Screening and Identification of Cellulose□Degrading Bacteria from The Mangrove Areas of Sembilang National Park South Sumatera

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Screening and Identification of Cellulose-Degrading Bacteria from The Mangrove Areas of Sembilang National Park South Sumatera

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***Abstract*— The aim of the present study was to isolate and characterize the cellulose-degrading bacteria from the mangrove areas of Sembilang National Park South Sumatera. The bacteria capable of growing in the liquid medium containing cellulose as the only source of carbon were isolated and their cellulolytic activity on CMC-containing media was confirmed by the congo red clearing zone assay. The isolates were identified based on colony and cell morphological characteristics and biochemical characteristics. The results of the present study show that 12 cellulose-degrading bacteria isolated from the mangrove areas of Sembilang National Park belonged to the species *Micrococus* sp.(S_1SS_1), *Acinetobacter* sp.(S_1SS_2), *Bacillus* sp. (S_1SS_3), *Bacillus* sp. (S_1TS_1), *Clostridium* sp.(S_1SS_4), *Aerococus* sp. (S_1TS_3) dan *Pseudomonas* sp. (S_1TS_5), *Pseudomonas* sp. (S_2TS_2) *Clostridium* sp. (S_2SS_3), *Pseudomonas* sp. (S_3SS_1), *Clostridium* sp. (S_3SS_3), and *Clostridium* sp. (S_3TS_1).**

***Keywords* : cellulose-degrading bacteria, identification, mangrove areas**

I. INTRODUCTION

Indonesian territory consists of 17,508 islands and has a long coastline of about 81,000 km , is the country that has the largest mangrove forest in the world. Extensive mangrove forests in Indonesia reached 4.25 million hectares is the largest mangrove in the world beyond Brazil (1.3 million ha) , Nigeria (1.1 million ha) and Australia (0.97 ha) [11]. One Indonesian mangrove areas which Sembilang National Park located in South Sumatera . According Suwignyo *et al*.[9], various types of mangrove genera that dominat in Sembilang National Park were *Rhizophora, Avicennia, Sonneratia* and *Bruguiera*. According to Noor *et al*.[6], the mangrove ecosystem is an interface between terrestrial and ocean ecosystem. Biodiversity level of mangrove ecosystem was high so that mangrove can grow well. Mangrove forest ecosystems that are capable of producing a high organic matter, 90 % of organic particles in the water coming from the mangrove vegetation and result in 35-60 % of nutrients that are beneficial to the growth of mangroves. Constituent components of the organic matter is cellulose, hemicellulose and lignin .

The importance of microbial diversity in mangrove ecosystems due to the presence of microbes capable of providing nutrients in a mangrove ecosystem that can grow well without organic fertilizer. Microbial diversity in mangrove ecosystems have each function in degrading organic materials for the growth of mangroves. Thus in conserving the mangrove ecosystem, information and benefit of microbes was required such as cellulose-degrading and lignin-degrading bacteria that play role in litter decomposition in a mangrove ecosystem benefits [8]. The aims of the research was to isolate and characterize cellulose-degrading bacteria in mangrove litter and soil, in Sembilang National Park, South Sumatera.

II. MATERIALS AND METHODS

Sample of mangrove litter and soil bacterial isolates were taken from a mangrove forest area Sembilang National Park, South Sumatera. Soil samples as top soil were taken from 20 cm depth at 3 stations with purposive sampling method. In each station, samples were taken at 5 points of soil sampling to represent each study sites. Samples taken in the form of litter collected for 1 day at amount 5 grams of finely ground leaf litter and 5 grams of soil in each erlenmeyer then put in 45 mL distilled water respectively. Serial dilutions to a concentration of 10^{-6} were performed. One mL sample was taken at the last 3 serial dilutions and grown on CMC (Caboxy Methyl Cellulose) medium for growth of cellulose-degrading bacteria in a petri dish with pour plate method, and then incubated at 37°C for 24-48 hours [2].

Selection is done by taking the pure bacterial isolates using a needle inserted in the center of the loop that has solid medium in a petri dish, and then incubated at 37°C for 2 days. Bacteria were grown on selective media cellulolytic after spilled congo red and 1M NaCl to form a clear zone is a zone of cellulose-degrading bacteria [2].

III. RESULTS AND DISCUSSION

Based on the research conducted showed cellulose-degrading bacteria isolates of mangrove litter and soil in Sembilang National Park area as in Table 1.

Thirty pure isolates pure taken from isolation of cellulose-degrading bacteria were isolated using CMC medium (Table 1), because CMC medium used is the best substrate to induce the synthesis of extracellular cellulolytic enzymes. CMC is a synthetic substrates that serve as model compounds of cellulose. CMC has many amorphous regions so soluble in water. CMC concentration used

was 1 %, according to research Narasimha *et al*. (2005), 1 % cellulose concentration is the optimum concentration for the production of cellulase enzymes. The medium used for bacterial growth substrate is a suitable medium for the growth of bacteria [2].

Table 1. Cellulose-degrading bacteria isolates

Station	Sample	Number of cellulose-degrading bacteria isolates
I $104^0$54'13,2''E $2^0$9'25,5"S	Litter	6
	Soil	5
II $104^0$53'41,4"E $2^0$5'43,6"S	Litter	4
	Soil	4
III $104^0$54'18"E $2^0$9'47,4"S	Litter	7
	Soil	4
Total		30

Table 2.
Screening result of cellulose-degrading bacteria

Station	Sample	Screening result of cellulose-degrading bacteria	Cellulose-degrading bacteria isolates code
I $104^0$54'13,2''E $2^0$9'25,5"S	Litter	4	S_1SS_1, S_1SS_2, S_1SS_3, S_1SS_4
	Soil	3	S_1TS_1,S_1TS_3, S_1TS_5
II $104^0$53'41,4"E $2^0$5'43,6"S	Litter	1	S_2SS_3
	Soil	1	S_2TS_2
III $104^0$54'18"E $2^0$9'47,4"S	Litter	2	S_3SS_1, S_3SS_3
	Soil	1	S_3TS_1
Total		12	

Screening of cellulolytic bacteria used selective medium are characterized by the formation of clear zone after spilled congo red dye that is used as an indicator of cellulolytic bacteria. Formation of a clear zone is due cellulolytic bacteria capable of hydrolyzing cellulose into simple compounds. The formation of clear zone indicates that the cellulose contained in the media is hydrolyzed by cellulase enzymes into simple compounds that cellobiose is then simplified into two molecules of glucose [6].

Clear zone can be formed by washing use 1M NaCl. Clear zone will be clearly after congo red addition. Congo red is the sodium salt of benzidinediazo bis - 1 - naphthylamine - 4-sulfonic acid ($C_{32}H_{22}N_6Na_2O_6S_2$) so that the dye will dissolve and leached by other sodium salts, such as NaCl . Thus , the clear zone is formed will be clearly. Formation of clear zone indicates that the bacteria are able to degrade cellulose [8].

Based on the characteristics obtained, each bacterial isolate can be grouped into several genera were identified [1] [4]. Each cellulolytic bacteria belongs to the genera *Micrococus, Ancinetobacter, Bacillus, Clostridium, Pseudomonas,* and *Aerococus*.

Isolates of bacteria belonging to the genus *Micrococus* is S1SS1. The bacterial isolates have characteristic shape cocci cells, negative staining and aerobic endospores. The characteristics of the genus *Micrococcus* spherical cell shape , nature of gram-positive, negative indole test, catalase test positive, does not produce acid fermentation of carbohydrates, is motile due to its flagella as locomotor and there is also nonmotil. This genus can be isolated from soil, water and food products. Isolates of bacteria belonging to the genus *Acinetobacter*[1]. Genus *Acinetobacter* discrete forms of cocci, gram-negative, non motile, growth requires oxygen (aerobic), not produce endospores, biochemical test results were positive in catalase test and negative on indole and H_2S test, can be isolated of soil, water and litter decomposition [4].

Other genera that found *Bacillus* sp. (S1SS3), *Bacillus* sp. (S1TS1), *Bacillus* sp. (S1SL2) , *Bacillus* sp. (S1SL4) and *Bacillus* sp. (S2TL1). The fifth *Bacillus* genera have characteristics as bacillus form, gram-positive, catalase test positive, and form endospores. Differences in biochemical test on each isolate would classify the genus *Bacillus* isolates at the species level. Genus *Bacillus* have characteristics such as bacillus shape, straight, long and short, gram-positive, motile but there are a few non- motile, form endospores oval, spherical, cylindrical and resistant to a wide range of conditions, aerobic and facultative anaerobic, catalase test positive, are pathogenic in vertebrates and invertebrates, its broad habitat, and can be found in water and soil [1] [4].

Isolates S1SS4 , S2SS3 , S3SS3 , S3TS1 , and S3TL1 belonging to the genus *Clostridium*. They have bacillus shaped *Clostridium* characteristics, anaerobic, gram positive, and form endospores. *Clostridium* discrete cell shape in general, but there are a few bacilli, cocci, gram-positive, motile with flagella peritric partially and sometimes non-motile, anaerobic, form endospores, and negative catalase test, found in water, soil, also in human skin. The difference in the physiological test each isolate in the genus *Clostridium,* enabling this genus can be grouped in the species level [1] [4].

Isolates S1TS3, S1TL5 and S3SL3 belong to the genus *Aerococus*. This genus generally have characteristics of gram-positive, non motile and catalase test negative. *Aerococus* belong to the gram- positive, non motile, facultative anaerobic and some are anaerobic, not produce catalase, so based on common characteristics of these three isolates were grouped in a single genus. However, the differences in the nature of the physiological test, *Aerococus* this genus can be further grouped based on species level [1].

Besides *Aerococus* also found the genus *Pseudomonas* isolates S1TS5, S2TS2, S3SS1, S1SL5, S2SL1, S2SL2 and S3SL2. These isolates differed in physiological tests can be grouped by allowing the species level . However , similarity of the characteristic of these isolates, like flagella are motile, gram negative and positive catalase test that are grouped into a single genus. This is consistent with [1] and [4] *Pseudomonas* has the characteristics bacillus form single or in groups, motile with flagella lies opposite, gram-negative, aerobic and facultative anaerobic, catalase test positive, can be found in soil, water and sea. Genus of bacteria most commonly found is the genus *Pseudomonas* as mention by Rao [8] that genus of microorganisms that can degrade cellulose and lignin is the genus *Pseudomonas*.

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Appendix 1 **Tabel 3. The results of characterization and identification of cellulose degrading bacteria**

Isolate Character		S_1SS_1	S_1SS_2	S_1SS_3	S_1SS_4	S_1TS_1	S_1TS_3	S_1TS_5	S_2SS_3	S_2TS_2	S_3SS_1	S_3SS_3	S_3TS_1
Macroscopic colony morphology		Plumose Echinulate Filamentous Ramose Convex Papilate White	Plumose Echinulate Comensal Undulate Umbonate Cream	Effuse Villous Crenate Undulate Convex Papilate Cream	Beaded Villous Crenate Lobate Raised with Concave Brown	Filliform Villous Circular Entire Effuse white	Plumose Beaded Circular Entire Convex Yellow	Filliform Villous Circular Entire Convex Orange	Filliform Villous Irregular & spreading Crenate Convex Rugose White	Spreading Echinulate Irregular & spreading Crenate Umbonate Cream	Plumose Beaded Circular, filamentous Crenate Raised with Concave Cream	Filliform Villous Circular Entire Convex Brown	Spreading Villous Irregular, spreading Crenate Convex Papilate Cream
Microscopic cell morphology		Coccus, Gram positive, did not produce spore	Coccus Gram negative, did not produce spore	Bacillus, Gram positive, produce spore	Coccus, Gram positive, produce spore	Bacillus, Gram positive, produce spore	Bacillus, Gram positive, did not produce spore	Bacillus, Gram negative, did not produce spore	Bacillus, Gram positive, produce spore	Bacillus, Gram negative, did not produce spore	Bacillus, Gram negative, did not produce spore	Bacillus, Gram positive, produce spore	Bacillus, Gram positive, produce spore
O_2 needed		Aerob	Aerob	Anaerob Fakultatif	Anaerob	Anaerob Fakultatif	Anaerob Fakultatif	Anaerob Fakultatif	Anaerob	Anaerob Fakultatif	Anaerob	Anaerob	Anaerob
Motility test		+	-	+	-	+	-	+	-	+	+	-	+
Biochemical test :													
Simmon's citrate test		+	+	-	-	+	+	-	+	-	+	-	+
Indole production		-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis		+	+	+	+	-	+	-	+	+	+	+	+
Urea hydrolysis		-	-	-	-	-	-	-	+	-	-	-	-
Methyl-red test		+	+	+	-	-	-	-	+	+	+	-	+
Voges Proskauer test		-	-	-	-	-	-	-	-	-	-	-	-
Catalase production		+	+	+	-	+	-	+	-	+	+	-	-
Glucose Fermentation	Gas	+	+	+	+	+	-	+	+	+	+	+	+
	Acid	-	-	-	-	-	+	+	-	-	-	-	+
Lactose Fermentation	Gas		+	+	+	+	+	+	+	+	+	+	+
	Acid		+	+	+	+	+	+	+	+	+	+	+
Sucrose Fermentation	Gas												
	Acid												
H_2S and gas production	Gas	+	+	+	+	+	+	+	+	+	+	+	-
	Sugar fermentation	+	+	-	-	-	-	-	-	+	-	+	+
	H_2S	-	-	-	-	+	+	-	-	-	+	-	-
CONCLUSION		***Micrococus* sp. (S_1SS_1)**	***Ancinetobacter* sp. (S_1SS_2)**	***Bacillus* sp. (S_1SS_3)**	***Clostridium* sp. (S_1SS_4)**	***Bacillus* sp. (S_1TS_1)**	***Aerococus* sp. (S_1TS_3)**	***Pseudomonas* sp. (S_1TS_5)**	***Clostridium* sp. (S_2SS_3)**	***Pseudomonas* sp. (S_2TS_2)**	***Pseudomonas* sp. (S_3SS_1)**	***Clostridium* sp. (S_3SS_3)**	***Clostridium* sp. (S_3TS_1)**

Notes: (+) : Positive (-) : Negative

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