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Application of lactic acid bacteria to improve the food safety of sago starch

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Abstract

Sago starch production in local industries is still carried out traditionally and uses poor-quality water. This production causes sago starch to be fermented spontaneously, resulting in sour sago and possibly contamination by pathogenic bacteria. Lactic acid bacteria (LAB) can produce lactic acid and are suitable for use as a starter. Adding LAB as a starter in sago starch fermentation is expected to reduce the number of pathogenic bacterial growths, thereby increasing food safety in sago starch. This research aimed to obtain LAB and evaluate their use in sago starch fermentation to improve food safety. LAB selection was conducted by testing the LAB tolerance ability to low pH and the adaptability of the LAB growth in sago starch. This study was carried out using and without a LAB liquid starter. The water source during the fermentation originated from drinking water and the sago starch industrial factory. The fermentation was carried out for ten days at room temperature with an observation every two days. The results showed that fermented sago starch using drinking water did not harbor *E. coli*, *Salmonella*, or *Shigella* bacterial contamination. In contrast, sago starch fermented using water from the factory harbored these bacterial contaminations. Adding LAB IL1 isolate as a starter in fermentation showed the ability to reduce the number of pathogenic bacteria in sago starch.

[Keywords: drinking water, *E. coli*, fermentation, lactid acid, *Salmonela* sp.]

Introduction

Sago (*Metroxylon* spp.) is an important socio-economic plant that has the potential to be developed (Partini et al., 2023). The potential for sago in Indonesia represents 50% of the world's total sago (Ahmad et al., 2016). Based on the Directorate General of Plantation (2019), the sago area in 2018 in Indonesia reached 311,964 ha, with the broadest distribution in Papua Province, 155,943 ha. According to Haryanto et al. (2015), the potential of sago in Indonesia is one of the assets of national food security. Sago is a carbohydrate-producing plant with higher productivity than other carbohydrate-producing plants such as rice, corn, sweet potatoes, and cassava (Nurul et al., 2023). However, sago production still needs to improve because most of the existing sago is still in natural sago forests that have not been cultivated optimally. The sago harvesting process is only carried out in areas easily accessible to the community (Mangallo et al., 2022).

Sago plants have a very high starch content. The starch comes from the pith in extracted sago stems (Ahmad et al., 2016). The sago extraction process in Indonesia is generally still carried out traditionally and semi-mechanically, and liNGle aNGention is paid to hygiene aspects (Kasi et al., 2019). Processing sago requires large amounts of water, and the water sources used are mostly taken from river banks. This causes an accumulation of sago starch waste, which impacts river water pollution (Rasyid et al., 2020). In addition, poor river water quality can induce the growth of pathogenic microbes on sago starch (Kasi et al., 2017).

The equipment used in Indonesia's sago processing process still needs improvement. If it is not cleaned, it can cause fungal spore contamination in sago starch (Jong, 2018), reduce the quality of sago starch, and cause foodborne disease (Pue et al., 2018). Sago hemolytic disease or SHD, is a disease thought to occur due to the presence of mycotoxins from the production of poorly processed sago starch, has been reported in Papua New Guinea (Seymour, 2021). Using clean water in the sago processing industry is expected to improve the quality of sago products so that they are save for consumption (Suseno et al., 2016). Apart from that, improving the quality of sago starch can also be done using biopreservative methods using lactic acid bacteria (Kasi et al., 2017).

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Lactic acid bacteria (LAB) are often used as biopreservation agents because they can provide a distinctive taste and texture and extend the shelf life of sago starch during the fermentation process. Lactic acid bacteria can produce metabolite products such as bacteriocins which can inhibit the growth of putrefactive and pathogenic bacteria (Rakhmanova et al., 2018). Lactic acid bacteria belong to a group of bacteria that produce lactic acid as a product of carbohydrate metabolism (Wang et al., 2021). The production of lactic acid by LAB will reduce the environmental pH value during the fermentation process, resulting in a sour taste and can stop the growth of pathogenic bacteria (Anggraeni & Yuwono, 2014). Lactic acid bacteria groups commonly applied to food fermentation include *Lactobacillus*, *Lactococcus*, and *Streptococcus* (Rakhmanova et al., 2018). As a starter in the food fermentation process, LAB is considered safe because it is non-pathogenic and does not produce toxins in food.

This research aims to evaluate the effect of LAB and the use of water quality on the quality of sago starch. The parameters observed were the quality of sago starch according to SNI and the quality of the fermentation liquid.

Materials & Methods

A total 11 LAB isolates used was obtained from the collection of the Microbial Bioprospection Laboratory, IPB Biotechnology Center. The other materials used in this research were sago starch from a sago factory in Tanah Baru, Bogor, bottled drinking water (BDW), and water from a sago factory in Tanah Baru, Bogor.

Lactic acid bacteria characterization

LAB isolates grown in MRS broth media were inoculated in solid MRS media containing 1% $CaCO_3$ and incubated at 37°C for 48 hours. LAB characterization includes gram staining and a catalase test with 3% H_2O_2. Pathogenicity tests were carried out based on Tallapragada et al. (2018) by observing the hemolysis activity of LAB on blood agar media.

LAB selection and characterization of selected isolates

LAB selection was conducted by testing LAB tolerance to low pH based on the method of Phong et al. (2017) with modifications. LAB cultures were inoculated in MRS broth media whose pH had been adjusted to pH 3 using 1 N HCl and then incubated for 2 hours at 37°C. LAB density was calculated at initial inoculation (T0) and 2 hours after inoculation (T2) using the cup counting method on solid MRS. The selected LAB isolates were then tested for total bacterial acid based on the modified method of Moore et al. (2011) and Suseno et al. (2016) by calculating the total amount of acid titrated using 0.1 N NaOH.

Adaptation of selected LAB isolates to sago starch was then carried out based on the method of Yusmarini et al. (2017) with modifications. Lactic acid bacteria cultures were inoculated in MRS broth media, and distilled water solution which was added of 1% sago starch each. The cultures were then incubated for 24 hours at 37°C. The LAB density was calculated at initial inoculation (T0) and 24 hours after inoculation (T24) using the Total Plate Count (TPC) method on solid MRS. Selected LAB isolates that could adapt were then measured for growth curves based on the method of Suseno et al. (2016) with modifications for preparing LAB liquid starter in the sago starch fermentation process.

Fermentation of sago starch

Fermentation was carried out using an LAB liquid starter and without a starter. The water used during fermentation is bottled drinking water (BDW) and water from the sago starch industrial factory (RW). A number of 300 g of wet sago starch was added with 100 mL of liquid LAB starter (1%) (v/v), whereas in the starter treatment, only 100 mL of water was added. Fermentation was carried out for 10 days at room temperature with observations every 2 days. The starch precipitate was then separated from the fermentation liquid and dried in the oven at 50°C for 50 hours. In order to reduce the particle size, the sago starch was blended following the method of Suseno et al. (2016) with modifications.

Characterization of fermentation liquid and quality of sago starch

The fermentation liquid characterization included pH measurements (AOAC, 1994) and total acid using modified method of Moore et al. (2011). Analysis of microbes included total microbes on Plate Count Agar (PCA), total molds and yeasts on Potato Dextrose Agar (PDA), total LAB on De Man Rogosa Sharp Agar (MRS), total coliform bacteria in Eosin Methylene Blue Agar (EMBA), and total *Salmonella* sp. and *Shigella* sp. on Salmonella Shigella Agar (SSA) (AOAC, 1995).

Characterization of the sago starch quality included measurements of pH (AOAC, 1994), total acid (modified method by Moore et al. (2011)), and microbial counts (AOAC, 1995). Water content was determined by calculating the weight of the sample lost after being heated in the oven at a temperature of 130°C for 1 hour (AOAC, 2000).

Data analysis

The resulting data was analyzed by testing variance using ANOVA. If the test of variance results in rejection of H0 then a further test with Duncan's Multiple Range Test is used.

Results & Discussion

LAB characterization

Lactic acid bacteria are Gram-positive bacteria with round or rod-shaped, do not have spores, catalase-negative, and can produce lactic acid as a product at the end of fermentation (Swain et al., 2014). A total of 11 bacterial isolates were designated as lactic acid bacteria isolates because they produced clear zones on MRS agar media to which 1% $CaCO_3$ had been added, they also did not have catalase, were classified as Gram-positive bacteria with rod-shaped morphology, and did not have hemolytic activity on blood agar media (Table 1). According to Meryandini et al. (2020), the clear zone formed around bacterial colonies on MRS agar media supplemented with $CaCO_3$ indicates the presence of calcium-lactate compounds originating from the reaction of $CaCO_3$ with lactic acid produced by fermentation of LAB isolates.

Lactic acid bacteria isolate that will be applied to food products need to be tested for pathogenicity by observing the hemolysis activity of LAB on blood agar media. The absence of a clear zone around the bacterial colony indicates that the LAB isolate is not pathogenic because it cannot hemolyze (γ-hemolysis), namely the inability of bacteria to lyse red blood cells on blood agar media (Yasmin et al., 2020). Tallapragada et al. (2018) stated that the absence of hemolysis activity is a safe prerequisite for selecting LAB isolates for food products because LAB must be non-pathogenic.

LAB selection and characterization of selected isolates

Lactic acid bacteria are known as food-grade bacteria because they are widely used in the food industry (Landete, 2016). Lactic acid bacteria can potentially be used as a fermentation starter because it can extend the shelf life of the product (biopreservation) (Zapaśnik et al., 2022). Suseno et al., (2016) stated that LAB can also be a starter in sago starch fermentation. The suitability of LAB isolates as a starter for sago starch fermentation in this study was determined based on cell viability under low pH conditions (Table 2), the highest total acid value (Table 3), and cell viability after adaptation to sago starch (Table 4). The resistance of LAB isolates to pH 3 showed varying tolerance (Table 2). Two of the eleven LAB isolates were chosen for further testing because they showed the best ability to tolerate pH 3. The IL1 and IL2 isolates maintained constant cell viability (7 log CFU mL^{-1}) for 2 hours of incubation at pH 3 (Table 2). Based on Shokryazdan et al. (2014) report, 9 LAB isolates belonging to the genus Lactobacillus had good tolerance to acidic conditions (pH 3).

The LAB IL1 and IL2 isolates were then tested for total acid on MRS media. The LAB IL1 isolate produced the highest total acid i.e. 16.7 mg mL^{-1}, after 24 hours of incubation (Table 3). According to Safitri et al. (2016), LAB in MRS media can produce a total acid value of up to 18.7 mg mL^{-1} after 30 hours of incubation. The adaptability of LAB isolates IL1 and IL2 to sago starch was also carried out to select the best isolate before being applied as a starter in sago starch fermentation. LAB IL1 isolates had higher cell viability after 24 hours of incubation, namely 9.1 log CFU mL^{-1} in MRS media, which had been added with 1% sago starch, and 6.7 log CFU mL^{-1} in distilled water media that

Table 1. Characterization of LAB

No	Isolate code	Gram staining	Morphology	Clear zone $CaCO_3$	Catalase	Clear zone blood agar
1.	IL1	Positive (+)	Basil	Positive (+)	Negative (-)	None
2.	IL2	Positive (+)	Basil	Positive (+)	Negative (-)	None
3.	IL3	Positive (+)	Basil	Positive (+)	Negative (-)	None
4.	IL6	Positive (+)	Basil	Positive (+)	Negative (-)	None
5.	IL7	Positive (+)	Basil	Positive (+)	Negative (-)	None
6.	IL9	Positive (+)	Basil	Positive (+)	Negative (-)	None
7.	IL12	Positive (+)	Basil	Positive (+)	Negative (-)	None
8.	S2	Positive (+)	Basil	Positive (+)	Negative (-)	None
9.	S4	Positive (+)	Basil	Positive (+)	Negative (-)	None
10.	S5	Positive (+)	Basil	Positive (+)	Negative (-)	None
11.	S8	Positive (+)	Basil	Positive (+)	Negative (-)	None

Table 2. Resistance of LAB isolates after 2 hours of incubation at pH 3

Isolate code	Total microbes (CFU mL^{-1})	
	T_0	T_2
IL1	1.3 x 10^7	1.4 x $10^{7a*)}$
IL2	1.3 x 10^7	1.2 x 10^{7a}
IL3	1.3 x 10^7	7.2 x 10^{4cd}
IL6	4.6 x 10^6	1.2 x 10^{4e}
IL7	1.1 x 10^7	1.7 x 10^{4e}
IL9	2.6 x 10^6	4.4 x 10^{5b}
IL12	2.5 x 10^6	9.0 x 10^{4c}
S2	1.1 x 10^7	8.1 x 10^{5b}
S4	1.4 x 10^7	3.6 x 10^{4d}
S5	2.0 x 10^7	1.4 x 10^{5c}
S8	2.8 x 10^6	1.3 x 10^{5c}

Note: T_0: incubation time for 0 hour; T_2: incubation time for 2 hours
*) Numbers in the same column followed by the same letter are not significantly different at the 5% test level

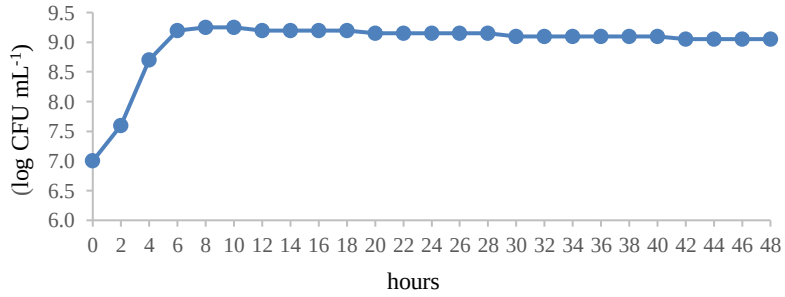

Figure 1. Growth curve of lactic acid bacteria isolate IL1 in MRS broth media

Table 3. Total acid of LAB isolates after 24 hours of incubation on MRS media

Isolate code	Total acid (mg mL^{-1})
IL1	16.7$^{a*)}$
IL2	14.4^{b}

*) Numbers in the same column followed by the same letter are not significantly different at the 5% test level

Table 4. Adaptation of LAB isolates after 24 hours of incubation on sago starch

Isolate code	Total microbes (log CFU mL^{-1})		
	T_{0A}	T_{24B}	T_{24C}
IL1	6.97	9.10$^{a*)}$	6.70^{a}
IL2	6.90	8.90^{b}	6.15^{b}

Note: T0A: incubation time for 0 hour in MRS broth media; T24B: incubation time for 24 hours in MRS broth media with sago starch; T24C: incubation time for 24 hours in distilled water media with sago starch
*) Numbers in the same column followed by the same letter are not significantly different at the 5% test level

had been added with 1% sago starch. (Table 4). Based on Suseno et al. (2016), LAB does not require adaptation during the sago starch fermentation process, so it can grow optimally because it is used to acidic environmental conditions, and the carbon source comes from sago starch. Based on the suitability of the isolate as a starter in sago starch fermentation, the LAB IL1 isolate was chosen as the starter for the fermentation process.

LAB IL1 isolate was then prepared as a liquid starter for fermentation. The preparation of the LAB liquid starter aims to prepare an inoculum that can shorten the fermentation time. Lactic acid bacteria liquid starter is added when the bacteria reach the exponential phase. Making LAB liquid starter begins by measuring the growth curve to determine the optimum growth time (Suseno et al., 2016). Lactic acid bacteria growth curve measurements were carried out on MRS broth media, and the number of colonies was counted using solid MRS media. The LAB IL1 isolates had an exponential phase from the 2nd to the 6th hour with a specific growth rate (μ) of 0.9 hour^{-1} (Figure 1), the same as reported by Kamara et al. (2016) for *Lactobacillus bulgaricus* or now known as *Lactobacillus delbrueckii* subsp. bulgaricus (Zheng et al., 2020). Therefore, the LAB IL1 inoculum that will be used for the following sago starch fermentation process is a 6-hour-old culture.

Characteristics of fermentation liquid

Sago starch fermentation is carried out using LAB liquid starter and without starter (spontaneous) using bottled drinking water (BDW) and water from the Sago factory (RW). The fermentation process using LAB or spontaneously can influence physical, chemical, and microbiological changes in the quality of the fermented product (Meryandini et al., 2019). The fermentation of sago starch produces a by-product of a fermentation liquid obtained from separating the starch from the liquid. The parameters tested on sago starch fermentation liquid in the study included pH value (Table 5), total acid (Table 6), and microbial population (Table 7).

During the fermentation process, the pH value of the fermentation liquid in each treatment from day 0 to day 10 of observation decreased on average (Table 5), while the total acid concentration increased (Table 6). The decrease in pH occurs due to the presence of organic acids such as lactic acid, acetic acid, and alcohol produced by LAB activity originating from the liquid starter or from sago starch itself during the fermentation process (Suseno et al., 2016; Yuliana et al., 2019). The increasing number of microbes during the fermentation process causes a decrease in the pH value and an increase in the total amount of acid (Yuliana et al., 2019; Meryandini et al., 2019).

Analysis of microbial populations in fermentation liquids includes calculating the total LAB in MRS media, total mold and yeast in PDA media, total TPC in PCA media, total *E. coli* bacteria in EMB media, and total *Salmonella* sp bacteria, and *Shigella* sp. on SSA media (Table 7). The LAB population in the fermentation liquid for each treatment increased on average from day 0 to day 8 of fermentation (Table 7). The LAB population in the fermentation liquid for each treatment increased on average until the 8th day of fermentation. It was stable until the 10th day of fermentation except for the BDW treatment with the addition of a starter, which experienced a slight decrease (Table 7). The increase in LAB on day 2 in the treatment without starter could come from LAB in sago starch itself, as was the result of research by Yuliana et al. (2019) on spontaneous fermentation of sorghum without the addition of starter. An increase in the LAB population of sago starch fermented liquid in treatments using starter and without starter was also reported by Suseno et al. (2016). The accumulation of organic acids in the fermentation liquid can increase the growth of LAB for a specific time. However, the reduced concentration of dissolved sugars and increasingly acidic pH values cause a decrease in the number of LAB.

Table 5. Changes in pH of sago starch fermentation liquid

Treatment	Fermentation liquid pH value on day						
	Control	0	2	4	6	8	10
BDW+ LAB	7.6	5.0$^{ab,a*)}$	5.0^{ab,a}	4.8^{ab,a}	4.5^{ab,b}	4.1^{ab,c}	4.0^{ab,c}
RW+ LAB	6.3	4.7^{b,a}	4.5^{b,a}	4.5^{b,a}	3.9^{b,b}	3.9^{b,c}	3.9^{b,c}

Note: Control: pH of the liquid measured in the treatment before adding wet sago starch; BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+LAB: Fermentation treatment using sago brew water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level

Table 6. Total acid concentration of sago starch fermentation liquid

Treatment	Concentration of total acid (mg mL^{-1}) on day						
	Control	0	2	4	6	8	10
BDW+LAB	2.7	3.6$^{ab,d*)}$	4.5^{ab,d}	7.2^{ab,c}	8.1^{ab,c}	9.9^{ab,b}	13.1^{ab,a}
BDW	2.7	3.6^{b,d}	4.5^{b,d}	5.4^{b,c}	6.3^{b,c}	9.0^{b,b}	12.6^{b,a}
RW+LAB	4.5	6.3^{a,d}	7.2^{a,d}	8.1^{a,c}	10.8^{a,c}	12.6^{a,b}	15.3^{a,a}
RW	3.6	5.4^{ab,d}	6.3^{ab,d}	8.1^{ab,c}	9.9^{ab,c}	11.7^{ab,b}	14.4^{ab,a}

Note: Control: Total liquid acid measured in the treatment before adding wet sago starch; BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+LAB: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level

Table 7. Dynamics of microbial populations in fermentation liquids

Microbes	Treatment	Number of microbes (CFU mL^{-1}) on day					
		0	2	4	6	8	10
Lactic acid bacteria	BDW+LAB	$1.6 \times 10^{5c,c*)}$	$2.4 \times 10^{5c,b}$	$3.4 \times 10^{5c,ab}$	$4.9 \times 10^{5c,a}$	$6.7 \times 10^{5c,a}$	$5.4 \times 10^{5c,a}$
	BDW	$1.7 \times 10^{4c,c}$	$1.3 \times 10^{5c,b}$	$5.3 \times 10^{5c,ab}$	$1.4 \times 10^{6c,a}$	$2.2 \times 10^{6c,a}$	$2.0 \times 10^{6c,a}$
	RW+LAB	$1.1 \times 10^{5a,c}$	$2.0 \times 10^{5a,b}$	$3.1 \times 10^{5a,ab}$	$6.4 \times 10^{5a,a}$	$6.7 \times 10^{5a,a}$	$5.6 \times 10^{5a,a}$
	RW	$3.0 \times 10^{4b,c}$	$1.5 \times 10^{5b,b}$	$1.7 \times 10^{5b,ab}$	$1.9 \times 10^{5b,a}$	$2.2 \times 10^{5b,a}$	$1.8 \times 10^{5b,a}$
Molds and yeast	BDW+LAB	$1.1 \times 10^{5a,c}$	$1.4 \times 10^{5a,bc}$	$7.1 \times 10^{5a,a}$	$5.6 \times 10^{5a,a}$	$3.0 \times 10^{5a,ab}$	$1.6 \times 10^{5a,bc}$
	BDW	$7.7 \times 10^{3a,c}$	$2.2 \times 10^{4a,bc}$	$8.0 \times 10^{5a,a}$	$8.3 \times 10^{5a,a}$	$8.9 \times 10^{5a,ab}$	$8.0 \times 10^{4a,bc}$
	RW+LAB	$1.8 \times 10^{5a,c}$	$4.1 \times 10^{5a,bc}$	$5.1 \times 10^{5a,a}$	$6.6 \times 10^{5a,a}$	$9.0 \times 10^{4a,ab}$	$7.0 \times 10^{4a,bc}$
	RW	$3.5 \times 10^{5a,c}$	$5.2 \times 10^{5a,bc}$	$6.8 \times 10^{5a,a}$	$9.1 \times 10^{5a,a}$	$2.9 \times 10^{5a,ab}$	$1.8 \times 10^{5a,bc}$
Total plate count	BDW+LAB	$4.9 \times 10^{4b,b}$	$5.7 \times 10^{4b,a}$	$6.7 \times 10^{4b,a}$	$2.0 \times 10^{5b,a}$	$1.1 \times 10^{5b,a}$	$2.9 \times 10^{4b,a}$
	BDW	$1.1 \times 10^{3ab,b}$	$5.7 \times 10^{4ab,a}$	$7.1 \times 10^{5ab,a}$	$9.3 \times 10^{5ab,a}$	$7.6 \times 10^{5ab,a}$	$6.0 \times 10^{5ab,a}$
	RW+LAB	$1.2 \times 10^{5a,b}$	$3.7 \times 10^{5a,a}$	$4.9 \times 10^{5a,a}$	$7.4 \times 10^{5a,a}$	$5.2 \times 10^{5a,a}$	$4.5 \times 10^{5a,a}$
	RW	$1.9 \times 10^{5a,b}$	$4.3 \times 10^{5a,a}$	$5.4 \times 10^{5a,a}$	$8.2 \times 10^{5a,a}$	$6.2 \times 10^{5a,a}$	$5.4 \times 10^{5a,a}$
Escherichia coli	BDW+LAB	NG	NG	NG	NG	NG	NG
	BDW	NG	NG	NG	NG	NG	NG
	RW+LAB	$1.2 \times 10^{5a,a}$	$1.4 \times 10^{5a,a}$	$5.3 \times 10^{5a,a}$	$1.5 \times 10^{5a,a}$	$1.4 \times 10^{5a,a}$	$1.3 \times 10^{5a,a}$
	RW	$2.5 \times 10^{2b,a}$	$5.1 \times 10^{4b,a}$	$6.3 \times 10^{4b,a}$	$7.7 \times 10^{4b,a}$	$2.2 \times 10^{5b,a}$	$1.1 \times 10^{5b,a}$
Salmonella sp. and *Shigella* sp.	BDW+LAB	NG	NG	NG	NG	NG	NG
	BDW	NG	NG	NG	NG	NG	NG
	RW+LAB	TFTC	TFTC	TFTC	NG	NG	NG
	RW	TFTC	TFTC	$1.3 \times 10^{3a,a}$	$2.4 \times 10^{3a,a}$	$1.8 \times 10^{3a,a}$	$1.1 \times 10^{3a,a}$

Note: BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+LAB: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter; NG: No growth; TFTC: Too few to count (<30 colonies mL^{-1})

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level in each microorganism test

The population of mold and yeast in the fermentation liquid in the BDW treatment with the addition of starter and without starter increased on average from day 0 to day 4 of fermentation, then on day 6 to day 10 it decreased (Table 7). The population of mold and yeast in the fermentation liquid in the AS treatment with the addition of starter and without starter increased from day 0 to day 6 and then decreased when observed on day 8 to day 10 of fermentation (Table 7). The amount of TPC in the fermentation liquid for each treatment increased on average from day 0 to day 6 and decreased from day 8 to day 10 of observation (Table 7). According to Dewi et al. (2014), the increase and decrease in microbial populations can be influenced by environmental conditions in the form of pH in an acidic atmosphere. Mold has an optimum pH value of 5-7 and can grow in the pH value range of 3-8.5, while yeast has an optimum pH value of 4-5 and can grow in the pH value range of 2.5-8.5.

E. coli, *Salmonella* sp, and *Shigella* sp. are bacteria that cause foodborne diseases (Muna & Khariri, 2020). The population of pathogenic bacteria in the fermentation liquid treated with BDW with starter or without starter from day 0 to day 10 showed no growth of *E. coli*, *Salmonella* sp., and *Shigella* sp. detected (Table 7). Bottled drinking water (BDW) used during fermentation represents the use of clean water that meets standards as raw material for the sago processing industry, namely that there must be no *E. coli* bacterial contamination by the Republic of Indonesia Minister of Health Regulation No. 492/32 of 2010 (Krisno et al. 2021). The use of BDW with starter and without starter during the sago starch fermentation process from day 0 to day 35 also showed no growth of *Salmonella* sp bacteria. and *Shigella* sp. in fermentation liquid (Suseno et al. 2016).

The population of pathogenic bacteria in the fermentation liquid treated with RW without starter from day 0 to day 10 showed that there was still contamination by pathogenic bacteria (Table 7). The number of *E. coli* bacteria in the fermentation liquid increased from day 0 to day 8 and then decreased on day 10 of observation (Table 7). *Salmonella* sp. and *Shigella* sp. fermentation liquid increased from day 0 to day 6, then decreased from day 8 to day 10 of observation (Table 7). During fermentation, the water obtained from the sago processing factory (RW) comes from murky and dirty river flows. According to Elfidasari et al. (2015), river water in the Bogor area is unsuitable for raw water because it was detected to contain pathogenic bacteria *Salmonella* sp., *Shigella* sp., and *E. coli*. According to Yuliana et al. (2019), spontaneous fermentation can trigger the growth of pathogenic bacteria and inhibit the growth of beneficial bacteria, such as LAB, in the fermentation liquid. LAB that grows in spontaneous fermentation is unstable and relatively slower.

Adding LAB starter to the fermentation is expected to maintain the stability of the LAB population in the fermentation liquid. A stable LAB population during the fermentation process impacts LAB's ability to produce enough organic acids quickly to inhibit the growth of pathogens in the fermentation liquid (Yuliana et al., 2019). *Escherichia coli* in the fermentation liquid treated with AS with starter added increased from day 0 to day 4, then decreased on day 6 to day 10 of observation (Table 7). The bacterial population of *Salmonella* sp. and *Shigella* sp. in the fermentation liquid treated using AS, which added starter from day 0 to day 4 of observation, namely <30 colonies mL^{-1}, and on day 6 to day 10, the observation showed no growth of these pathogenic bacteria (Table 7). Adding LAB IL1 starter to AS during the sago starch fermentation process can reduce pathogenic bacteria more quickly than without adding a starter to the fermentation liquid (Table 7).

The role of LAB in improving the safety quality of sago starch

The wet sago starch samples in this study were obtained from the sago processing unit in Tanah Baru, Bogor, which is a small-scale sago processing unit. The sago processing unit has not implemented proper sanitation and has not complied with the requirements for good food production methods. The condition of the sago processing production room is in an open space and close to a river where there are still piles of rubbish. The sago processing machines and equipment used are still simple. Workers also do not maintain personal and environmental hygiene during the sago processing process. This has the potential to cause physical and microbiological contamination and affect the quality and food safety of the sago starch products produced (Fatimah et al., 2022). Sago starch fermentation is carried out as an effort to improve the quality and safety of sago starch (Suseno et al., 2016).

When used as a food ingredient, sago starch produced from the fermentation process must be on good quality so that it is safe for consumption. The quality of sago starch was analyzed through several test parameters including water content (Table 8), pH value (Table 9), total acid (Table 10), and microbial population (Table 11). Based on the quality standard SNI 3729:2008, a good water content in sago starch is a maximum of 13% (BSN, 2008). The water content of fermented sago starch in the treatment using BDW with the addition of starter met SNI standards on the 8^{th} and 10^{th} day, namely 12.4% and 10.5%, while without the addition of the starter the water content met the SNI standards on the 10^{th} day, namely 12.5% (Table 8).

The water content of fermented sago starch in the treatment using AS with the addition of starter and without starter respectively met SNI standards on the 10th day, namely 11.5% and 13% (Table 8).

The decrease in water content during the fermentation process occurs due to starch degradation by microorganisms which reduces the ability of the material to retain water. High or low water content values can affect the product's shelf life (Suseno et al., 2016). Anggraeni and Yuwono (2014) found that the longer the fermentation, the lower the water content value in fermented sweet potato flour.

The pH value of fermented sago starch in each treatment decreased on average from day 0 (5.1-6.0) to day 10 (4.0-4.7) (Table 9). In contrast, the total acid concentration in each treatment increased during the fermentation process, as indicated by the increase in NaOH volume as titration, from day 0 (0.5 mL of 1 N NaOH per 100 g) to day 10 (1.5-2.0 mL of 1 N NaOH per 100 g) (Table 10). The acidity of the sago starch produced is influenced by organic acids bound to starch molecules during the fermentation process so that the pH of sago starch becomes acidic (Suseno et al., 2016). Based on the SNI 3729:2008 quality standard, a good degree of acidity in sago starch is a maximum of 4.0 mL NaOH 1 N per 100 g (BSN, 2008), while the pH value of sago starch is not included in the SNI quality requirements. However, based on sago industry standards in Malaysia, a good pH value for sago starch is a minimum of 4.0 (Karim et al., 2008). Therefore, the pH value and total acid of fermented sago starch meet the good quality standards.

Table 8. Water content of fermented sago starch

Treatment	Water content (%) on day					
	0	2	4	6	8	10
BDW+LAB	$18.4^{b,a*)}$	$16.1^{b,b}$	$15.1^{b,c}$	$13.5^{b,d}$	$12.4^{b,e}$	$10.5^{b,f}$
BDW	$18.5^{ab,a}$	$17.5^{ab,b}$	$15.1^{ab,c}$	$14.6^{ab,d}$	$13.5^{ab,e}$	$12.5^{ab,f}$
RW+LAB	$19.0^{ab,a}$	$18.5^{ab,b}$	$17.0^{ab,c}$	$15.8^{ab,d}$	$13.2^{ab,e}$	$11.5^{ab,f}$
RW	$19.4^{a,a}$	$1.87^{a,b}$	$17.4^{a,c}$	$16.0^{a,d}$	$15.5^{a,e}$	$13.0^{a,f}$

Note: BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+LAB: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level

Table 9. Changes in pH in fermented sago starch

Treatment	pH value of flour on day					
	0	2	4	6	8	10
BDW+LAB	$5.1^{c,a*)}$	$4.4^{c,ab}$	$4.3^{c,bc}$	$4.1^{c,bc}$	$4.0^{c,bc}$	$4.0^{c,c}$
BDW	$5.2^{c,a}$	$4.2^{c,ab}$	$4.2^{c,bc}$	$4.2^{c,bc}$	$4.2^{c,bc}$	$4.1^{c,c}$
RW+LAB	$5.1^{b,a}$	$5.0^{b,ab}$	$4.6^{b,bc}$	$4.6^{b,bc}$	$4.5^{b,bc}$	$4.3^{b,c}$
RW	$6.0^{a,a}$	$6.0^{a,ab}$	$5.8^{a,bc}$	$4.8^{a,bc}$	$4.8^{a,bc}$	$4.7^{a,c}$

Note: BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter RW+LAB: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level

Table 10. Total titratable acidity in fermented sago starch

Treatment	Total titratable Acidity (mL NaOH 1 N / 100 g material) on day					
	0	2	4	6	8	10
BDW+BAL	$0.5^{a,e*)}$	$1.5^{a,d}$	$1.5^{a,c}$	$1.5^{a,c}$	$2.0^{a,b}$	$2.0^{a,a}$
BDW	$0.5^{a,e}$	$1.0^{a,d}$	$1.5^{a,c}$	$1.5^{a,c}$	$1.5^{a,b}$	$2.0^{a,a}$
RW+BAL	$0.5^{a,e}$	$1.0^{a,d}$	$1.0^{a,c}$	$1.5^{a,c}$	$1.5^{a,b}$	$2.0^{a,a}$
RW	$0.5^{a,e}$	$0.5^{a,d}$	$1.0^{a,c}$	$1.0^{a,c}$	$1.5^{a,b}$	$1.5^{a,a}$

Note: BDW+BAL: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+BAL: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level

Analysis of the microbial population on fermented sago starch includes calculating the total number of LAB on MRS media, total mold and yeast on PDA media, the number of TPC on PCA media, total *E. coli* on EMB media, and total *Salmonella* sp and *Shigella* sp. on SSA media (Table 11). The LAB population in fermented sago starch with starter addition treatment had a greater number of LAB compared to fermented sago starch treatment without starter addition. According to Suseno et al. (2016), LAB trapped in the cavities of sago starch structure during fermentation plays a beneficial role in inhibiting the growth of pathogenic bacteria, reducing the number of molds and yeasts, and reducing total microbes. Apart from that, LAB can also extend shelf life, improve food safety and the quality of the fermented products produced. This is related to the presence of compounds resulting from LAB metabolism such as lactic acid, acetic acid, exopolysaccharides and bacteriocins which are antimicrobial substances (Ariyana et al., 2018).

The population of mold and yeast in fermented sago starch in each treatment increased on average from day 0 to day 6, then on day 8 to day 10 it decreased (Table 11). Likewise, the amount of TPC in fermented sago starch in each treatment increased on average from day 0 to day 6 and then decreased from day 8 to day 10 of observation. Based on the SNI 3729:2008 quality standard, safe microbial contamination in sago starch is a maximum of 4 log CFU g^{-1} for the amount of mold and a maximum of 6 log CFU g^{-1} for the amount of TPC (BSN, 2008). The fermented sago starch mold and yeast population in the BDW treatment with the addition of starter and without starter met SNI standards on the 8^{th} and 10^{th} day because it showed no growth of mold and yeast. The population of fermented sago starch mold and yeast in the treatment using RW with the addition of starter met SNI standards on the 10^{th} day (4 log CFU g^{-1}), whereas in the RW treatment without adding starter it did not meet SNI standards because it exceeded the limit for the maximum amount of mold and yeast contamination. The amount of TPC in fermented sago starch in each treatment during the fermentation process still meets SNI quality standards because it is in the range of <30 colonies g^{-1} to 4.9 log CFU g^{-1}.

Wet sago starch obtained before fermentation comes from sago processing units that have not implemented good sanitation procedures during the processing process. Wet sago starch is obtained from extraction using river water as raw material. This can be the cause of mold growth on sago starch (Fatimah et al., 2022). According to Pue et al. (2018), mold and yeast contamination can also occur due to spontaneous fermentation. Pathogenic fungi that can cause disease in humans such as the molds *Aspergillus flavipes* and *Penicillium citrinum*, as well as the yeasts *Geotrichum candidum* and *Candida tropicalis* have been found in spontaneously fermented sago starch. According to Suseno et al. (2016), spontaneous fermentation can also produce sour sago starch which is not suitable for consumption because it is contaminated with pathogenic bacteria.

The population of pathogenic bacteria in fermented sago starch treated with BDW with starter or without starter from day 0 to day 10 showed that there was no growth of *E. coli*, *Salmonella* sp., and *Shigella* sp. detected (Table 11). This has met the quality standards for safe microbial contamination in sago starch, which is a maximum of 1 log CFU g^{-1} for the amount of *E. coli* according to the SNI 3729:2008 quality standard and negative per 25 g for the amount of *Salmonella* sp. contamination on starch based on PerBPOM No. 13 of 2019 (BSN, 2008; BPOM, 2019). The population of pathogenic bacteria in fermented sago starch treated with RW with added starter or without starter still showed the presence of pathogenic bacterial contamination.

The number of *E. coli* bacteria in fermented sago starch treated with RW without starter still did not meet the SNI safe standard, namely 4.3 log CFU g^{-1} on the 10^{th} day of observation (Table 11). The bacterial population of *Salmonella* sp. and *Shigella* sp. in fermented sago starch from day 4 to day 6, observations were <30 colonies g^{-1}, then on day 8 to day 10, observations showed no growth of pathogenic bacteria. The population of *E. coli* in fermented sago starch treated by RW with starter addition increased from day 0 to day 4 and did not meet the SNI safe standard, which was 5.7 log CFU g^{-1}. However, on the 6^{th} to 10^{th} day of observation, the number of *E. coli* in fermented sago starch decreased drastically and met the SNI safe standard (<30 colonies g^{-1}) so that no *E. coli* growth was detected. The bacterial population of *Salmonella* sp. and *Shigella* sp. in fermented sago starch treated with RW which added starter on the 4^{th} day of observation (less than 30 colonies g^{-1}) and on the 6^{th} to 10^{th} day of observation showed no growth of the pathogenic bacteria.

Based on the results of the microbial population dynamics, the treatment of adding LAB starter to RW during the fermentation process can reduce the number of molds and yeasts, total microbes, and pathogenic bacteria more quickly in fermented sago starch (Table 11). The use of BDW or clean water during the fermentation process can reduce pathogenic bacteria in fermented sago starch (Table 11). Suseno et al. (2016) showed that there was no growth of *Salmonella* sp. and *Shigella* sp. in sago starch fermented with BDW treatment with added starter and without starter during the sago starch fermentation process from day 0 to day 35.

Table 11. Dynamics of microbial populations in fermented sago starch

Microbes	Treatment	Number of microbes (CFU g^{-1}) on day					
		0	2	4	6	8	10
Lactic acid bacteria	BDW+LAB	$9.8 \times 10^{2ab,c*)}$	$1.6 \times 10^{3ab,abc}$	$1.8 \times 10^{3ab,ab}$	$2.1 \times 10^{4ab,a}$	$3.5 \times 10^{5ab,ab}$	$2.9 \times 10^{5ab,bc}$
	BDW	NG	NG	$4.9 \times 10^{4c,ab}$	$7.1 \times 10^{4c,a}$	TFTC	NG
	RW+LAB	$7.6 \times 10^{3a,c}$	$2.0 \times 10^{4a,abc}$	$2.2 \times 10^{4a,ab}$	$6.2 \times 10^{4a,a}$	$2.0 \times 10^{5a,ab}$	$1.6 \times 10^{5a,bc}$
	RW	NG	$2.4 \times 10^{4bc,abc}$	$2.5 \times 10^{4bc,ab}$	$1.4 \times 10^{5bc,a}$	$2.6 \times 10^{3bc,ab}$	NG
Molds and yeast	BDW+LAB	$1.0 \times 10^{3bc,b}$	$1.4 \times 10^{4bc,ab}$	$2.8 \times 10^{5bc,a}$	$2.4 \times 10^{5bc,a}$	NG	NG
	BDW	NG	NG	$7.0 \times 10^{4c,a}$	$8.8 \times 10^{4c,a}$	NG	NG
	RW+BAL	$2.9 \times 10^{4a,b}$	$1.1 \times 10^{5a,ab}$	$1.6 \times 10^{5a,a}$	$6.5 \times 10^{4a,a}$	$3.5 \times 10^{4a,b}$	$1.1 \times 10^{4a,b}$
	RW	TFTC	$2.5 \times 10^{3ab,ab}$	$1.7 \times 10^{3ab,a}$	$9.3 \times 10^{4ab,a}$	$6.2 \times 10^{4ab,b}$	$5.1 \times 10^{4ab,b}$
Total plate count	BDW+LAB	TFTC	$1.1 \times 10^{4b,ab}$	$1.8 \times 10^{4b,ab}$	$2.2 \times 10^{4b,a}$	TFTC	TFTC
	BDW	$3.6 \times 10^{2b,c}$	$8.7 \times 10^{2b,ab}$	$4.3 \times 10^{4b,ab}$	$6.9 \times 10^{4b,a}$	$4.1 \times 10^{2b,bc}$	$3.3 \times 10^{2b,bc}$
	RW+BAL	$9.2 \times 10^{3a,c}$	$1.1 \times 10^{4a,ab}$	$1.6 \times 10^{4a,ab}$	$7.9 \times 10^{4a,a}$	$7.4 \times 10^{4a,bc}$	$6.5 \times 10^{4a,bc}$
	RW	TFTC	$2.9 \times 10^{3ab,ab}$	$5.3 \times 10^{3ab,ab}$	$4.4 \times 10^{4ab,a}$	$2.3 \times 10^{4ab,bc}$	$2.0 \times 10^{4ab,bc}$
Escherichia coli	BDW+LAB	NG	NG	NG	NG	NG	NG
	BDW	NG	NG	NG	NG	NG	NG
	RW+LAB	$1.6 \times 10^{4a,a}$	$5.7 \times 10^{5a,a}$	$5.2 \times 10^{4a,a}$	TFTC	NG	NG
	RW	NG	$1.4 \times 10^{3a,a}$	$2.4 \times 10^{4a,a}$	$4.8 \times 10^{4a,a}$	$6.6 \times 10^{4a,a}$	$2.2 \times 10^{4a,a}$
Salmonella sp. and *Shigella* sp.	BDW+LAB	NG	NG	NG	NG	NG	NG
	BDW	NG	NG	NG	NG	NG	NG
	RW+LAB	NG	NG	TFTC	NG	NG	NG
	RW	NG	NG	TFTC	TFTC	NG	NG

Note: BDW+LAB: Fermentation treatment using bottled drinking water with added lactic acid bacteria starter; BDW: Fermentation treatment using bottled drinking water without starter; RW+LAB: Fermentation treatment using sago factory water with added lactic acid bacteria starter; RW: Fermentation treatment using sago factory water without starter; NG: No growth; TFTC: Too few to count (<30 colonies g^{-1})

*) Numbers followed by the same letter in the first notation indicate values that are not significantly different in the same column at the 5% test level and numbers followed by the same letter in the second notation indicate values that are not significantly different in the same row at the 5% test level in each microorganism test

Conclusion

Lactic acid bacteria isolate IL1 is a selected LAB isolate that has the potential to be used as a starter for fermentation because it has good tolerance to acidic conditions. The addition of LAB as a starter in sago starch fermentation has an effect on decreasing the pH value and increasing the total amount of acid. Acid accumulation can suppress the number of pathogenic microbial growth in sago starch thereby increasing food safety assurance for the product. The use of clean water in sago starch fermentation can also prevent the growth of pathogenic microbes in the sago starch produced.

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