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The Existence And Characterization Of Potential Fermentative Bacteria From Fresh Sago Pith (Metroxylon Sagu ROTTB.)

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Abstract – Sago is one of the largest agricultural commodities in West Sumatra. The presence of starch and fiber in sago pith is the carbon source of indigenous fermentative bacteria. The study was conducted to know the existence of indigenous fermentative bacteria and determine their potential character as lactic acid bacteria. This research was conducted using the survey method and analyzed descriptively. The result showed the presence of fermentative acid bacteria in sago pith (56 x 10^5 cfu/g). Isolate SG06 has a higher potential index of fermentative bacteria and was selected as a potential indigenous fermentative bacteria.

Keywords – sago pith; acid fermentative; indigenous; lactic acid bacteria; characterization

I. INTRODUCTION

Sago (*Metroxylon sago* Rottb.) is one of the agricultural commodities that is widespread in Indonesia. The distribution of sago plant areas is widespread in several provinces, including Aceh, Riau, West Sumatera, Kalimantan, Sulawesi, and Papua [1]. Mentawai Islands and Padang Pariaman are sago production areas in West Sumatra Province with an area of 1,461 ha and high production amount of 1,205 tons in 2019 and 1,060 tons in 2020 [2]. Sago is a potential food crop rich in carbohydrate sources; starch is the main product produced through the extraction of sago pith and can be utilized in food and non-food applications.

As a carbohydrate source with high productivity, sago pith has two main constituent components; starch and fiber. Starch has the highest proportion at 64% and fiber at 35% [3], indicating a high concentration of carbon and nitrogen sources in sago pith. Starch and fiber are non-complex carbohydrate sources widely available in plants and used by indigenous bacteria. According to Hastuti *et al. [4],* the high amount of organic matter available allows it to be utilized by indigenous fermentative bacteria to support the plant metabolism.

Acid fermentative bacteria consist of lactic acid and acetic acid bacteria. Lactic acid bacteria are a group that produces lactic acid as the primary product during fermentation. Meanwhile, acetic acid bacteria with the characteristics of obligate aerobic bacteria can rapidly oxidize the alcohol and sugar into acetic acid due to the fermentation processes [5]. Tenriawaru *et al.* [6] added that the presence of indigenous acid-producing bacteria is also influenced by the degree of acidity and sour smell in the sago pith. Acidic conditions in sago pith affect the quality of the sago starch, which has a sour smell and quickly browns [7]. In

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these conditions, lactic acid bacteria can inhibit the browning process and growth of pathogenic bacteria [8]. Based on this, exploring the presence of indigenous fermentative bacteria is needed to diversify food products.

Several studies reported the bacteria was found in sago plants; nitrogen-fixing bacteria associated with plants, indigenous cellulolytic bacteria, amylolytic and acid producers. Sago-associated bacteria from the sago plant are *Bacillus* sp., *Agrobacterium* sp., *Flexibacter* sp., *Burkholderia* sp., *Paenibacillus* sp., and indigenous bacteria found in the bark is *Enterobacter* sp.[9]. Indigenous cellulolytic and amylolytic bacteria from sago waste are *Burkholderia* sp., *Bacillus* sp., *Acinetobacter* sp., *Lactobacillus plantarum,* and *Serratia* sp. [4, 10, 11]. Meanwhile [7] the lactic acid indigenous bacteria *Lactobacillus* sp. was obtained from the soaking water of starch extraction. The indigenous bacteria acid producer also found in the sago pith [6] *Lactobacillus* sp., *Gluconobacter* sp., and *Dysgomonas* sp. In general, the study of indigenous bacteria in sago has been carried out, but exploring the indigenous fermentative bacteria in the sago pith still needs to be discovered. Therefore, this study aims to determine the presence of natural microflora and potential fermentative bacteria in the sago pith.

II. Research Methods

2.1. Collection of sample

Samples of sago pith were obtained from Padang Pariaman Regency, VII Koto Sungai Sarik Sub-district in Padang City, West Sumatera Province, Indonesia. The characteristics of sago pith were 8^{th} and ready to be harvested. The sampling was done by purposive sampling. The sago pith sample was placed into a sterile plastic bag and stored in a cool box containing ice gel for transport to the Research and Microbiology Laboratory, Faculty of Mathematics and Natural Science, Andalas University to be prepared for isolation.

2.2. Existence of indigenous microflora from sago pith

The existence of indigenous microflora was observed by colony bacteria that grew on medium GPA (Glucose Peptone Agar), and the presence of fermentative acid bacteria was observed by the colony that grew on medium GPA+$CaCO_3$ (Glucose Peptone Agar + Calcium Carbonate) [12]. The existence of fermentative bacteria signed with the formation of a clear zone around the colony after 24 hours of incubation. Method to differentiate and confirm the colony bacteria lactate or acetate group used Ethanol + $CaCO_3$ Agar. Only acetic bacteria can grow and form clear zone around the colony [13]. The smallest with the largest clear zone from each different colony grew on the GPA+$CaCO_3$ medium and were selected to be cultured using the streak method in GPA. The isolates obtained were coded with the initial SG (sago) and the number **of isolates after.**

2.3. Evaluation of fermentative index from selected acid fermentative bacteria

Isolates of selected indigenous bacteria were grown on 10 ml of GPB (Glucose Pepton Broth) media with the same cell density of 10^7 cfu/g for 24 hours. 1 ml culture isolates were grown on GPA + CaCO3 media with the pour plate method and incubated at 37 ^oC for 48 hours [14]. Observations of the halo zone were carried out after 48 hours. The Fermentative Index of indigenous bacteria was calculated by comparing the diameter of the clear zone formed by the colony of bacteria and the diameter of the colony of bacteria [10].

2.4. Characterization of potential fermentative bacteria isolates

The isolates were characterized morphologically by observing the shape, elevation, color, margin, and surface of the colony of each isolate. Characterization was also carried out by observing cell morphology with Gram staining and biochemical tests (Catalase test with H_2O_2 3%, KOH 3%, TSIA, and Motility test in Sulfid Indol Motility Agar medium).

2.5. Data Analysis

The data in this study were the presence of bacteria, morphological character (Microscopic and Macroscopic) and Fermentative Index. The data was analyzed with descriptive qualitative method.

III. Result And Discussion

3.1. The existence of indigenous microflora from sago pith

Based on the results of microflora exploration in non-prickly sago pith from Pariaman, there was a indigenous bacteria in GPA and fermentative bacteria in GPA + $CaCO_3$ (Table. 1).

TABLE I. THE PRESENCE OF MICROFLORA IN FRESH SAGO PITH (*M.SAGU*)

Microflora	**Total Colony (… x 10^5 cfu/g)**
Total bacteria (GPA)	165
Fermentative bacteria (GPA+$CaCO_3$)	56

The presence of indigenous microflora in the sago pith was observed in GPA (165 × 10^5 cfu/g). The high content of substrate components in sago pith serves as a carbon source. According to Santoso *et al*. [15] sago pith is composed of 65% starch and 35% fiber.In addition, Tenriawaru *et al*. [6] found a high index of the diversity of indigenous bacteria in the pith, which are dominated by bacteria that produce acid.

Meanwhile, the presence of fermentative indigenous bacteria was detected by the formation of clear zones around the colony in GPA+$CaCO_3$ (Fig 1). The total fermentative bacteria in sago pith is 56 x 10^5 cfu/g. Fermentative bacteria that can metabolize sugar as a carbon source and secrete organic acid as a primary metabolite into the medium to hydrolyze $CaCO_3$ formed clear zones around the colonies [20].

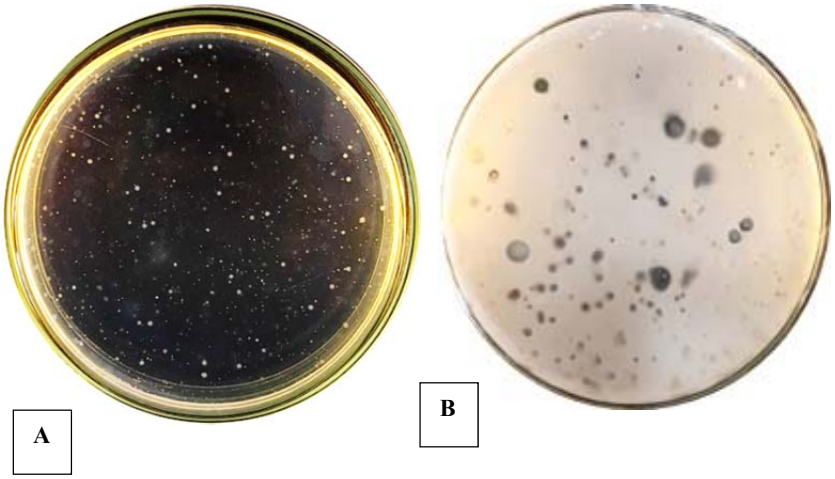

Fig1. The presence of sago pith indigenous microflora in specific medium.

Notes: A. GPA, B. GPA+$CaCO_3$

The presence of fermentative bacteria is related to the amount of acid produced. Shibata *et al*. [16] sago pith contains a high amount of amylose and starch, making it an ideal substrate for fermentation to produce large amounts of lactic acid. In addition, Tenriawaru *et al*. [6] stated that the high number of diverse acid-producing bacteria from sago pith is influenced by acidic pH, rich nutrients, and vitamin C, which can stimulate the presence of specific bacteria and inhibit others bacteria.

3.2. Evaluation of Fermentative Index

Six isolates of fermentative bacteria are characterized the fermentative index (Table 2).

TABLE II. THE FERMENTATIVE INDEX OF POTENTIAL ISOLATE FROM GPA+$CACO_3$

Isolate code	Fermentative index
SG01	2,50
SG02	1,63
SG03	1,19
SG04	1,96
SG05	2,30
SG06	2,80

The Fermentative Index showed the enzymatic activity of bacteria in degrading glucose into organic acids based on the ratio the diameter colony and clear zone. According to Periadnadi [17], fermentative bacteria form the clear zones as the result of acid hydrolyzing in the medium. The addition of $CaCO_3$ plays an important role as a neutralizer of organic acids around the colony; $CaCO_3$ also plays a role in balancing the pH, which affects the amount of organic acid production [18]. Production of organic acids in fermentative bacteria is influenced by the optimum activity of the *lactic dehydrogenase* enzyme, which is stable at neutral conditions. If the pH is in an acidic state, it supports the activation of the *pyruvate dehydrogenase* enzyme to convert *pyruvate* into ethanol [19].

Three isolates showed the highest fermentative index: SG06, SG01, and SG05. The high of fermentative activity is refers to the high amount of acid produced. According to Nurmiati [20], the width of the diameter of the clear zone produced by the bacterial colony describes the ability of bacteria to produce high amounts of acid. This indicates that these three isolates of indigenous bacteria could be used as potential candidates for fermentative bacteria capable of producing organic acids. Based on Kamsina *et al. [21],* bacterial isolates with a fermentative index of ≥ 2.5 can be categorized as fermentative potential bacteria.

A confirmation test was needed on the ability of bacteria to hydrolyze carbon sources into organic acids in Ethanol + $CaCO_3$, which used to identify the ability of bacteria to oxidize alcohol to acetic acid. There is an absence of activity from the bacteria oxidizing ethanol to acetic acid, characterized by the absence of colonies capable of growing on the medium. This showed that the three isolates are classified as lactic acid bacteria, Lynch *et al*. [22] stated that bacteria with a high ability to oxidize alcohols, aldehydes, and sugars to acetic acid and gluconic acid under aerobic conditions are types of acetic acid bacteria. Generally, acetic acid bacteria are found in substrates containing sugars, acids, and alcohol, such as fruits and fermented foods [23]. The three isolates of indigenous bacteria are included in the BAL group with the highest fermentative index as potential lactic acid bacteria. The SG06 isolate with a high fermentative index (2.80) is a potential fermentative bacterial candidate.

3.3. Characterization of potential fermentative bacteria

Selected fermentative isolates continued to be characterized by partial morphology (macroscopic, microscopic, and biochemical) (Table 3). The morphology of indigenous bacteria colonies close to the morphological characteristics of lactic acid bacteria, Kasi *et al.*,[24] found in some isolates of indigenous lactic acid bacteria isolated from sago extraction wastewater has morphological characteristics of round colony shapes, smooth surfaces, flat colony margins and pigmentation of milky white and yellowish beige colors.

TABLE III. PARTIAL CHARACTERS OF POTENTIAL FERMENTATIVE BACTERIA FROM SAGO PITH

No	Characteristics	Isolate		
		SG01	SG05	SG06
1	**Macroscopy**			
	a. Colony shape	Circular	Circular	Irregular
	b. Colony margin	Entire	Entire	Undulate
	c. Colony elevation	Convex	Flat	Raised
	d. Colony colors	Milky white	White	Yellowish
	e. Colony size	Small	Moderate	Moderate
	f. Colony surface	Smooth	Smooth	Rough
2	**Microscopy**			

	a. Cell shape	Bacilli	Bacilli	Bacilli
	b. Gram	+	-	+
	c. Endospore	-	-	-
	d. Motility	-	-	-
3	**Biochemical**			
	a. Catalase	-	+	-
	b. KOH 3%	-	+	-
	c. TSIA (B/S)	A/A	A/A	A/A
	d. Glucose	+	+	+
	e. Sucrose	+	+	+
	f. Lactose	+	+	+
	g. H_2S	-	-	-
	h. Gas	+	+	-
4	Fermentative index	2,50	2,30	2,80

Notes : (+) : positive, (-) : negative, B: *butt*, S: *slant*, A/A:*acid/acid*.

The three isolates showed the same cell morphology, i.e., a rod with different characteristics. SG01 and SG06 isolates are Gram-positive, and SG05 is Gram-negative (Fig 2.). Based on morphological, physiological, and biochemical characterizations adapted to the key determination of bacterial identification in Bergey's Manual of Systematic Bacteriology, Second Edition [25], the SG01 and SG06 isolates were found have similarities with the *Lactobacillus* genus. The similarity of morphological and physiological characters refers to *Lactobacillus* with morphological characteristics of colony growth. [26] The morphological characteristics of *Lactobacillus* are: bacilli, Gram-positive, non-endospore-forming, negative catalase, non-motile, aerotolerant, anaerobic, and complex fermentors in rich carbohydrates, amino acids, and vitamins.

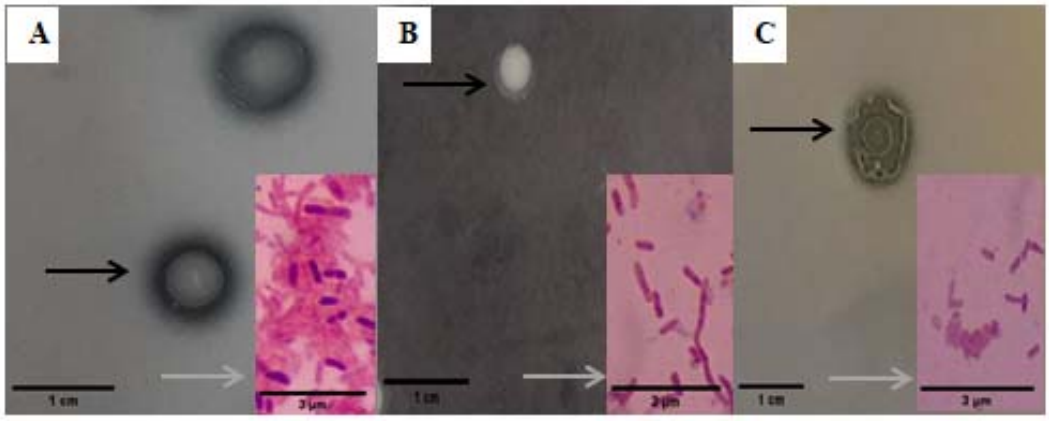

Fig 2**.** Morphological characters of three indigenous bacterial isolates from sago pith.

Notes: A.SG01, B.SG05 and C.SG06

Generally, the *Lactobacillus* is spread through in rich of carbohydrate sources, including plant parts that contain starch, organic waste derived from plants, and fermented food. Some species act as starter cultures for food fermentation because the acids produced able to inhibited the growth of pathogenic microorganisms [26]. Based on this, it is one indicator that supports the discovery of *Lactobacillus* on substrates from sago stems. The research of Tenriawaru et al. [6] found *Lactobacillus* in sago pith. Kasi et al. [24] also found the presence *Lactobacillus* sp. in sago extraction soaking water.

The SG05 isolate morphological results and biochemical and physiological tests adjusted in Bergey's Manual Of Systematic Bacteriology Second Edition [24] refers to the group of bacteria genus *Enterobacter*. The similarity of morphological and physiological characters possessed by the genus *Enterobacter* with isolates obtained is in the form of morphological characters of

colony growth. *Enterobacter* colonies usually have a slightly slimy characteristic, have the morphological structure of gram-negative bacilli cells, are positive for catalase and negative for oxidase, and are non-motile. Meanwhile, *Enterobacter* has the distinctive physiological characteristics of being able to ferment carbohydrates in the form of glucose, lactose, and sucrose with the production of acids and gases in fermentation media [27].

Enterobacter is generally widely spread in nature, one of which is in plants; this genus is often found to act as a nitrogen-fixing bacteria in plants. In this study, the SG05 bacterial isolates is refers to *Enterobacter*. Shrestha *et al.* [16] found *Enterobacter* is one of the indigenous bacteria dominant in sago plants, especially in the starch, stem, and root parts.

IV. Conclusion

The sago pith has indigenous fermentative bacteria. The SG06 isolate, which refers to the *Lactobacillus* genus was chosen as the potential isolate of the indigenous bacteria with the greatest fermentative index. The characteristics of isolate are gram-positive, catalase-negative, non-motile, and non-endospore-forming.

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