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Isolation and Identification of Cellulase- Producing Encophytic Bacteria From Yellow Root Plants (*Arvangelisia flava* (L.) Merr) From Enggano Island

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ABSTRACT
Endophytic bacteria are bacteria that live in plant tissue without causing disease in their host. In general, endophytic bacteria enter through stomata or wounds in plants by producing cellulase enzymes to degrade cellulose in plant cell walls that contain cellulose, one of which is yellow root (Arcangelisia flava (L.) Merr). The purpose of this study was to isolate endophytic bacteria from yellow roots from Enggano Island, Bengkulu Province, and to identify morphologically, Gram staining and biochemical tests as well as testing the potential of endophytic bacteria in producing cellulase enzymes. Isolation was carried out by the patch method with surface sterilization, using 70% alcohol and 5.25% sodium hypochlorite and then put on NA media that had been treated with Nystatin. The isolates obtained were then tested for their ability to produce cellulase enzymes by scratching them onto CMC media, then measuring the forming of clear zone. The results showed that from 29 isolates, 26 of them were able to degrade cellulose. AKEBG26 and AKEBG25 isolates had a higher ability to hydrolyze cellulose with cellolulitic potential index (IP) were about ±2.90 and ±1.51. Identification based on gram staining and biochemical tests of 8 endophytic bacterial isolates that had the potential to produce cellulase were closely related to 3 genera, namely Bacillus, Amphibacillus, and Micrococcus.

Keywords: *Arcangelisia flava, cellulase, endophytic bacteria, Enggano*

1. INTRODUCTION

Endophytic bacteria are microbes that are associated with plants without disease symptoms. Endophytic bacteria grow in the vascular tissue, host plant organs, and even their seeds [1]. Each plant can potentially be a host for endophytic bacteria. Endophytic variations differ in each plant species and their diversity depends on conditions of the environment and the host plant [2]. The yellow root (*Arcangelisia flava* (L.) Merr.) is a liana plant, and belongs to the Menispermaceae family which has long been used by the community as a medicinal plant and antibacterial [3]. Endophytic bacteria associated with plants are known to have the potential to produce extracellular enzymes such as cellulases which can be used in biotechnology, industry, and pharmacy [4]. Endophytic bacteria enter the host through wounds that occur at the lateral root junction or wounds caused by microbes or phytopathogenic nematodes, these bacteria can also enter through root hairs and spaces between epidermal cells. In an invasive process, endophytic bacteria perform enzymatic degradation of the plant cell sheath. This process of enzymatic activity includes the production of endoglycanase, pectinase, and cellulase which help them enter plants [5]. Cellulase enzymes are enzymes that can hydrolyze cellulose by breaking the β 1,4 glycosidic bonds in cellulose cellodectrin, cellobiose, and other cellulose derivatives into simple sugars [6]. Several studies have been carried out that endophytic bacteria can

produce cellulose enzymes, this is evidenced by [7], which states that the results of isolation of endophytic bacteria from Mangrove, obtained several genera that produce cellulase enzymes such as *Bacillus, Staphylococcus, Streptomyces, Myroides, Stenotrophomonas, Lysinbacillus, Achromobacter, Pseudobactrum, Serratia, and Klebsiella*. Based on this, a study was conducted on Isolation and Identification of CellulaseProducing Endophytic Bacteria from Yellow Root Plants (*Arcangelisia flava* (L.) Merr) from Enggano Island.

2. MATERIALS AND METHODS

1.1. Location

Yellow root plants (Arcangelisia flava (L.) Merr) were collected from Enggano Island from Malakoni Village. Preparation, isolation and identification bacteria were carried out at the Biology Laboratory of FMIPA, Universitas Bengkulu.

1.2. Research Procedure

By taking the roots, stems, and leaves of yellow roots. The isolation of endophytic bacteria was carried out by two methods, namely peace pant and the grinding methods, by first sterilizing the surface using 70% alcohol and sodium hypochlorite, then growing it on NA media which had been added nystatin [8]. The isolates obtained were purified, then identified by colony morphology, then tested for their ability to produce cellulase enzymes using CMC media. The screening was carried out using a 0.5% congo red as an indicator. Then waited for 15 minutes and rinsed using 1 M NaCl. The presence of cellulase enzyme activity is indicated by a clear zone formed around the colony [9]. The cellulolytic index is calculated using the formula [10].

Furthermore, bacterial isolates that have the ability to produce cellulase enzymes were identified by Gram staining and biochemical tests consisting of sugar, motility, citrate, catalase, and urea tests. The isolate characters obtained were then identified according to Bergey's Manual of Determinative Bacteriology 9th edition [11]. The data obtained were then analyzed descriptively based on the morphological characteristics of the colony, gram staining, and biochemical tests.

3. RESULT AND DISCUSSION

Based on the research that has been done, several endophytic bacterial isolates were obtained from yellow root plants from Enggano Island, Enggano District, North Bengkulu Regency, Bengkulu. The isolation results from the roots, stems, and leaves of yellow roots obtained 55 isolates with 19 isolates from the roots, 27 from the stems, and 9 from the leaves (Table 1) which were then grown in NA media that had been treated with nystatin and carried out with 5 repetitions for the isolation stage. from any isolated plant part.

Table 1. Calculation of endophytic bacterial colonies isolated from yellow root plants

Organ of plant	Number of Colonies					Total Colony
	R1	R2	R3	R4	R5	
Root	6	4	2	6	1	19
Stem		13	5	6	3	27
Leaf	6	3				9

The total isolates isolated from the yellow root plant were 55 isolates with 19 isolates from the roots, 27 isolates from the stems, and 9 isolates from the leaves. Usually, endophytic bacteria are abundant in roots and descend on stems and leaves. This can be caused by roots that are in the soil so that soil bacteria can be found and most endophytic bacteria enter through the soiled tissue so that the number of roots is more in the roots [12]. But sometimes the highest number of endophytic bacteria is in the stem. This is because photosynthetic products are transported from the leaves down to the roots through the tree trunks before reaching the roots, so they are used by endophytic bacteria as a source of nutrition [13]. A total of 55 isolates were then purified and 29 isolates of endophytic bacteria were isolated from the yellow root plant. The pure colonies obtained were coded as AKE1 to AKE29. Twentynine isolates were then carried out morphological characterization with parameters of edge, appearance, elevation, and colony color (Table 2).

The results of tests carried out among 29 isolates obtained 23 isolates that were able to degrade cellulose. Cellulolytic is a process of breaking cellulose into smaller compounds or units such as glucose using cellulase enzymes [14]. The largest cellulolytic potential index of 29 isolates was AKE26, which is a genus Bacillus with an indeks cellulolytic 2.90 (Figure 1). In this study, 8 best isolates were selected for further testing.

The test of the ability of endophytic bacteria in producing cellulase enzymes was carried out using the spotting method and measuring the cellulolytic index (Table 3). Bacterial isolates were subjected to a qualitative test of their ability to produce cellulose enzymes by calculating the cellulolytic index. Based

Table 2. Morphological characterization of endophytic bacteria isolates

Isolate code	Species Name	Characteristic of bacterial morphology			
		Elevation	Form	Margin	Color
AKE1	*Amphibacillus sp 1*	Flat	Irreguler	Undulate	Cream
AKE2	*Bacillus sp 1*	Flat	Irreguler	Lobate	Yellow
AKE3	*Bacillus sp 2*	Flat	Filamentous	Filamentous	Cream
AKE4	*Bacillus sp 1*	Flat	Filamentous	Filamentous	White
AKE5	*Pseudomonas sp 1*	Flat	Filamentous	Filamentous	White
AKE6	*Amphibacillus sp 2*	Flat	Filamentous	Filamentous	White
AKE7	*Bacillus sp 3*	Flat	Filamentous	Filamentous	White
AKE8	*Amphibacillus sp 2*	Flat	Filamentous	Filamentous	White
AKE9	*Bacillus sp 4*	Flat	Filamentous	Undulate	Yellow
AKE10	*Bacillus sp 1*	Flat	Irreguler	Undulate	White
AKE11	*Amphibacillus sp 2*	Raised	Irreguler	Undulate	White
AKE12	*Staphylococcus sp 1*	Flat	Irreguler	Flat	White
AKE13	*Micrococcus sp 1*	Raised	Irreguler	Undulate	Yellow
AKE14	*Micrococcus sp 1*	Convex	Irreguler	Lobate	Yellow
AKE15	*Micrococcus sp 1*	Convex	Irreguler	Lobate	Yellow
AKE16	*Micrococcus sp 1*	Flat	Irreguler	Lobate	White
AKE17	*Micrococcus sp 1*	Flat	Irreguler	Undulate	White
AKE18	*Micrococcus sp 1*	Flat	Filamentous	Filamentous	Yellow
AKE19	*Bacillus sp 5*	Raised	Irreguler	Undulate	White
AKE20	*Bacillus sp 5*	Convex	Irreguler	Undulate	White
AKE21	*Bacillus sp 6*	Raised	Irreguler	Undulate	White
AKE22	*Bacillus sp 6*	Flat	Circular	Flat	White
AKE23	*Bacillus sp 1*	Raised	Irreguler	Undulate	Yellow
AKE24	*Bacillus sp 3*	Convex	Irreguler	Undulate	Yellow
AKE25	*Bacillus sp 1*	Raised	Irreguler	Lobate	White
AKE26	*Bacillus sp 1*	Raised	Irreguler	Undulate	White
AKE27	*Bacillus sp 7*	Convex	Irreguler	Lobate	White
AKE28	*Bacillus sp 1*	Raised	Irreguler	Undulate	Yellow
AKE29	*Bacillus sp 5*	Convex	Circular	Undulate	Yellow

Table 3. Cellulolytic index of 23 endophytic bacteria isolated from yellow roots

Number	Isolate code	Cellulolytic index	Number	Isolate code	Cellulolytic index
1.	AKE1	0,47	16.	AKE16	0,52
2.	AKE2	0,44	17.	AKE17	1,23
3.	AKE3	0,70	18.	AKE18	-
4.	AKE4	0,75	19.	AKE19	0,34
5.	AKE5	0,43	20.	AKE20	0,74
6.	AKE6	0,74	21.	AKE21	0,48
7.	AKE7	0,34	22.	AKE22	0,88
8.	AKE8	0,45	23.	AKE23	0,41
9.	AKE9	-	24.	AKE24	0,40
10.	AKE10	0,21	25.	AKE25	1,51
11.	AKE11	-	26.	AKE26	**2,90**
12.	AKE12	0,27	27.	AKE27	0,36
13.	AKE13	0,54	28.	AKE28	0,39
14.	AKE14	0,21	29.	AKE29	1,21
15.	AKE15	0,42			

on the cellulolytic index value, of the 29 endophytic bacterial isolates, 23 of them were able to produce cellulase enzymes. AKE26 isolate had the highest cellulolytic index of 2.90 mm. Figures and tables should be placed either at the top or bottom of the page and close to the text referring to them if possible. The clear zone in the figure shows the presence of hydrolytic activity by the extracellular cellulase enzyme excreted by endophytic bacterial isolates with a certain diameter. The hydrolysis product is a simple monosaccharide sugar and there is no complex bonding with the red congo. The hydrolytic cellulase enzymes produced by endophytic bacteria have an important role in the penetration

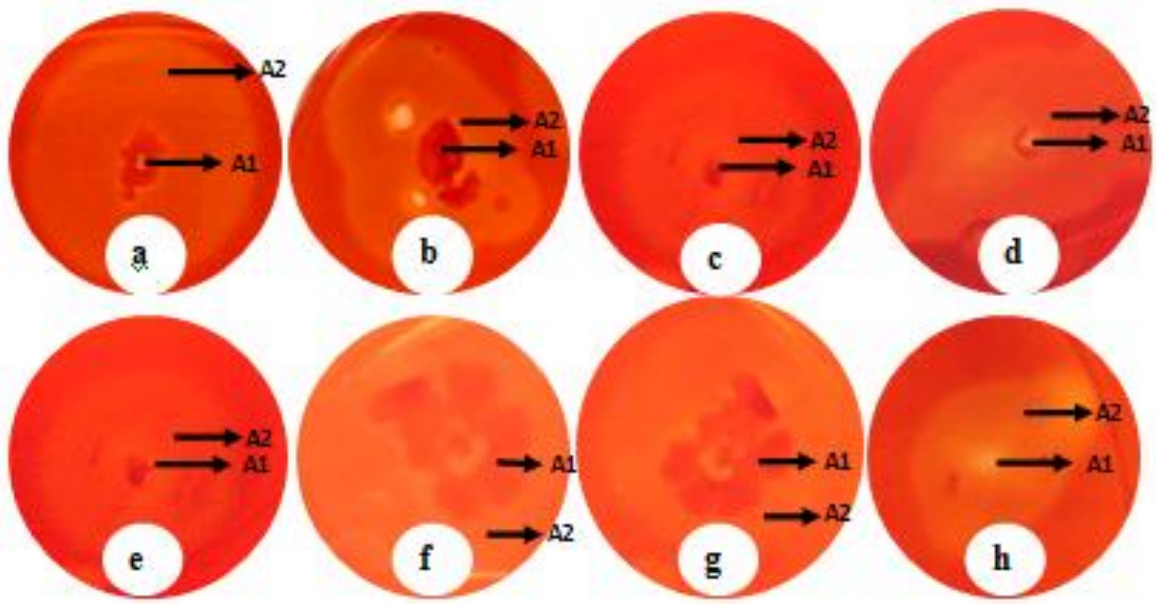

Figure 1. The results of 8 selected endophytic bacterial isolates that can produce cellulase enzymes after being incubated for 24 hours on CMC media and given congo red, (a) AKE26, (b) AKE25, (c) AKE17, (d) AKE29, (e) AKE22, (f) AKE4, (g) AKE20, (h) AKE6, A1 = bacterial isolate, A2 = clear zone.

Table 4. The results of gram staining observations and biochemical tests of endophytic bacterial isolates from the yellow root plant (Arcangelisia flava (L.) Merr) from Enggano Island

No.	Isolate code	Gram Staining	Shape and Arrangment of cell	Biochemical test							
				C	Ci	Mo	U	Sugars			
								G	M	L	S
1.	AKE4	Positif	Streptobacil	+	+	+	-	+	+	+	+
2.	AKE6	Positif	Streptobacil	-	-	+	-	+	+	+	+
3.	AKE17	Positif	Monococcus	+	+	+	-	+	+	+	+
4.	AKE20	Positif	Streptobacil	+	+	+	+	+	+	+	+
5.	AKE22	Positif	Streptobacil	+	+	+	-	+	-	+	+
6.	AKE25	Negatif	Staphylococcus	+	+	+	-	+	+	+	+
7.	AKE36	Positif	Monococcus	+	+	+	-	+	+	+	+
8.	AKE29	Positif	Monococcus	+	+	+	+	+	+	+	+

mechanism of endophytic bacteria in plants. The existence of cellulase enzymes secreted by endophytic bacteria makes it easier for endophytic bacteria to enter plants [15]. Isolates that can produce cellulose enzymes are then tested for biochemistry and gram staining to see the genera of 23 endophytic bacterial isolates that are capable of producing cellulose enzymes. The biochemical tests carried out were the catalase test, citrate test, motility test, urease test, and sugar test which included glucose, maltose, lactose, and sucrose (Table 4).

Gram staining was also carried out to see the isolates obtained were included in the gram positive or negative groups. The result of gram staining showed that the best 8 isolates were gram positive (Figure 2).

Based on the results of morphological observations, Gram staining, and biochemical tests, 8 isolates were closely related to the genera Bacillus, Micrococcus, and Amphibacillus. AKE4, AKE20, AKE22, AKE25, AKE26, and AKE29 are Gram positive bacteria. Biochemical tests that have been

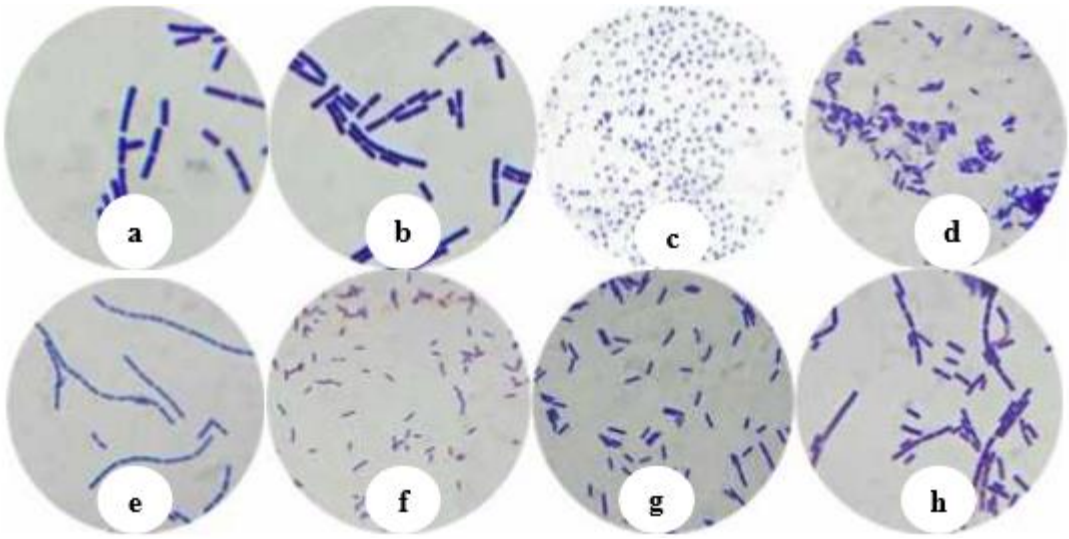

Figure 2. Gram staining of 8 endophytic bacterial isolates that can produce cellulase enzymes with a magnification of 1000x using a binuclear microscope, (a) AKE4, (b) AKE6, (c) AKE17, (d) AKE20, (e) AKE22, (f) AKE25, (g) AKE26, (h) AKE29.

carried out have shown the physiological properties of bacterial isolates. The results of the catalase test showed that the bacteria could produce the catalase enzyme, the citrate test showed that the bacteria used citrate as the main carbon source, and could be motile. The six isolates have similarities with the genus Bacillus which refers to Bergey's Manual of Determinative Bacteriology 9th Edition, which states that the genus Bacillus has rod-shaped characters, are classified as Gram positive, and motile bacteria.

AKE17 isolate has a morphology with flat elevation, with irregular margin shape and irregular surface and the color of the isolate is yellow. Biochemical tests show that AKE17 isolates have the ability to produce catalase enzymes, this means that they can convert hydrogen peroxide into water and oxygen, motile, and can use citrate as the main carbon source. Included in Gram positive bacteria with a round shape (coccus). This isolate has similarities with the genus Micrococcus which refers to Bergey's Manual of Determinative Bacteriology 9th Edition, which states that the Micrococcus genus has the characteristics of round cells, measuring 0.5-2.0. Cells are single, tetraded, clustered, Gram positive in character, and rarely motile.

AKE6 is an isolate that has morphological characters with the appearance of filamentous colonies, flat elevation, filamentous margins, and white colony color. The results showed that the results of the catalase test were negative, Gram positive and motile. Referring to Bergey's Manual of Determinative Bacteriology, Edition 9 explains that the bacteria of the genus Amphibacillus have the characteristics of bacillus cells, single cell arrangement, in pairs and sometimes forming short chains, Gram positive, can form spores in extreme environments, the catalase test is negative and motile. Endophytic bacterial isolates that produce cellulase enzymes, after being given gram staining and biochemical tests, were identified as belonging to 3 different genera.

4. CONCLUSION

Total of 29 endophytic bacterial isolates were isolated from yellow root plants, 23 of them are able to produce cellulase enzymes. The isolate with the highest index of cellulose enzyme was AKE26 which is a genus Bacillus with an IP of 2.90. Based on gram staining and biochemical test, 8 selected isolates showed close relationship with Bacillus, Micrococcus, and Amphibacillus.

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