

Isolation and Identification of cellulolytic bacteria from mangrove sediment in Bangka Island

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Abstract. Cellulolytic bacteria is bacteria which hydrolyze cellulose to reducing sugars. This research aims to obtain cellulolytic bacteria from the sediment of mangroves in Bangka island. Research was conducted from March to August 2017. Sampling was conducted at Sungailiat, and Tukak Sadai, South of Bangka. Bacteria was isolated using 1% Carboxymethyl Cellulosa (CMC). The isolation resulted in four isolates from Sungailiat and nine isolates from Tukak Sadai. Total five isolates, namely *Bacillus pumilus*, *Pseudomonas* sp., *Bacillus amyloliquefacien*, *Bacillus alvei*, *Bacillus coagulans* were identified. The best isolates that produced cellulose was *Pseudomonas aeruginosa*.

1. Introduction

Estuaries ecosystem is a reservoir of microorganism which usually produces enormous and unique metabolites. Mangrove is an ecosystem found in tropical and sub-tropical regions. This ecosystem provides ecological benefits such as protection against tsunami danger, and oxygen supplier. Mangroves can store large quantities of organic carbon and sediments which mainly derive from litter falls and underground roots of mangrove plants [1]. Microbes in mangroves play an important role in the organic carbon cycle. Cellulolytic bacteria are bacteria which capable of degrading organic cellulose to simple sugar derivatives [2].



Cellulolytic bacteria obtained from nature are expected to be useful for degrading cellulose for both industrial and environmental conservation applications. Cellulolytic bacteria are abundant in mangrove ecosystem. They easily be found in mangrove forests [2, 3, 4]. The bacteria are usually *Pseudomonas fluorescens* var *cellulose*, *Cellulomonas fimi*, *Bacillus subtilis*, *Clostridium thermocellum*, *Acetobacter xylinum* [1].

Bangka Island is an area with mangrove-covering area for about 48.090 hectares. Nowadays, there is no exploration was recorded in this specific area. Contrastingly, study by Prihanto and Wakayama suggested that the ocean and coastal region is mainly unexplored source for enzymes [5]. In this area is also the location of tin mining. It activities surely affecting the physical and chemical characteristic of the mangrove ecosystem. Hence, by exploring this area, the unique enzyme will be achieved.

The objectives of this study was to investigate the potency of the mangrove sediment in Bangka Island as a source of cellulolytic bacteria. Further detail of the isolates would be identified using molecular method.

2. Materials and methods

2.1. Sampling sites

Mangrove sediment were collected from Sungailiat and Tukak Sadai, South Bangka district (figure 1.). Sample taking was done on the depth of 10-20 cm. The samples were packed and brought to the Laboratory using ice box by maintaining its temperature at 4°C.

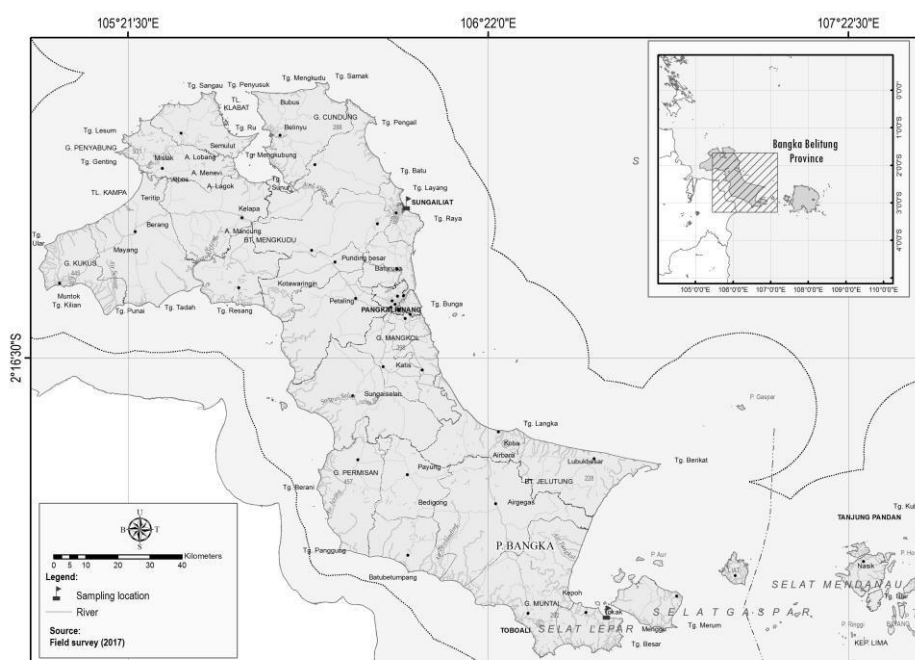


Figure 1. Sampling location in Sungailiat and Tukak Sadai.

2.2. Culture and screening of cellulolytic bacteria

Prior to culture, Mangrove sediments weighed and proper diluted. The diluted samples were grown onto 1% CMC (Carboxy Methyl Cellulose) medium with a pour plate method. One loop of bacteria is scratched on the medium by forming a line with approximately one cm. The culture was incubated for 72 hours at 30 ° C. The Lugol solution (2g of potassium iodine and 1g iodine in 300 ml of aquadest) was flooded to cover the entire medium and is allowed to stand for a minute. The isolates

formed clear zone accounted as cellulose-degrading bacteria. Further identifications were performed by using two different methods namely, Microbact and 16s rDNA method.

2.3. Biochemical characterization of bacteria

Biochemical characterization was performed using Microbact system following company manual procedures. In order to determine the bacterial identity, their biochemical characters would be compared to Bergey's Manual of Determinative Bacteriology.

2.4. Identification of bacteria molecular 16S rRNA

The protocol for the the identification of best producer of cellulose, the methods by Prihanto *et al.*, (2016) was used with slight modification. DNA isolation was performed using the procedure of the DNA Isolation Kit (Wizard of Genomic DNA Purification Kit from Promega). PCR composition with a total volume of 20 µl / tube consists of 6 µl ddH₂O, 10 µl PCR kit GoTaq® Green Master Mix (10 x taq polymerase buffer, dNTP, MgCl₂, primer, Taq DNA polymerase, ddH₂O), 1 µl forward primer, primary reverse 1 µl and 2 µl isolated DNA samples.

Primers for this analysis were 20F (52 - GTAATCGTCGGCCAGTA GAGTTTGATCCTGGCTC-32) and 1510R (52 - CAGGAA ACAGCTATGACCGGCTACC TTGTTACGACT-32). The sequence was BLAST (*Basic Local Alignment Search Tool*) analyzed. Phylogenetic tree was constructed using online phylogenetic analysis Phylogeny.fr.

3. Result and discussion

Thirteen isolates were successfully isolated from two locations. Nine bacterial isolates (TS1, TS2, TS3, TS4, TS5, TS6, TS7, TS8, TS9) were collected from Tukak Sadai and four isolates (SL1, SL2, SL3, SL4) from the Sungailiat mangrove samples. All thirteen isolates were further screened for its capability to hydrolyze CMC. The screening analysis suggested that only five isolates were cellulose positive bacteria (table 1).

Table 1. Screening of cellulose producing bacteria.

Isolates Code	Screening result	Diameter (mm)
TS1	+	3.4
TS2	+	4.6
TS3	-	-
TS4	-	-
TS5	-	-
TS6	-	-
TS7	+	4.2
TS8	-	-
TS9	-	-
SL1	-	-
SL2	+	3.6
SL3	+	4.1
SL4	-	-

From the result it can be concluded that five isolates were cellulose positive. Three isolates from the location of Tukak Sadai (TS1, TS2, TS7) and two isolates from Sungailiat (SL2, SL3). All positive isolates were further analyzed using microbact analysis system. This analysis based on the biochemical characters of the isolate. The result for the analysis was provided in table 2.

Table 2. Results of biochemical characterization and test of bacterial isolates.

Parameter	Result				
	TS1	TS2	TS7	SL2	SL3
Gram	+	+	+	+	+
Cell form	rod-shaped	rod-shaped	rod-shaped	rod-shaped	rod-shaped
Motility	-	+	-	-	-
Oxidase	-	+	-	-	-
Catalase	+	-	-	-	+
Citrate	-	+	-	-	-
TSIA	As/As,G-H2S-	Alk/As,G-H2S-	As/As,G-H2S-	As/As,G-H2S-	As/As,G-H2S-
VP	+	-	+	+	-
Spore	+	-	+	+	-
Nitrate	+	-	-	+	-
Lysin	+	+	-	+	+
Ornithin	-	-	-	-	-
H ₂ S	-	-	-	-	-
Glucosa	+	+	-	+	+
Mannitol	+	-	-	+	-
Xylose	-	-	-	-	-
ONPG	-	+	+	-	+
Indole	-	-	-	-	-
Urease	-	-	-	-	-
V-P	-	-	-	-	-
TDA	-	-	-	-	-
Gelatin	-	-	-	-	-
Malonat	-	-	-	-	-
Inositol	-	-	+	-	-
Rhamnose	-	-	-	-	-
Sucrosa	+	-	-	+	-
Lactosa	-	-	-	-	-
Arabinose	+	+	-	+	+
Adonitol	-	-	-	-	-
Raffinose	-	-	-	-	-
Salicin	-	-	-	-	-
Arginin	-	-	-	-	-
Catalase	+	-	+	+	-
Coagulase	-	-	-	-	-
Hemolysis	β	α	β	β	α
Novobiosin sensitivity	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-

Casein hydrolysis	+	-	+	+	-
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The microbact analysis revealed that the five bacteria for TS1, TS2, TS7, SL2 and SL3 were predicted as *Bacillus pumilus*, *Pseudomonas* sp., *Bacillus amyloliquefacien*, *Bacillus alvei* and *Bacillus coagulant*. The best cellulose producer was *Pseudomonas* sp. Hence, only this isolate were further confirmed by using 16s rDNA analysis. The result of the identification revealed that *Pseudomonas* sp. was new strain of *Pseudomonas aeruginosa*. It has 91% similar identity of *Pseudomonas aeruginosa* strain ZJHG29. Hence, we named this isolate as *Pseudomonas aeruginosa* UBB. Phylogenetic tree of this isolate was depicted in figure 2.

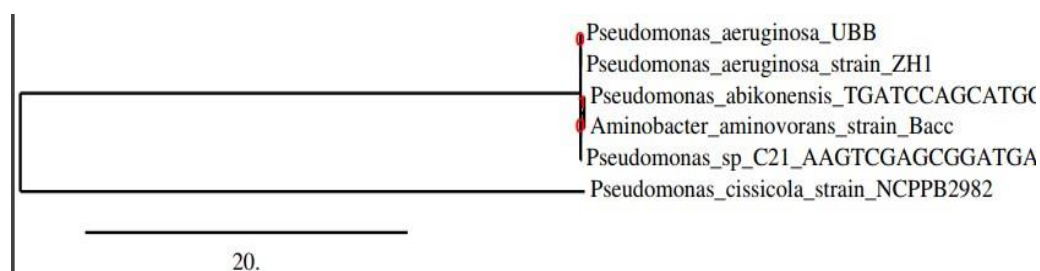


Figure 2. Phylogenetic tree of *Pseudomonas aeruginosa* UBB.

Bacillus bacteria in mangroves is important to degrade organic matter into nutrients. This is supported by the statement which explains that *Bacillus* bacteria are a phosphate decomposer. Phosphate (P) decomposing bacteria play a role in the re-provision of phosphate compounds in the seagrass ecosystem by decomposing the litter. The bacteria will enzymatically decompose the cellulose material through the active role of proteolytic, cellulolytic and chitinolytic enzymes [9]. The Bacillaceae (60 %) family is the predominant family in mangrove rhizospheres in the southern part of the Gulf of Kachchh, Gujarat and followed by the Rhodobacteraceae family constituting of 11.43 %; Micrococcaceae constitutes 8.57%; and the Nocardiaceae and Pseudoalteromonadaceae families constitute 5.71 % [10].

Cellulolytic bacteria have an important role in the nutrients providing for mangrove. Bacteria are an important source of cellulase with a variety of industrial and biotechnological applications. Non-haemolytic bacterial strains tolerant of various environmental pollutants (heavy metals and organic solvents) are identified as *Bacillus* sp [11].

The *Bacillus* genus were dominant of cellulolytic bacteria found in the mangroves ecosystem of Bangka Island in the biochemical characterization. They are *Bacillus pumilus*, *Bacillus amyloliquefacien*, *Bacillus alvei*, and *Bacillus coagulant*. *Bacillus* sp effectively reduced the crude fiber of palm oil cake from 17.74 % to 5.8 % through a fermentation process [12, 13]. *Bacillus pumilus* which was isolated from the mangrove ecosystem was identified with the potential production highest in chitinase [14]. *Bacillus pumilus* bacteria can be utilized in rumen cow fermentation to enrich the culture media of zooplankton [15].

The results of 16s rDNA analysis revealed that the bacteria is *Pseudomonas aeruginosa* UBB. The bacteria was closely related to the *Pseudomonas aeruginosa* strain ZH1. *Pseudomonas aeruginosa* may be present in contaminated mangrove sediments as in the opinion [12], that the *Pseudomonas aeruginosa* strain KVD-HM52 was isolated from oil contaminated mangrove sediments which showed significant antifungal activity against *Fusarium oxysporum* wilt disease in tomato plants using the purified RL biosurfactant .

4. Conclusion

Cellulolytic bacteria can be found in Sungailiat and Tukak Sadai island. Five bacteria, namely *Bacillus pumilus*, *Pseudomonas* sp., *Bacillus amyloliquefacient*, *Bacillus alvei*, *Bacillus coagulant*. Molecular identification revealed that the best producer for cellulose is *Pseudomonas aeruginosa* UBB..

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