Isolation and Characterization of Indigenous Lactic Acid Bacteria from Sago Wastewater

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ABSTRACT

Sago extraction processing into sago flour is generally still done in a traditional or semi-mechanical way, with less attention to aspects of hygiene, and resulted sago fermentation spontaneously. This study aimed to isolate and characterize indigenous lactic acid bacteria (LAB) from sago wastewater which obtained directly from the traditional sago processing industry in Malangke Barat, South Sulawesi. The sago wastewater was stored for 1, 3 and 7 days of fermentation. The research was conducted on the LAB isolation and purification on MRS agar medium supplemented with 0.5% CaCO₃. Two purified putative LAB isolate were each obtained from 1 day fermentation and 3 days fermentation. The LAB isolate from 1 day fermentation has milky white colors and smooth-round colonies, while LAB isolates from 3 days fermentation has creamy colors and smooth-round colonies. Both of the isolate were gram positive-rods, non-motile and negative catalase reaction.

Keywords : characterization, isolation, Lactic acid bacteria, sago wastewater.

Introduction

Sago palm (*Metroxylon sagu* Rottb.) is one of the most potential carbohydrate-producing crops in supporting Indonesian food security program [1]. Sago palm has many advantages over other carbohydrate-producing crops especially for its higher yield (15-25 ton dry starch/ha/year) [2]. This palm can grow along riverbanks and in swampy areas where are not suitable for other crops. The potential of sago in Indonesia is huge. Approximately 60% of the world's sago are in Indonesia. The dispersion of sago plants in Indonesia especially in Papua, West Papua, Maluku, Sulawesi and Riau islands.

The processing of sago extraction from plant into sago starch is generally done in a traditional or semi-mechanical ways with less attention to hygiene aspects. It requires large quantities of water, mostly taken from river waters [3]. River waters that used have poor quality so it can induce the growth of pathogenic microbes in sago starch. Besides that, Banu *et al.* [4] mentions that from sago extraction processing, 94% of waste generated in the form of liquid waste. Sago waste-water that directly channeled into the river can disrupt the flow of the river and cause contamination.

Lactic Acid Bacteria (LAB) is a bacteria that can inhibit the growth of pathogenic bacteria [5]. LAB produces antimicrobial compounds that inhibit during the fermentation process and remain inside foodstuff thus inhibiting the growth of harmful bacteria and pathogens [6]. LAB could isolated from land, water, waste-water and solid waste even from animal and human digestive tracts [7]. Suseno [8] said that LAB utilization isolated from soaking water of sago starch extract can be used as sago fermentation starter to improve the quality of sago starch by biopreservative method. So far, sago wastewater has not been widely utilized. In addition, information of potential bacteria in sago wastewater remains limited. In this research, isolation and characterization of indigenous LAB is conducted from sago

wastewater which obtained directly from traditional sago processing industry in Malangke district, South Sulawesi.

Materials

Materials that been used is sago wastewater obtained from from traditional sago processing industry in Malangke district, South Sulawesi. Fresh sago wastewaters were collected and stored in sterilized bottled. Sampled was brought to laboratory for further analysis. Samples were stored to fermentation for 1, 3 and 7 days in room temperature.

Methods

As much as 1 ml sago wastewater from sample that stored for 1 day was put into 9 ml of physiological saline solution (NaCl) 0.85%. For isolation and enumeration of microorganisms, samples were serially diluted (ranging from 10^{-1} to 10^{-5}) and plated on MRS (de Man Rogosa Sharpe) agar medium supplemented with 0.5% CaCO_3 for LAB screening [9]. Plates were incubated at room temperature ($30 \pm 2^\circ\text{C}$ respectively) for 2 days. The colonies which showed zone of clearance were maintained and purified on to MRS agar medium supplemented with 0.5% CaCO_3 for further experiments.

The purified isolates were gram-stained for morphological identification under microscope. The motility test was performed by taking the isolate with stitching wire and stuck on agar slants medium, then incubated for 2 days at temperature 37°C . The result of motile isolate is indicated by spreading isolate to all medium causing the medium to become feculent. While catalase reaction test is done by transfer a small amount of isolate colonies to a surface of clean dry glass using a loop, then a drop of 3% H_2O_2 is placed on to the glass. When air bubbles are formed, then the catalase is positive.

Results and Discussion

Isolation of LAB is done from 3 sago wastewaters samples according to storing time or fermentation (1, 3 and 7 days respectively). When the samples were stored the spontaneous fermentation occurs. The result of LAB isolation from sago wastewater showed the presence of LAB isolate colonies that forms clear zones on MRS agar supplemented with CaCO_3 (Figure 1). One of the LAB isolates criteria is could produce acids that will react with calcium carbonate in medium and form clear zones. The number of colonies obtained at each of fermentation time was presented on Table 1.

Figure 1. LAB isolates from sago wastewater forms clear zones on MRS agar supplemented with CaCO_3

Table 1. Numbers of LAB cell isolated from sago wastewater on serial dilution (cell/mL)

Fermentation time	Dilution	Numbers of cell
1 day	10^{-1}	Uncountable
	10^{-2}	4.1×10^4
	10^{-3}	1.2×10^5
	10^{-4}	1.5×10^5
	10^{-5}	0
3 days	10^{-1}	1.0×10^1
	10^{-2}	4.0×10^2
	10^{-3}	1.1×10^4
	10^{-4}	1.0×10^4
	10^{-5}	0
7 days	10^{-1}	0
	10^{-2}	1.0×10^2
	10^{-3}	3.0×10^3
	10^{-4}	0
	10^{-5}	0

Enumeration of LAB isolates colonies showed that numbers of LAB cells were decreased from fermentation 1 day to fermentation 7 days (Table 1). Fungi contaminations is one of the factors of lack of acquires isolation. Another factor is the growth of acid-producing bacteria other than LAB will increase and inhibit the LAB growth on sago wastewater. Isolate colonies that form clear zone were purified several times until single colony is obtained. Two purified putative LAB isolate were each obtained from 1 day fermentation and 3 days fermentation, respectively. The LAB isolate from 1 day fermentation has milky white colors and smooth-round colonies, while LAB isolates from 3 days fermentation has creamy colors and smooth-round colonies (Figure 2). Gram-stained for both isolates shows gram positive-rods. The LAB isolates from 1 day fermentation was short-rod, then the LAB isolates from 3 days fermentation was long-rod (Figure 3). Motility test and catalase reaction test for both isolates showed non-motile and negative catalase reaction.

Figure 2. Single colony of LAB isolates from sago wastewater (A) 1 day fermentation; and (2) 3 days fermentation.

Figure 3. Gram-stained for both LAB isolates from sago wastewater (A) 1 day fermentation; and (2) 3 days fermentation.

The LAB isolates with characteristics were gram positive, negative catalase reaction and rod shape belongs to *Lactobacillus* genus [8]. *Lactobacillus plantarum* and *Lactobacillus fermentum* were endogenous bacteria in the process of fermentation of starch-containing foodstuff such as corn, cassava and rice [10]. Sago wastewater still contains amount of starches that were included in the washing process.

In conclusions, we found the presence of LAB in sago wastewater. The isolates will be identified further by inhibition to pathogenic bacteria (*E. coli* and *S. aureus*) and molecular test. The indigenous isolate of LAB could be utilized to improve the quality of sago starch and act as probiotics.

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