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Isolation and Screening of Cellulolytic Bacteria from Mangrove Waters in Mandeh, West Sumatera Province, Indonesia

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Abstract— Microbial communities in mangrove ecosystems produce cellulase enzymes, essential for breaking down cellulose, which are used in various applications. The objective of the research is to isolate and purify cellulolytics from the mangrove waters in Mandeh coast, West Sumatera Province, Indonesia. Thirteen sites were used for this study, and water samples were chosen at random. The findings demonstrated that 16 isolates exhibited cellulolytic activity, and 38 isolates that grew in nutrient agar were created by the purified bacteria. The common bacteria found have a high colony elevation, an undulating colony edge, an uneven colony form, and cream pigmentation. The most prevalent form of bacteria is the bacilli cell, and 16 of the 38 isolates exhibited cellulase activity after inoculation. The cellulolytic index (CI) ranged from 0.36 to 4.82, and the highest CI was PUA-18 (4.82), followed by PUA-38 (4.6), PUA-28 (3.74) and PUA-21 (3.61).

Keywords—Bacteria; Cellulase; Cellulolytic Isolation; Mangrove; Screening.

I. INTRODUCTION

The varied microbial communities found in the mangrove ecosystem, which is distinguished by high primary and secondary production, generate enzymes that are used in industry, agriculture, fisheries, and animal husbandry. [1]–[3]. Mangrove litter acts as the main organic food source for aquatic organisms, providing cellulose that supports the growth of cellulolytic microorganisms that produce cellulase enzymes. These cellulolytic bacteria, essential for breaking down cellulose, are typically found in cellulose-rich environments such as plants. [4].

Microbes produce extracellular enzymes to decompose complex organic nutrients into simpler components that can be absorbed by cells as nutrients. As a plentiful source of glucose, cellulose necessitates the enhancement of its decomposition by cellulolytic bacteria. Typically, cellulase is composed of a combination of three enzymes: exoglucanases, endoglucanases, and β-glucanases. [5]. Furthermore, cellulolytic bacteria that secrete cellulase enzymes are useful as crude degradations of animal feed raw materials [6]. Microbial enzymes tend to be more stable and possess a greater variety of properties compared to those obtained from plants and animals. In fisheries, breaking down cellulose in fish feed ingredients can enhance fish growth by improving feed digestibility. The populations and activities of these microbes are affected by the soil's physical and chemical traits, as well as the oceanographic conditions of the waters. [7].

Quantification of microbial populations as phosphate solvents, amylase producers, and cellulase enzyme producers can provide important information about organic matter decomposition and mineralization to increase the fertility and productivity of the mangrove ecosystem [8]. Previous studies have isolated ninety-nine bacteria from coastal mangrove sediment in Logending

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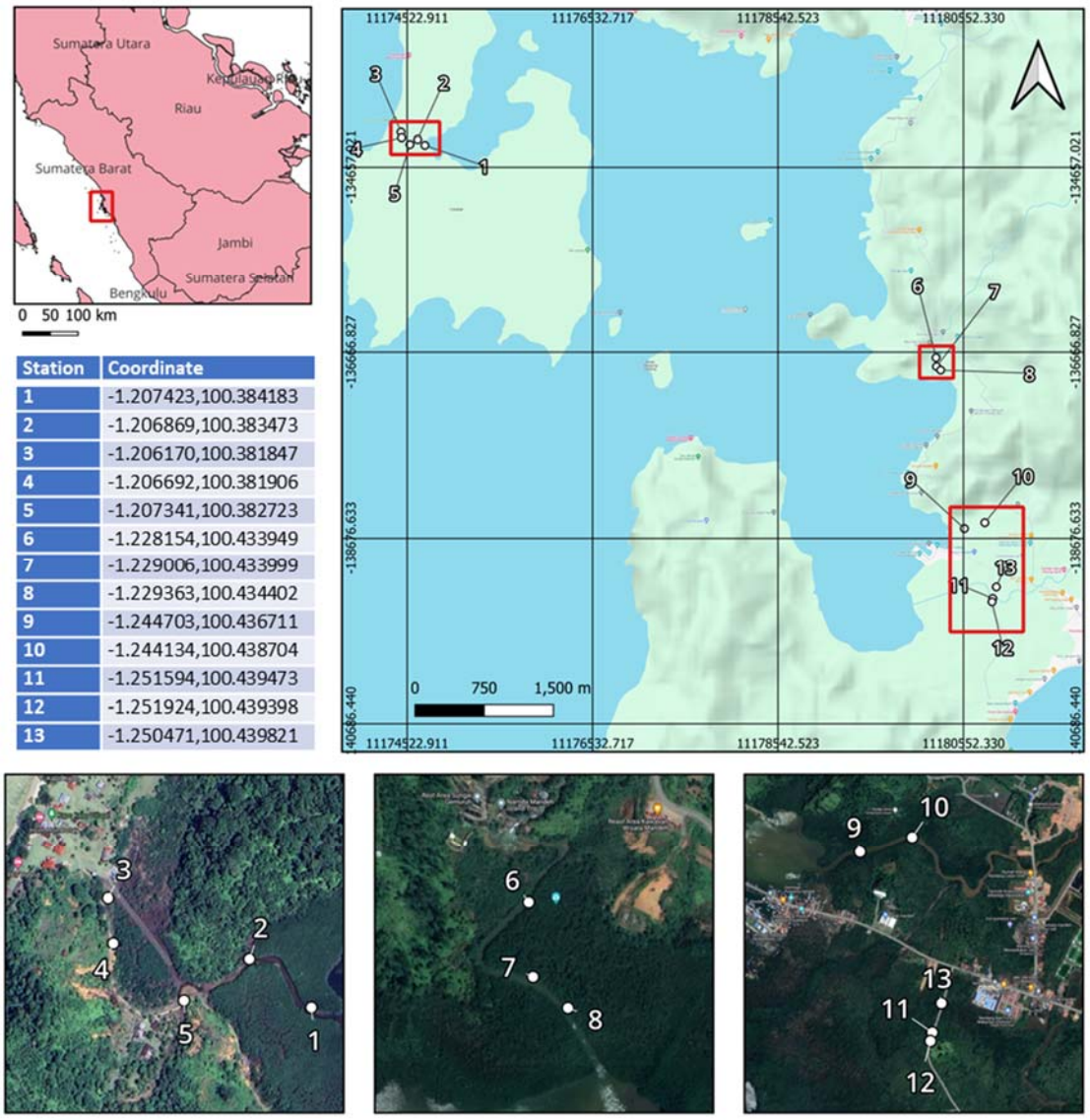
Beach, Kebumen, Indonesia, with 87.87% exhibiting cellulolytic activity and cellulolytic index values ranging from 0.25 to 13.96 [9]. In the mangrove ecosystem of Kuala Simbur village, three bacterial isolates showed the highest cellulolytic activity indices: MS06 (9.73), MS08 (5.41), and MS02 (5.07), resembling Bacillus, Cellulomonas, and Micrococcus genera [10]. Additionally, from mangrove soil on the northern coast of Aceh Province, Indonesia, 22 out of 39 bacterial isolates demonstrated cellulase activity, with cellulolytic indices ranging from 0.31 to 4.82, and BTM533 (4.82) and BTM622 (2.09) being the highest [11]. However, there is no information about the presence and activity of cellulolytic bacteria in mangrove ecosystems in West Sumatra Province. Therefore, the objectives of the present study are screening and determining potential cellular bacteria from the mangrove ecosystem, Mandeh, West Sumatra.

II. Research Methods

2.1. Water Sampling in Mangrove Ecosystems

Water samples were collected from the mangrove ecosystems at 13 sampling stations, with five samples taken from the mangrove forest of Kapo-Kapo island and eight samples from the Mandeh coast. Stations 1 through 10 are located along the boating route, and Stations 11 to 13 are inland (Fig. 1). Variations in water characteristics among mangrove areas were considered in selecting sampling locations.



Station	Coordinate
1	-1.207423,100.384183
2	-1.206869,100.383473
3	-1.206170,100.381847
4	-1.206692,100.381906
5	-1.207341,100.382723
6	-1.228154,100.433949
7	-1.229006,100.433999
8	-1.229363,100.434402
9	-1.244703,100.436711
10	-1.244134,100.438704
11	-1.251594,100.439473
12	-1.251924,100.439398
13	-1.250471,100.439821

Fig. 1. Sampling location

Subsequently, the collected water samples were combined to create composite samples. A total of 100 ml of water was collected using sterilized equipment and transferred into sterilized plastic sample containers. Finally, the water samples were transported to the laboratory in a cool box for analysis of cellulolytic bacteria characterization and their cellulase activities.

2.2. Isolation of aquatic bacteria and morphological identification

Each water sample was aseptically pipetted into volumes of 0.1 mL and 0.01 mL. Subsequently, aliquots of these volumes were pour-plated onto nutrient agar plates using sterile techniques. The plates were subsequently incubated at 30°C for 24 hours to allow bacterial growth.

After incubation, agar plates were inspected for bacterial growth, and individual colonies were visually assessed for morphological traits including size, shape, color, and texture. Colonies exhibiting characteristics that were not found on other plates are then aseptically selected and transferred to fresh agar plates for further purification. The bacterial isolates were then purified using the streak quadrant method on nutrient agar plates and incubated at 30°C for 24 hours. Then, streaking was repeated to obtain a single and pure isolate colony.

2.3. Screening of cellulolytic bacteria

The pure isolates of cellulolytic bacteria obtained were tested qualitatively to determine their abilities to produce cellulase enzymes using the spot technique. The isolates were inoculated in the selective medium for cellulolytic bacteria, which consisted of 10 g/l of CMC, 0.2 g/l of $MgSO_4 \cdot 7H_2O$, 0.5 g/l of KH_2PO_4, 0.75 g/l of KNO_3, 0.02 g/l of $FeSO_4$, 0.04 g/l of $CaCl_2$, and 1.5 g of bacto agar, and incubated at 37°C for 48 hours, and the cellulolytic activity was carried out using the Congo Red method.

The plates were stained with 1% (w/v) Congo Red and allowed to stand for 15 minutes to observe clear zone formation [12]. Subsequently, the plates were washed with 2M NaCl and incubated for 24-48 hours to complete the formation of the clear zone. The formation of a clear zone around the colony indicated that CMC had decomposed. The diameter of the clear zone formed was measured with a digital caliper. The cellulolytic index (CI) was calculated by comparing the value of the clear zone diameter with the colony diameter [13]. The value of CI is categorized as low when ≤ 1 cm, medium at 1-2 cm, and high when ≥ 2 cm. The CI for each purified isolate obtained was calculated using the equation below [14]:

III. Result And Discussion

3.1. Isolation of aquatic bacteria

Thirty-eight bacterial isolates were obtained after a 24-hour incubation at 30°C with varying morphological characters (Tab. 1).

TABLE I. MORPOLOGICAL FEATURE OF ISOLATES

Station	Isolate	Macroscopic character				Microscopic character	
		Colony shape	Colony margin	Colony elevation	Colony pigment	Gram	Cell shape
1	PUA-1	irregular	undulate	flat	white	+	bacilli
	PUA-2	irregular	undulate	flat	cream	+	bacilli
2	PUA-3	round	entire	flat	white	+	bacilli
	PUA-4	round	entire	raised	white	+	bacilli
	PUA-5	round	entire	raised	white	+	bacilli
	PUA-6	irregular	lobate	raised	cream	+	bacilli
3	PUA-7	irreguler	undulate	raised	white	+	bacilli
	PUA-8	round	entire	raised	white	+	bacilli
	PUA-9	irreguler	undulate	raised	cream	+	bacilli

4	PUA-10	irregular	lobate	umbonate	white	-	bacilli
	PUA-11	irregular	undulate	raised	cream	+	bacilli
	PUA-12	irregular	undulate	raised	white	+	bacilli
5	PUA-13	round	entire	umbonate	pale yellow	+	bacilli
	PUA-14	irregular	lobate	flat	white	+	bacilli
	PUA-15	round	entire	umbonate	pale yellow	+	bacilli
	PUA-16	round	entire	raised	cream	+	bacilli
6	PUA-17	irregular	undulate	raised	white	+	bacilli
7	PUA-18	irregular	lobate	convex	white	+	bacilli
	PUA-19	irregular	undulate	flat	white	+	bacilli
	PUA-20	irregular	undulate	raised	white	+	bacilli
8	PUA-21	irregular	rhizoid	flat	white	+	bacilli
	PUA-22	irregular	undulate	flat	white	+	bacilli
9	PUA-23	round	entire	raised	pale yelow	-	bacilli
	PUA-24	irregular	rhizoid	flat	cream	+	bacilli
	PUA-25	irregular	undulate	flat	white	variable	streptobacilli
	PUA-26	irregular	undulate	flat	cream	+	cocci
10	PUA-27	irregular	undulate	umbonate	white	variable	bacilli
	PUA-28	irregular	undulate	raised	pale yellow	+	bacilli
	PUA-29	irregular	undulate	flat	cream	+	bacilli
11	PUA-30	round	filamentous	umbonate	white	+	bacilli
	PUA-31	irregular	lobate	raised	white	+	bacilli
	PUA-32	irregular	rhizoid	flat	cream	+	bacilli
12	PUA-33	irregular	rhizoid	flat	cream	+	bacilli
	PUA-34	irregular	lobate	raised	white	+	bacilli
13	PUA-35	irregular	lobate	flat	white	+	bacilli
	PUA-36	irregular	undulate	flat	white	+	bacilli
	PUA-37	round	entire	raised	white	+	bacilli
	PUA-38	irregular	undulate	raised	yellow	+	staphylococci

The cellulolytic bacteria isolated from various locations (Station 1 to 13) in the mangrove river reveal significant diversity in their macroscopic and microscopic characteristics. Despite a common trend of Gram-positive bacilli across most locations, there is notable variability in colony morphology. Irregular colony shapes with undulate margins are prevalent, though round shapes with entire margins are also frequently observed. Colony elevation ranges from flat to raised and umbonate, while colony colors vary from white and cream to pale yellow and yellow. Specific locations exhibit unique characteristics; for instance, PUA-10

from Station 4 is a Gram-negative isolate, and PUA-23 from Station 9 is another Gram-negative with a distinctive pale-yellow colony color. Locations like Station 5 and Station 10 display a broader range of colony color diversity, suggesting environmental influences on bacterial traits.

Dewiyanti's research [11] identified predominant traits among 39 isolates, including irregular shapes with undulate edges, raised elevations, and cream pigmentation. Circular and filamentous shapes with lobate and entire edges were also noted, with bacilli cell forms and Gram-positive bacteria being common in both rehabilitated and unrehabilitated mangroves.

3.2. Screening of cellulolytic bacteria

A qualitative enzyme activity test was utilized to identify the bacteria that produce cellulase enzymes by displaying the clear zones produced by each isolate. Screening for cellulolytic bacteria Based on qualitative cellulase tests, 16 of the 38 purified isolates displayed clear zones (Tab. 2).

TABLE II. SCREENING RESULT OF ISOLATES

Station	Isolate	Cellulase activity	Avg colony dia (mm)	Halo dia (mm)	Index
1	PUA-1	+	8.06	11.61	0.44
	PUA-2	-	6.61		
2	PUA-3	-	6.90		
	PUA-4	-	7.07		
	PUA-5	+	6.92	12.07	0.75
	PUA-6	-	3.56		
3	PUA-7	-	5.26		
	PUA-8	-	4.80		
	PUA-9	-	3.69		
4	PUA-10	-	9.31		
	PUA-11	-	6.74		
	PUA-12	-	5.19		
5	PUA-13	-	3.95		
	PUA-14	-	3.93		
	PUA-15	+	5.74	20.32	2.54
	PUA-16	-	5.77		
6	PUA-17	-	5.36		
7	PUA-18	+	3.09	18.44	4.98
	PUA-19	+	6.48	14.80	1.29
	PUA-20	-	4.08		
8	PUA-21	+	3.29	15.15	3.61
	PUA-22	+	5.87	12.59	1.15

9	PUA-23	+	3.33	4.52	0.36
	PUA-24	+	3.47	7.93	1.29
	PUA-25	+	8.35	11.60	0.39
	PUA-26	-	2.89		
10	PUA-27	+	6.22	11.39	0.83
	PUA-28	+	4.12	19.53	3.74
	PUA-29	-	6.70		
11	PUA-30	+	3.71	6.40	0.73
	PUA-31	+	4.22	12.23	1.90
	PUA-32	+	4.84	8.49	0.75
12	PUA-33	+	3.62	13.26	2.66
	PUA-34	+	3.45	9.84	1.86
13	PUA-35	+	7.17	13.35	0.86
	PUA-36	-	3.67		
	PUA-37	-	4.73		
	PUA-38	+	3.54	19.84	4.60

The screening results of cellulolytic bacterial isolates from Stations 1 to 13 reveal significant diversity in cellulase activity and cellulolytic index (CI), indicating a correlation between environmental conditions and bacterial efficiency. High cellulolytic activity, indicated by high CIs (≥ 2), was observed in isolates from Stations 5, 7, 8, 10, 12, and 13, such as PUA-15, PUA-18, PUA-21, PUA-28, PUA-33, and PUA-38, suggesting these locations have optimal conditions for cellulolytic bacteria. Medium CI values (1-2) were found in isolates from Stations 1, 2, 9, and 11, like PUA-19, PUA-22, PUA-24, PUA-31, and PUA-34, indicating moderate cellulolytic activity. Stations 3, 4, and 6 had no cellulase-positive isolates, reflecting less favorable conditions for cellulolytic bacteria. The varied CI values across different stations highlight the widespread presence and differing efficiency of cellulolytic bacteria in the mangrove ecosystem.

The cellulolytic activity is indicated by the ability of bacteria to hydrolyze CMC substrates. Bacterial colonies that hydrolyze CMC will form a clear zone (halo zone) around the colony after being soaked in 1% (w/v) Congo red, which does not color the bacteria but the culture media. Congo red staining is commonly used in many studies to screen cellulase microorganisms [15]. The clear zone was produced by 1% Congo red solution because it cannot bind to the media without β1,4glycosidic bonds contained in cellulose polymers [16]. Each isolate has different clear zone (halo zone) sizes, which indicates that the activity of cellulolytic bacteria with the largest clear zone diameter also has high cellulase enzyme activity.

According to Demissie [13], the CI is low when the value of CI ≤ 1, medium when 1-2, and high when ≥ 2. The difference in CI values could be caused by different types of isolates with the ability to produce cellulase enzymes. The greater the hydrolysis capacity, the higher the cellulase enzymes production by isolate culture [17]. The ability of bacteria to degrade cellulose varies based on the type of bacterium strain. Sometimes, cellulase activity was not detected in various liquid media containing CMC and other cellulose materials, thus showing the enzyme concentration produced by this strain is very low, or the ability of the strain to secrete cellulase is weak [18].

The study isolated 38 cellulolytic bacteria, with 16 exhibiting cellulolytic activity, indicating a significant presence of these microorganisms in the mangrove ecosystem. This result surpasses the 7 isolates found in Tropical mangrove, North Malaysia (Naresh et al., 2019), and matches the 22 isolates reported from the mangroves of South Bangka, Indonesia [7]. Dewiyanti et al.

[11] identified 22 cellulolytic bacteria out of 39 isolates from the Northern Coast of Aceh Province, Indonesia, while Batubara et al. [10] obtained 8 isolates from Desa Kuala Simbur. The higher number of isolates in the current study may be attributed to the specific environmental conditions of the study area, which support a more diverse microbial community, highlighting the microbial richness of mangrove ecosystems.

Extracellular enzyme production and activity interacted with the quantity and quality of substrates such as soil temperature, moisture, organic carbon, nitrogen, and pH soil. Soil moisture and temperatures will affect plant growth and soil organic matter input, affecting soil microbe communities and activities [19]. These showed that enzymatic processes by microorganisms are associated with their environment. Furthermore, organic matter in the marine ecosystem is affected by extracellular enzymes [20].

IV. Conclusion

In conclusion, the study identified and characterized bacterial isolates from various locations, including Station 1 to Station 13, based on their morphological and cellulolytic properties. Most isolates exhibited irregular colony shapes, undulate margins, and were predominantly gram-positive bacilli. Screening for cellulase activity across these locations revealed a range of cellulolytic index values. Noteworthy isolates include PUA-28 from Station 10 with an index of 3.74, PUA-18 from Station 7 with an index of 4.98, PUA-21 from Station 8 with an index of 3.61, and PUA-38 from Station 13 with an index of 4.6. These results highlight significant variability in cellulase activity among the isolates, suggesting potential candidates for further studies in organic matter decomposition and ecosystem productivity enhancement.

V. Acknowledgment

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