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PEATLAND BACTERIA AS ALTERNATIVE SOURCES FOR CELLULASE

(BAKTERI GAMBUT SEBAGAI SUMBER ALTERNATIF SELULASE)

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Abstract

This research has been conducted to isolate and identify cellulolytic bacteria from peatland in Pontianak, Kalimantan Barat. We found that 9 of 18 bacterial isolates possessed cellulolytic activity. Active isolates showed hydrolytic zone around bacterial colonies on agar plates when they was grown on media containing of 1% of carboxymethyl cellulose (CMC). Three isolates, namely *Yersinia pseudotuberculosis* RAG21, *Yersinia pseudotuberculosis* RAG25, and *Proteus penneri* RAG31 displayed largest diameter ratio of hydrolytic zone to colony, which were 2.73±0.15, 2.61±0.15, and 4.14±0.30 respectively. The cellulase produced and exhibit activity in acidic medium containing humic substances.

Keywords: peatland bacteria, humic, cellulase, *Yersinia pseudotuberculosis*, *Proteus penneri*

Abstrak

Riset ini dilakukan untuk mengisolasi dan mengidentifikasi bakteri dari tanah gambut Kota Pontianak, Kalimantan Barat. Sebanyak 9 dari 18 isolat bakteri diketahui memiliki aktivitas selulolitik. Isolat aktif tersebut menunjukkan terbentuknya zona hidrolisis di sekitar koloni bakteri ketika ditumbuhkan pada media mengandung karboksimetil selulosa 1%. Tiga isolat yaitu *Yersinia pseudotuberculosis* RAG21, *Yersinia pseudotuberculosis* RAG25, dan *Proteus penneri* RAG31 menunjukkan rasio diameter zona hidrolisis terhadap koloni, dengan rasio sebesar 2,73±0,15, 2,61±0,15, dan 4,14±0,30 secara berturut-turut.

Kata Kunci: bakteri gambut, humat, selulase, *Yersinia pseudotuberculosis*, *Proteus penneri*

INTRODUCTION

Bioethanol grows more consideration to replace non-renewable petroleum-based fuels as major energy resources. Bioethanol was produced through fermentation carbohydrate substrates such as sucrose, starch (1) and cellulose (2, 3). Cellulose is the most promising substrate due to its abundance in most of plant biomass. However, extensive pretreatment in acid is needed to hydrolyze β-glycosidic bond in cellulose (4).

β-glycosidic bond can be hydrolyzed in mild condition by cellulase (5). Cellulase is hydrolytic enzyme produced by fungi and few bacteria. Bacteria were minor sources of cellulase, but often they provide cellulase possessing interesting characteristic, for example heat resistance or extreme pH resistance (6, 7). These characteristic are significant for cellulase implementation in bioethanol industries. Cellulase-producing bacteria can be isolated from soil. Cellulomonas sp.

isolated from soil in Indonesia produced cellulase which was able to hydrolyze simple cellulose substrate such as carboxymethyl cellulose, avicell, and whatman paper, or more complex substrate from agro-industrial wastes (8).

A peatland is an area is an area with or without vegetation with a naturally accumulated peat layer at the surface (9). Peat is highly organic content and very acidic environment which allow only limited number of bacteria to grow (10, 11). However, few bacteria was able to inhabit peatland, notably *Arthrobacter* sp., *Aquaspirillum* sp., *Cellulomonas* sp., *Curtobacterium* sp. dan *Rhodococcus* sp. (12). These bacteria lived by degrading organic substances in peat using hydrolytic enzyme, for example cellulase (13, 14), xylanase dan protease (15).

In this researched, we explored peatland in Pontianak, Indonesia to isolate cellulase-producing bacteria. We expected to find acidic resistances which was able to produce cellulase.

MATERIALS AND METHODS

Materials

Materials that were used in this research are peptone, yeast extract, iodine (I_2), potassium iodide (KI), natural humic water, and sets of biochemical test kits for bacterial identification. Natural humic water was collected during samples collection in runnel which located 1 m from sample collection spot. Set of biochemical test kits was provided by Clinical Laboratory Unit Kalimantan Barat Province, Indonesia. Other chemical was purchased from Merck Millipore. Solid and broth mediums for bacterial growing were modified from nutrient agar medium (NA) (16). First, one part nutrient medium mixed with nine part of distilled water and supplemented with 1%CMC (NA^{-1}+1%CMC). Second, nutrient medium enriched by natural humic water (NG). Third, one part NG mixed with nine parts of natural humic water (NG^{-1}). Fourth, NG^{-1} supplemented with 1% of CMC (NG^{-1}+1%CMC).

Procedure

1. Bacteria isolation from peat

Peat was collected from peatland at April 17th 2014 in Parit Tokaya village, South Pontianak District, Pontianak, Indonesia. It was dug 5-10 cm underground and then was put on aseptic bottle. The bottles were kept on ice bottle for transferring onto laboratory. During sample collection, temperature and pH were measured and was about 31°C and 4.01.

In the laboratory, peat was suspended onto serial dilution of aseptic natural humic water. The suspensions were inoculated on solid NG medium and incubated for 5 days. Each day during incubation colonies formed were observed. Each distinctive colony was purified for the next step.

2. Cellulase-producing bacteria screening

Screening of cellulase-producing bacteria was carried out according to previous method on variety of medium (17). Bacteria were grown onto solid medium and incubated for 2 days. After 2 days, the medium was flooded by gram iodine reagent for 3-5 minutes. Colony of cellulase-producing bacteria formed clear hydrolytic zone around the colony. Colony and hydrolytic zone diameter were measured. Cellulase activity was determined based on ratio of hydrolytic zone diameter to colony diameter.

3. Identification of cellulase-producing bacteria

Promising cellulase-producing bacteria were identified based on macroscopic and microscopic examination, as well as biochemical characterization. Microscopic test included gram staining and shape. Biochemical tests included carbohydrate utilization, catalase activity, oxidase activity, aerobic and anaerobic fermented oxidation test of glucose, indole, H2S, urease, citrate utilization, motility, decarboxylase activity and growth test in TCBS medium. The result of

macroscopic, microscopic and biochemical characters were compared to the identification keys on *Bergey's Manual of Systematic Bacteriology*.

RESULTS

Bacteria Isolation from Peatland

Based on shape, diameter size, elevation, and margin, we isolate 18 bacteria from peatland in Pontianak, Indonesia. Isolates were encoded RAG01 RAG01, RAG02, RAG03, RAG19, RAG20, RAG21, RAG22, RAG23, RAG24, RAG25, RAG26, RAG27, RAG28, RAG29, RAG30, RAG31, RAG32, and RAG33. Mostly isolates were aerobic, while only RAG02 isolate was anaerobic and grew under medium. The shape, majorly circular, with colony diameter around less than 1 mm to 4 mm (Table 1).

Table 1 Morphology of isolate colonies

Code	Shape	Diameter	Color	Margin	Elevation
RAG01	circular	Less than 1,0 mm	White	Entire	Convex
RAG02	circular	Less than 1,0 mm	White	Entire	Convex
RAG03	circular	2,0 mm	White	Entire	Convex
RAG19	circular	2,0 mm	Cloudy	Wavy	Convex
RAG20	circular	3,0 mm	White	Filiform	Convex
RAG21	circular	2,0 mm	White	Wavy	Convex
RAG22	circular	3,0 mm	Cloudy	Entire	Convex
RAG23	circular	3,0 mm	Cloudy	Entire	Convex
RAG24	Irregular	4,0 mm	Yellowish white	Filiform	Convex
RAG25	Circular	Less than 1,0 mm	White	Entire	Convex
RAG26	Circular	3,0 mm	White	Entire	Convex
RAG27	Circular	3,0 mm	Translucent	Entire	Convex
RAG28	Irregular	3,0 mm	Yellowish white	Filiform	Convex
RAG29	Circular	4,0 mm	White	Filiform	Convex
RAG30	Irregular	1,0 mm	White	Entire	Convex
RAG31	Circular	3,0 mm	Cloudy	Entire	Convex
RAG32	Circular	Less than 1,0 mm	White	Entire	Convex
RAG33	Circular	2,0 mm	White	Entire	Convex

Cellulase-producing bacteria screening

The result showed that 9 of 18 isolates were able to perform cellulase activity after incubated in solid NG^{-1}+1% namely, RAG01, RAG02, RAG20, RAG21, RAG23, RAG25, RAG29, RAG31, and RAG33. The highest ratio of hydrolytic zone diameter to colony diameter was possessed by RAG31 that was 4.14±0.30 (Table 2).

Table 2 Ratio of Hydrolytic Zone Diameter to Colony Diameter

Isolates	Ratio of Hydrolytic Zone Diameter to Colony Diameter	
	Average	Standard Deviation
RAG01	2,51	0,04
RAG02	2,70	0,12
RAG20	1,00	0,00
RAG21	2,73	0,15
RAG23	1,00	0,00
RAG25	2,61	0,15
RAG29	1,20	0,25
RAG31	4,14	0,30
RAG33	1,67	0,14

Identification

Identification results of three isolates, namely RAG21, RAG25 and RAG31, involved morphology and biochemical characteristic were displayed in Table 2. The data was compared to Bergey's Manual of Determinative Bacteriology, so we can conclude that RAG31 was *Proteus penneri*, while RAG21 and RAG25 were *Yersinia pseudotuberculosis*.

Table 3 Identification of Bacteria

Morphology and Biochemical Characteristic	RAG21	RAG25	RAG31
Gram Stain	negative	negative	negative
Cell shape	basil	basil	basil
Motility	immotile	immotile	motile
Catalase	positive	positive	positive
Oxydase	negative	negative	negative
Catabolism of Carbohydrates and derivative			
a. Glucose	positive	positive	positive
b. Lactose	negative	negative	negative
c. Maltose	positive	positive	positive
d. Mannitol	negative	negative	negative
e. Sucrose	positive	positive	positive
Citrate test	negative	negative	negative
Indol test	negative	negative	negative
Urea test	positive	positive	positive
H_2S test	negative	negative	negative
Decarboxylase test			
a. Lysine	negative	negative	negative
b. Arginine	negative	negative	negative
c. Ornithine	negative	negative	negative

DISCUSSION

This research was preliminary effort to explore cellulase produced by peatland bacteria. Our finding showed about a half of all isolates were produced cellulase. Active isolates produced cellulase

and released it to surrounding medium, so it might perform hydrolytic activity to break β-glycosydic bond in CMC to meet their requirement of carbon sources. This activity was observed by clear hydrolytic zone around isolates colony (Figure 1), while the rest of medium showed dark red color as the result of CMC-iodine complex formation (17). But, two of our cellulase producing isolates, RAG20 and RAG23 showed hydrolytic zone that were precisely fitting the colonies. We assumed that cellulase produced was not released on the surrounding environment. It possibly was bound on bacteria plasma membranes (18) or possessed very huge complex structure enzyme (cellulosome) (19), so it was not able to diffuse on the medium.

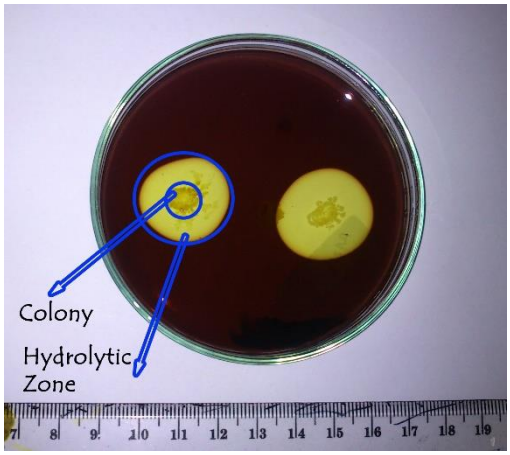

Figure 1: Hydrolytic zone

Three isolates were investigated thorough, namely *Yersinia pseudotuberculosis* RAG21, *Yersinia pseudotuberculosis* RAG25, and *Proteus penneri* RAG31. These isolates were also grown in media NA^{-1}+1%CMC to screen their cellulase-producing activity. The results showed that isolates were not not able to produce cellulase without supplementation of natural humic water. We expected humic substances play role in cellulase production. Our result was confirmed by previous report that humic substances might affect metabolism of bacteria and stimulated their growth (20-22). Some mechanism to explain humic substances interaction to organism has been investigated (23). Yet, their specific mechanism is still unclear.

Our investigation discovered peatland bacteria as alternative sources for cellulase. Cellulase was produced and exhibited activity in the NG^{-1}+1%CMC media contained humic substances. The media was acidic with 4 in pH. It is clear indication that cellulase worked in acidic environment.

CONCLUSION

In this research we isolated 9 cellulase-producing bacteria from peatland in Pontianak, Indonesia. The cellulase produced and exhibit activity in acidic medium containing humic substances. Further investigated are needed to characterize cellulase produced.

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