-craft. Mangrove also has another function, i.e., to protect the seashore from the abration and for spawning of some sea biota [1]. Commonly, the people who live at the Waai seashore, Ambon island, Moluccas, often neglect the role of mangroves in the ecosystem. They cut the mangrove

and use as a burned wood. The remainder of the cut mangrove stem will be decayed for a long time and become a waste product at Waai seashore. The decayed mangrove stem will become the waste product which contains carbohydrate compounds, i.e., cellulose and amylum, as same as the waste product from the agriculture industry, i.e., corn peel, sago pith and coffee skin. Some indigenous cellulolytic bacteria species live in the decayed mangrove stem waste product and could degrade the cellulose to more simple compound. The cellulolytic bacteria can be used to degrade the waste product that contains cellulose. Besides, there are also some live indigenous

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amylolytic bacteria species and they could degrade the amylum compound to more simple compound, so it could dissolve easily. Based on this fact, it is important to isolate and identify the indigenous cellulolytic and amylolytic bacteria in decayed mangrove stem in order to use the bacteria for biodegradation process.

The research result of Apun et al. [2] proved that in the sago pith waste product from Pusa Sarawak, Malaysia, an indigenous amylolytic and cellulolytic bacteria—*Bacillus amyloliquefaciens* was found. The research result of Hastuti et al. [3] has also reported that in the sago waste product from Susupu, North Moluccas, lived some indigenous amylolytic as well as cellulolytic bacteria species, i.e., *Serratia liquefaciens*, *Bacillus licheniformis* and *Bacillus cereus*. *Acinetobacter iwoffi*, a cellulolytic bacteria species, was found in soil [4].

Mangrove at Waai seashore, Ambon island, was dominated by genera *Sonneratia*, because the substrate and environmental condition were match with the habitat of this genera. The environment of Waai seashore was match with the habitat of indigenous cellulolytic and amylolytic bacteria in the decayed mangrove stem (*Sonneratia* spp.) that contains cellulose and amylum. Cellulose usually is needed as nutrition by some organism, i.e., fungi, protista and bacteria [5]. Cellulolytic bacteria is a group of bacteria that have cellulase enzyme, and this enzyme plays a role in the degradation process of cellulose to become the glucose. The cellulose degradation ability could be different among each cellulolytic bacteria species [6]. Each amylolytic bacteria's ability to amylum degradation could be different between each other, so it is important to analyze the cellulose and amylum degradation ability of the cellulolytic and amylolytic bacteria isolated from decayed mangrove stem. This research was done to identify and determine whether the bacteria was cellulolytic or amylolytic and to determine its cellulase and amylase activity.

2. Materials and Methods

2.1 Materials

Decayed mangrove stem from Waai seashore, 0.1% peptone solution, nutrient agar medium, nutrient broth medium, carboxymethyl cellulose (CMC) plate medium, amylum agar (AA), plate medium, 0.1% Congo red solution, 1 M NaCl solution, CMC sigma medium, amylum broth medium were used in this experiment.

2.2 Methods

The decayed mangrove stem samples were taken from 10 locations with 5 g each. Then, 25 g of mixed-up samples was diluted in 225 mL of 0.1% peptone solution, and homogenized in 100 rpm shaker incubator for 24 h. The homogenized samples were then diluted at 10^{-1} to 10^{-6} , inoculated on nutrient agar (NA) plate medium and incubated in 37 °C for 24 h.

Each colony that grew on NA plate was isolated and determined their cellulose hydrolytic ability on CMC streak medium and in 37 °C for 24 h. After incubation on CMC medium, each colony was stained using 1% Congo red for 15 min and then washed with 1 M NaCl [2, 7]. Clear zone in surrounding of the bacteria colony indicates that the bacteria have an ability to hydrolyze the cellulose. For amylolytic bacteria identification, the samples were incubated on AA medium and stained with iodine solution. Those identifications were using Microbact™ system 12B/24E identification kits. The cellulolytic activity determination was analysed using the glucose DNS method and the amylolytic activity determination was analysed using spectrophotometry method.

3. Results and Discussion

3.1 Isolation of the Indigenous Bacteria from Decayed Mangrove Stem Waste Product

The isolation result of indigenous bacteria shows that there are eight bacteria isolates encoded as b, f, k, m, C, D, K and L. Each bacteria isolate was

determined to know its cellulolytic and amylolytic activities.

3.2 Determination of the Cellulolytic and Amylolytic Bacteria Species

Congo red staining results of each bacteria colony on CMC media showed that there are four cellulolytic bacteria isolates, of which each colony was surrounded by a clear zone (Fig. 1). Iodine test results showed that there are four amylolytic bacteria colonies, of which each colony was surrounded by a clear zone (Fig. 2).

The analysis result show that there are four cellulolytic bacteria species, i.e., *Micrococcus luteus*, *Bacillus cereus*, *Planococcus citreus* and *Bacillus pumilus*. Besides, there are four amylolytic bacteria species, i.e., *Bacillus firmus*, *Nitrobacter* sp., *Bacillus mycoides* and *Pseudomonas aeruginosa*. The identification results for each cellulolytic and amylolytic bacteria isolates were shown in Table 1.

3.3 The Cellulase and Amylase Enzyme Activity Analyses Result

The cellulase activity of each cellulolytic bacteria was analyzed to know the bacteria species that have the highest cellulase activity. The amylase activity of each amylolytic bacteria was also analyzed to know the bacteria species that have the highest amylase activity. The cellulolytic bacteria species that have the highest cellulolytic activity is *Bacillus cereus* with cellulase activity value of 47.307 IU/g (Table 2). The amylolytic bacteria species that have the highest

amylolytic activity is *Nitrobacter* sp. with the amylase activity value of 79.983 IU/g (Table 3).

Based on the cellulase and amylase activity analyzes result, *Bacillus cereus* has the highest cellulase activity value and *Nitrobacter* sp. has the highest amylase activity value.

The decayed mangrove stem at the shore would contribute to the environment pollution. However, the decayed mangrove stem waste product could be degraded by the soil insects, and then continued by the indigenous bacteria that live in the decayed mangrove

Fig. 1 Cellulolytic bacteria colonies on CMC medium. Black arrow points the clear zone surrounding the cellulolytic colony.

Fig. 2 The amylolytic bacteria colonies on AA Medium. Yellow arrow points the clear zone surrounding the amylolytic colony.

Table 1 The cellulolytic and amylolytic bacteria species from decayed mangrove stem waste product at Waai seashore, Ambon island.

Number	Isolate code	Species	Character	
			Cellulolytic	Amylolytic
1	C	<i>Micrococcus luteus</i>	+	-
2	D	<i>Bacillus cereus</i>	+	-
3	K	<i>Planococcus citreus</i>	+	-
4	L	<i>Bacillus pumilus</i>	+	-
5	b	<i>Bacillus firmus</i>	-	+
6	f	<i>Nitrobacter</i> sp.	-	+
7	k	<i>Bacillus mycoides</i>	-	+
8	m	<i>Pseudomonas aeruginosa</i>	-	+

Table 2 The cellulase activity of each cellulolytic bacteria.

Number	Isolate code	Species	Cellulase activity (IU/g)
1	C	<i>Micrococcus luteus</i>	41.501
2	D	<i>Bacillus cereus</i>	47.307
3	K	<i>Planococcus citreus</i>	35.922
4	L	<i>Bacillus pumilus</i>	44.517

Table 3 The amylase activity of each amylolytic bacteria.

Number	Isolate code	Species	Amylase activity (IU/g)
1	b	<i>Bacillus firmus</i>	65.604
2	f	<i>Nitrobacter</i> sp.	79.983
3	k	<i>Bacillus mycoides</i>	55.026
4	m	<i>Pseudomonas aeruginosa</i>	77.554

stem. The decayed mangrove stem contains compounds, i.e., cellulose and amylum. These compounds are needed by some bacteria species that have an ability to degrade cellulose and amylum. This research result showed that there are four indigenous cellulolytic bacteria species, i.e., *Micrococcus luteus*, *Bacillus cereus*, *Planococcus citreus* and *Bacillus pumilus*. *Bacillus cereus* is potential for cellulose hydrolysis shown by its highest cellulase activity (47.307 IU/g) compared to other isolates.

The last research result about indigenous cellulolytic bacteria species in sago waste product at Susupu, North Moluccas also proved that *Bacillus cereus* is an indigenous cellulolytic bacteria [3]. Commonly, the *Bacillus* genera could be found in the soil and water, including sea water [8]. This research also proved that *Bacillus cereus* is an indigenous bacteria that lives in decayed mangrove stem waste product at Waai seashore, Ambon island. The cellulolytic bacteria could produce a cellulase enzyme. This enzyme is diffused in the substrate that contains cellulose to degrade the cellulose to cellobiose, and then the cellobiose was degraded to be glucose-1-phosphate [9]. Glucose-1-phosphate is more soluble than cellulose, so this biodegradation process could help to eliminate the waste product that contains cellulose.

There are four indigenous amylolytic bacteria species, i.e., *Bacillus firmus*, *Nitrobacter* sp., *Bacillus mycoides* and *Pseudomonas aeruginosa*. *Bacillus firmus* and *Bacillus mycoides* could be found in the

soil and salty soil [8]. In this research, these bacteria is also found as an indigenous bacteria that lives in decayed mangrove stem waste product at seashore soil Waai, Ambon island. Amylolytic bacteria could produce an amylase enzyme that takes a role as a biocatalyzer in biodegradation process of amylum to maltose and then the maltose is degraded to be glucose by maltose enzyme. Glucose is more soluble than amylum, so this biodegradation process could help to eliminate the waste product that contains amylum. *Bacillus mycoides* could produce a maltose enzyme [8], so this bacteria has an ability to degrade maltose to be glucose. Each amylolytic bacteria species has a difference amylolytic activity value, and *Nitrobacter* sp. has the highest amylase activity value of 79.983 IU/g.

The cellulolytic bacteria as well as the amylolytic bacteria species that found in the decayed mangrove stem waste product at Waai seashore, Ambon island, could be used in the biodegradation process of cellulose and amylum compounds. These bacteria species could also be used for degrading another waste product that contains cellulose and amylase. Based on this fact, actually the decayed mangrove stem waste product problem could be solved by using the indigenous cellulolytic and amylolytic bacteria species. So, it is important to protect the seashore environment from pollutants, especially toxic substances, i.e., detergent, oil, etc., so that the indigenous bacteria which take a role in the waste product degradation could live optimally.

4. Conclusions

There are four cellulolytic bacteria species, i.e., *Micrococcus luteus*, *Bacillus pumilus*, *Planococcus citreus* and *Bacillus cereus*. *Bacillus cereus* has the highest cellulase activity value of 47.307 IU/g compared to other isolates. There are four amylolytic bacteria species, i.e., *Bacillus firmus*, *Nitrobacter* sp., *Bacillus mycoides* and *Pseudomonas aeruginosa*. *Nitrobacter* sp. has the highest amylase activity value of 79.983 IU/g compared to other isolates.

Based on this research results, the authors recommend *Bacillus cereus* for bioremediation process of waste products that contain cellulose compound, and *Nitrobacter* sp. for bioremediation process of waste products that contain amylose compound.

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