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Characteristics of Indigenous Bacterial Isolates from Cocoa Plantations in Meko Village, Central Sulawesi, with Ability to Degrade Cellulose

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A B S T R A C T

The characteristics of indigenous bacterial isolates in cocoa farms differ based on the study's location and methodology. More research is required to completely comprehend these bacterial isolates' diversity and features. This study intends to assess the cellulose-degrading capacity of bacterial isolates from cocoa plantations in Meko Village, Central Sulawesi. The shape of the bacterial colony, the shape of the colony's edge, elevation, colony size, color, and texture are considered during macroscopic morphological identification and microscopic morphological identification after gram stain. Afterward, the gram stains the bacteria to determine the type of cell wall. Hydrolysis capacity was then identified macroscopically by observing colony shape, colony periphery, colony color, colony appearance and height, and hydrolysis capacity on media in a petri dish (HC). Upon microscopic examination of cell shape and bacterial wall type (Gram-positive and gram-negative). Based on the research findings and identification of bacteria capable of degrading cellulose in cocoa soil, it can be inferred that among the 28 examined isolates, six isolates have the most excellent HC index values in KL62 isolates. In contrast, GL66, KL23, and KL62x isolates have medium HC index values.

1. INTRODUCTION

1.1. Research Background

After palm oil and rubber, cocoa is currently the third major plantation commodity in the province of Central Sulawesi. Cocoa has a significant impact on the economy as a foreign exchange earner and as a means of bolstering the prosperity of farmers [1]. A cocoa farm in Meko Village, Central Sulawesi that has not utilized inorganic fertilizers or chemical pesticides for the past ten years increases the likelihood of microbial diversity.

Land suitability measures a land's utility, especially its potential for cocoa farming. Climate and soil conditions influence the appropriateness of land for cocoa farming. The soil's physical qualities and chemical properties can be used to identify soil variables. Physical soil attributes include soil texture, aeration, and drainage, while chemical soil properties include soil pH, soil fertility, and organic matter content [2].

Biomass on the soil surface of cocoa plantations, comprising stems, stumps, branches, bark (litter), seeds, and leaves from lignin- and cellulose-rich vegetation, can be used as organic fertilizer with the help of cellulose-degrading microbes [3]. Cellulose decomposition necessitates the presence of bacteria that release cellulase enzymes. Cellulase enzymes hydrolyze cellulose by severing 1,4 ß-glucoside linkages on long cellulose strands. In an aerobic environment, cellulose decomposes into glucose and carbon dioxide, which combine to form developing cells, whereas

cellulose decomposes into alcohols and organic acids in an anaerobic environment [4]. Cellulose-degrading bacteria are one of the microorganisms that aid in the breakdown of cellulose into simpler chemicals, such as those found in the remainder of wood and plant decay.

Consequently, it is vital to perform research to establish morphological traits and the cellulose-degrading capacity of bacteria. This study aimed to isolate cellulose-degrading bacterial species from cocoa soil and evaluate their capacity to digest cellulose.

1.2. Literature Review

Cellulose is a homopolymer molecule composed of -D-Glucopyranlose and (1,4)-Glycoside in plant tissue. Cellulose comprises carbon (44.44%), hydrogen (6.17%), and oxygen (49.39%). Between 40 and 50% of the dry weight of lignocellulose materials are composed of cellulose. Differences in cellulose content are influenced by growth location, biomass type, plant age, stem location, and environmental conditions. Wood, nonwood, marine fauna, and bacterial cellulose are the four classifications of cellulose based on their origin. Cellulose is mostly derived from woody plant sources. Both broadleaf wood (hardwood) and needle-leaf wood must be considered in any discussion of wood (softwood). The primary distinction between needlewood and broadleaf wood resides in the composition of its constituent cells. Needle-leaf wood has a more consistent structure than broadleaf wood, which has a more complicated structure. This helps determine the physical characteristics of wood and other biomass. The relationship between the anatomical structure and wood's physical qualities is close and positive [5].

Acetobacter xyllinum, *Bacillus spp., Bacillus anthracis, B. endophyticus* (KM289167), *B. funiculus, B. thuringiensis, B. cereus, B. toyonensis, Virgibacillus chiquenigi, Acinetobacter baumannii,* and *Lactobacillus pantheries* [6] are bacterial germs that decompos Cellulolytic bacteria are typically found in cellulose-rich environments, such as mangrove ecosystems, waste, leaves, and worn wood, as well as in species that may utilise cellulose as a food source. The term cellulolytic refers to the breakdown of cellulose into smaller molecules or glucose units. *Achromobacter, Angiococcus, Bacillus, Cellulomonas, Cytophaga, Clostridium, Flavobacterium, Microbacterium, Pseudomonas, Poliangium, Sorangium, Sporocytophaga, Vibrio, Cellfalcicula, Citrobacter, Serratia, Klebsiella, Enterobacter,* and *Aeromonas* are some genera of bacteria with cellulolytic capabilities. These bacteria can digest cellulose due to their ability to create enzymes with varying characteristics that work in tandem [7].

1.3. Research Objective

This study aims to determine the ability of bacterial isolates that can degrade cellulose.

2. MATERIALS AND METHODS

2.1. Media Preparation

Making solid cellulolytic media for bacterial isolation and screening is by dissolving Agar 15 g, $NaNO_3$ 2 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.02 g, $MnSO_4.7H_2O$ 0.02 g, $FeSO_4$. $7H_2O$, 0.02 g, $CaCl_2.2H_2O$ 5 g, 1% CMC and Congo red 0.03 g in one liter of aquades set at pH 7. After it is stirred, neutral pH is set and then sterilized at 121ºC for 15 minutes. After the substrate is sterilized, then pour it on the substrate.

2.2. Culture Preparation

A bacterial isolate culture is generated on the Cellulase test medium by taking as many as one ose of bacteria grown on the test media and then identifying each isolate with its test code.

2.3. Morphological Characteristics

Macroscopic morphological identification by looking at the shape of the bacterial colony, the shape of the periphery, elevation, colony size, color and texture. Then proceed with gram staining to see the type of bacterial cell wall. First, by making a smear on the glass of the object. After that, drip purple crystals for one minute, then rinse with aquades. Continue by dripping lugol solution for one minute then rinse with acetone:alcohol (70:30) until the color in the glass of the object fades. Finally, drip carbol fuchsin dye for one minute then rinse with aquades. After that observe under a microscope with 400x magnification and 1000x magnification.

2.4. Hydrolase Capacity Test

This HC test measures an isolate's capacity to breakdown cellulose in its surrounding environment. The ratio of HC (hydrolysis capacity / HC value) is the diameter of the clear zone divided by the diameter of the colony that creates the clear zone [8].

$$HC\ Index\ = \frac{Diameter\ of\ Clear\ zone - Colony\ Diameter}{Colony\ Diameter}$$

This test utilizes a solid CMC medium. With an ose needle, a pure isolate culture is collected and then placed in the center of CMC media. The HC ratio was determined after 72 hours of incubation in the incubator at 30º Celcius.

Differences in cellulose remodeling in known media inside the clear zone indicate the capacity of bacteria to break down cellulose. The better the ability of bacteria to break down cellulose, the greater the area of the clear zone that can be measured. Cellulose-degrading bacterial isolates as either strong, moderate, or weak degraders. Strong degraders are bacteria with a hydrolysis capacity (HC) > 3, whereas those with HC values between 1 and 3 are classified as medium, and those with HC values 1 as weak cellulose degraders [9]. The ratio between the diameter of the clear zone and the diameter of the fungal colony is used to measure the extent of the fungus' cellulolytic capability [10].

2.5. Gram Staining

Gram staining to see the type of bacterial cell wall. First, make a smear on a glass object after that drop the purple crystals for one minute then rinse with distilled water. Continue by dripping Lugol solution for one minute then rinse with acetone:alcohol (70:30) until the color on the object glass fades. The last drop of carbol fuchsin dye for one minute then rinse with distilled water. After that, observe under a microscope with a magnification of 400x and a magnification of 1000x.

2.6. Analytical methods

Bacterial isolates tested for hydrolysis capacity were then identified macroscopically observed on media in a petri dish by looking at colony shape, colony periphery, colony color, colony appearance and elevation, and hydrolysis capacity (HC). While microscopically observing cell shape and type of bacterial wall (Gram-positive and gram-negative).

3. RESULT AND DISCUSSION

3.1. Hydrolysis capacity

In this study tested 28 isolates obtained from cocoa plantations that for 10 years had never used pesticides and inorganic fertilizers, 26 bacteria were found that play a role in degrading cellulose shows the ability to degrade cellulose qualitatively (listed in table 1). The best results were shown in isolates of Kl62, GL66, KL23, and KL62x

Table 1. Screening of Hydrolysis Capacity

Isolate Code	Diameter of Clear Zone	Colony Diameter	HC Index
KL62	19	4	3.5
GL66	8	4	1
BL45	10	8	0.15
GL54	11	10	0.1
JL65	7	5	0.4
BL51x	32	25	0.28
GL67	17	14	0.21
BL612x	11	8	0.375
KL66L	19	10	0.9
KL31	16	13	0.23
KL23	5	2	1.5
KL68	14	9	0.67
KL24	16	12	0.3
KL68x	14	10	0.4
JL23	14	8	0.75
GL61x	16	11	0.45
BL46	27	13	0.77
BL53	11	8	0.375
JL23	14	11	0.27
GL66	8	6	0.33
KL62x	15	5	2
BL45	12	8	0.5
JL65	13	8	0.625
GL68	-	8	-
GL610a	14	10	0.4
BL612	14	8	0.75
BL51	12	7	0.71
BL54	22	16	0.375
GL63	-	-	-
BL43	20	14	0.429
BL34x	20	14	0.429
JL52	11	6	0.83

Bacteria that grow on Carboxyl Methyl Cellulose (CMC) and Congo Red media are red and surrounded by clear zones. The clear zone may be indicated as the ability of a bacterium to hydrolyze cellulose [11].

Bacterial cultures that have high cellulase enzyme activity can decompose cellulose substrates into simple glucose compounds with indicators of clear zones forming around the colony [12]. CMC media decomposed by cellulase enzymes if dripped by the colorless congo red indicator. This bond occurs in a non-covalent way. This shows that there is a degradation process of β-D-glucan in agar media. The red color also indicates the presence of residual cellulose that is not hydrolyzed. Congo red is the sodium salt of benzidine-diazo-bis-1, naphthylamine-4, and sulfonic acid, so it is dissolved and leached by sodium chloride salt (NaCl) [13].

Carboxyl Methyl Cellulose (CMC) substrates in cellulolytic bacterial growth media can decompose through biocatalysis, namely cellulase enzymes or CMCase. Bacteria that can grow on these media prove that the bacterial isolate is cellulolytic bacteria. CMC media contains cellulose used by cellulolytic bacteria for carbon sources in their growth [14].

Bacteria grown in that medium enriched with 1% CMC is a bacterium that can utilize cellulose as a medium and nutrient for its life. However, it is necessary to ascertain the ability of bacterial isolates to degrade cellulose. The method to find out is through screening using congo-red. A total of 28 isolates showed the presence of clear zones with hydrolysis capacity index (HC) ≥3 (high category). In comparison, the use of congo-red showed 26 isolates had similar hydrolysis capacity, having isolates with high HC values that were highest in sediment samples.

In isolates of cellulose-degrading bacteria that have high HC values, gram-staining tests are carried out. The staining results on bacterial isolates from Tembelok mangroves with HC values of more than 3 showed that 50% were gram-positive bacteria, while some were gram-negative [15].

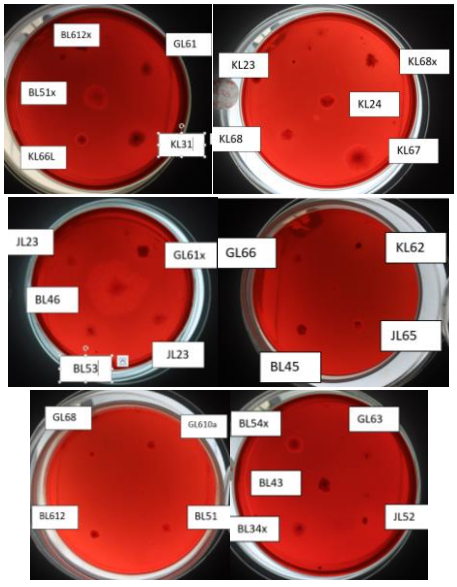

Figure 1. Qualitative Test of Hydrolysis Capacity of bacterial isolates that can degrade cellulose

Figure 1. Shows each bacterial isolate in degrading cellulose by comparing the colony's area and the clear zone's area. The clear zone appears red from congo red which appears to fade around the bacterial colonies indicating CMC hydrolyzed by cellulase enzymes secreted by the test bacteria.

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3.2. Macroscopic Morphological Identification

Bacteria with the highest IS are characterized by macroscopic morphology with the Colony Morphology Code presented in Table 2.

Table 2. Colony Morphology Code of Bacteria

Code	Form	Margin	Surface	Appearance	Color	Elevate
KL62	Circle	Undulate	Slimy	Blur	Pale Yellow	Convex
GL66	Irregular	Undulate	Rough	Cloudy	White	Flat
KL23	Circular	Undulate	Slimy	Blur	Yellow	Convex
KL62x	Spindle	Entire	Slimy	Blur	Pale Yellow	Raised

3.3. Microscopic Morphological Identification

Microscopically after gram staining, the isolate with the highest HC index, rod-shaped isolates KL62, GL66, KL23, and KL62x were seen (Figure 2).

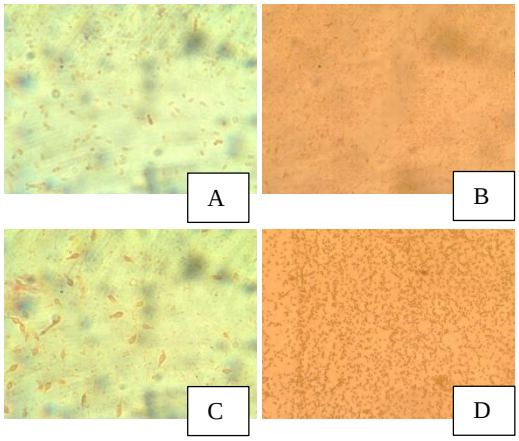

Figure 2. Morphology of bacterial cells by gram staining (A. KL62, B. GL66, C. KL23 and D. KL62x)

Microscopy of the four isolates with the best cellular index, KL62 isolates have a gram-negative rod shape. GL66 isolates are gram-negative rod-shaped. KL23 isolates have a positive gram bar shape, and KL62x isolates have a positive gram bar shape.

4. CONCLUSION

Based on the research results and identification of bacteria that can degrade cellulose in cocoa soil, it can be concluded from 30 isolates tested that six isolates have the highest HC index values in KL62 isolates which are medium indicated by GL66, KL23, and KL62x isolates.

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