Amylolytic activity of bacterial strains isolated from sago pulp of the traditional sago industry in Palopo, South Sulawesi

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Amylolytic Activity of Bacterial Strains Isolated from Sago Pulp of the Traditional Sago Industry in Palopo, South Sulawesi

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Abstract. Sago is a plant widely cultivated in Indonesia with a planting area of 1.128 million ha. Sago waste, especially sago pulp, has enough starch so that it can be used as a substrate for amylolytic bacteria. Amylase enzymes produced by bacteria have the potential to be applied in the industrial sector, food, medicine, and also used to recycle waste. This study aimed to obtain amylolytic bacteria isolated from the sago pulp industry in Palopo, South Sulawesi and observe the amylase activity of these bacteria. Amylolytic bacteria were isolated from sago pulp using selective media. The pure isolate was then filtered and the ability to produce the amylase enzyme was observed. Nine amylolytic bacterial isolates were filtered based on qualitative tests. Four bacterial isolates, 2B, 2D, 1C, and 4A showed high amylolytic index, 9.00 mm, 7.28 mm, 5.75 mm, and 4.67 mm, respectively. The highest amylase activity based on quantitative tests was shown by isolates 2B (3.62 units/mL). Based on the results of the study, it was shown that the bacteria isolated from sago pulp have good potential to produce amylase.

INTRODUCTION

Sago (*Metroxylon sago*) is an important source of carbohydrates in addition to starches and tubers. Sago is a native plant of Southeast Asia, one of the countries that has the largest area of sago plantation is Indonesia. Sago planting area in Indonesia reaches 1,128 million ha or around 51.3% of the total 2,201 million ha of the world sago area [1]. Besides being used as a food ingredient, sago is processed into flour that can be commercialized. The high amount of sago production causes an increase in the amount of sago waste. Sago pulp is solid waste that comes from processing sago starch from sago trees. Sago pulp waste has a starch content of 65.7%, 21% lignin, 20% cellulose. Sago pulp content depends on the age of the tree, species, and processing [2].

Sago pulp has the potential to be used as a source of amylolytic bacteria obtained. It can be used as a substrate for bacterial growth, while it is used to produce useful products, such as fermented sugar, enzyme production, organic acids, and ethanol because they contain high organic matter, such as starch. Sago porridge can be hydrolyzed to reduce sugar by bacteria that have degrading activities, such as amylolytic bacteria. In previous studies, there were 133 bacterial isolates derived from sago pulp, 120 isolates had amylolytic potential with a range of amylolytic ratios of 1.00-7.50 [3]. Another study stated that there were 23 amylolytic bacterial isolates obtained from sago pulp [4]. *Lactobacillus* sp., *Pseudomonas* sp., *Bacillus* sp., *Sreptomyces* sp. are bacteria that have potential to produce amylases [5]. Therefore, this research focused on obtaining amylolytic bacteria from sago pulp in Palopo, South Sulawesi that can be further used for amylase production and can support bioconservation of sago pulp into economically valuable products.

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MATERIALS AND METHODS

Isolation and Screening of Amylase Enzyme-Producing Bacteria

The samples were obtained from sago waste of sago industry in Palopo, South Sulawesi. The samples were taken from piles of sago waste, precisely the bottom closest to the ground. Measured environmental parameters, such as temperature and pH. Isolation of amylolytic bacteria was carried out using starch media (1% soluble starch, 0.2% yeast extract, 0.5% peptone, 0.05% $MgSO_4$, 0.05% NaCl, 0.015% $CaCl_2$, and 2% bacteriological agar) with method plate [3, 5]. All plates were incubated at 30 °C for 48 h. Screening isolates used iodin 1% by flooding the isolates inoculated in another petri dish, and a clear zone indicated positive value. The colonies isolated were photographed (front and back), then grouped according to their origin. Pure isolates were preserved in 30% glycerol and stored at -80 °C [6].

Screening of Potential Amylase Enzyme Production Bacteria

The isolates were selected based on their ability to produce amylase enzyme by inoculating the isolates into the liquid starch medium. After incubating for 48 h at 30 °C, the density of the culture was examined by using a spectrophotometer with a wavelength of 540 nm and then equalized the concentration of each sample. A total of 20 µl of the sample was taken and dropped into a paper disc then plated on agar media and incubated for 24-48 h at a room temperature. After incubation, each petri dish was then flooded by 1% iodine. Each isolate was carried out three times as many replications. The clear zone was indicated that the isolates have good potential to produce enzyme [7]. The amylolytic index was obtained by dividing the diameter of the clear zone by the diameter of the colony and using the following Equation 1 [3].

$$S = \frac{\pi}{4}\left(d_c^2 - d_{co}^2\right) \tag{1}$$

Note: S = amylolytic index (mm^2), d_c = diameter of clear zone (mm), d_{co} = colony diameter (mm)

Growth Curve and Extraction of Enzymes from Amylolytic Bacteria

One loop of each selected isolate was inoculated into 25 mL of liquid starch media then incubated in shaker incubator 150 rpm, at 37 °C for 24 h. Five mL of the bacterial culture was taken to observe the optical density at 540 nm. After equalization, 1 mL of culture was taken and transferred to 50 ml of new media, incubated at 37 °C, 150 rpm. Five mL of the samples were taken at 24, 48, 72, and 92 hours. The absorbance was measured at a wavelength of 540 nm [8].

Bacteria culture on liquid media compared the cell density using spectrophotometry at 540 nm. Then, 1 mL of the liquid was taken and inoculated in new media then incubated at 30 °C in a shaker incubator, 150 rpm. Three mL of bacterial fermentation broth at 48, 72 and 96 hours was centrifuged for 10 min at 10000 rpm. The bacterial free supernatant was used as a source of crude amylase enzyme then taken to analyze the enzyme activity [9].

Determination of Crude Amylase Enzyme Activity

Amylase activity was tested using the DNS method [10]. One mL of crude amylase filtrate from centrifugation (supernatant) was put into a test tube, then added liquid starch media with 1% sago as a source of starch and then incubated at 37 °C for 15 min. One g/100 mL of starch solution was added to the blank tube (as blank) and then incubated 15 min, 37 °C without added the enzyme. One mL of the DNS reagent was added in each test tube to stop the reaction after 15 min. The tube was heated to boiling for 5 min, added 1 mL of K-Nartrate solution, cooled with running water for 15 min and 1 mL of distilled water added. Each solution in the test tube was then determined by the color intensity using a spectrophotometer at 540 nm. The absorbance value was plotted with a glucose standard curve. Each test sample was repeated three times for enzyme activity.

Amylase activity was determined through the DNS method using sago starch as a substrate. One unit of enzyme activity was defined as the amount of µmol glucose produced from the hydrolysis of starch by 1 mL of crude amylase extract during the incubation period. The enzyme activity (U/mL) was calculated used Equation 2.

$$AE = \frac{C}{BM\ \text{glocose} \times t} \times \frac{H}{E} \quad (2)$$

Note: AE = enzyme activity (Unit/mL of enzyme filtrate), C = glucose concentration, BM glucose = 180, t = incubation time (10 min), H = total enzyme-substrate volume (2 mL), and E = enzyme volume (1 mL)

Data Analysis

Data from amylolytic index were analyzed using an analysis of variance (ANOVA) test with 0.5% confidence interval to examine the effect of treatment. The analysis was then followed by a Tukey test with 0.5% confidence interval to discover which treatment would be influential or significantly different from other treatments.

RESULTS AND DISCUSSION

Isolation and Screening for Amylolytic Bacteria

Bacterial isolates from sago pulp have the ability to produce amylase enzymes; it was expressed as the appearance of a clear zone around the colony (Fig. 1). The reaction that occurred between starch and iodine would produce a dark purple color, whereas in the clear zone the dark purple color was not found so that the reaction indicated the hydrolysis of starch by these bacterial isolates. A clear zone indicated that though hydrolysis of starch may occur at different percentage starch composition of the medium. Concentration of 1% was the most suitable. This case is related to the previous study that observed maximum enzyme activity in the range of 1 – 1.5 % of starch concentration [11] and percentage inhibition in the α-amylase activity beyond starch percentage concentration of 1.25% [12].

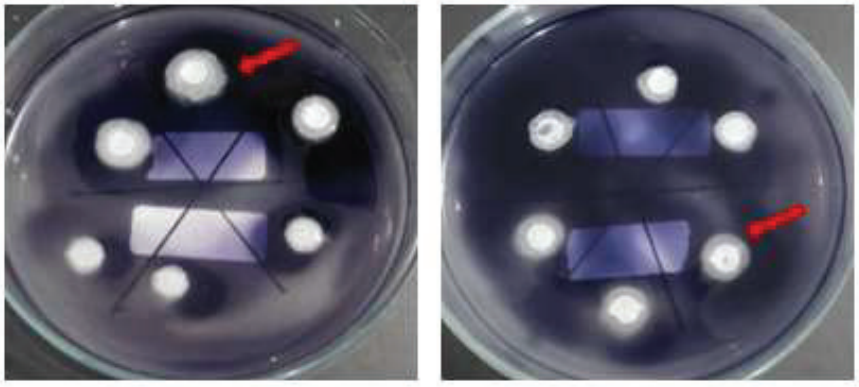

FIGURE 1. The clear zone formed from starch hydrolysis by amylolytic bacteria (arrow)

TABLE 1. Amylolytic index of bacterial isolates from sago pulp

Isolates[1]	Amylolytic index (mm)[2]
1B	1.27 ± 0.09^{ab}
1D	0.90 ± 0.40^{a}
1E	0.90 ± 0.40^{a}
1C	5.75 ± 0.17^{ab}
2A	1.21 ± 0.58^{ab}
2B	9.00 ± 6.16^{b}
2D	7.28 ± 2.96^{ab}
4A	4.70 ± 3.62^{ab}

[1]Isolates were grown in starch broth for 48 h at 30 °C

[2]All numbers are the means amylolytic index with different superscript letters that are significantly different ($p<0.05$)

Eight bacterial isolates that showed the clear zone indicated various amylolytic indeces (Table 1). There were four bacterial isolates with the high amylolytic index, namely 2B, 2D, 1C, and 4A. Qualitatively, isolate 2B had the highest amylolytic index of all the isolates with the amylolytic index was 9.004 mm. Several factors affected the work of enzymes that were not under the optimization of the enzymes produced by each isolate, for example, pH and temperature, which significantly impacted the enzymes. In measuring pH, the neutral pH and room temperature (30 °C) used were not yet identified for their optimum pH and temperature [13]. The pH of an enzyme solution could affect overall catalytic activity in various ways, and most of the enzymes were the most stable at physiological pH (7.4), but some of them showed maximum pH under slightly lower or higher conditions [14].

Growth Curve and Amylase Activity of Potential Bacterial Isolates

The most optimal production of amylolytic bacteria occurred in the last phase of exponential growth [8]. The increased enzyme production was associated with increased cell growth, which indicated that amylolytic bacteria actively used amylase during the growth phase. Based on the bacterial growth curve (Fig. 2) that has been obtained, it was known that at 0 hours, the bacteria were still in the phase of adaptation to the growth media. The data showed that at 24 h, all of the isolates were in the exponential phase. While at the 48 h, the isolates began to the stationary phase. This phase was indicated by a plateau in the number of living bacterial cells, rate of cell division and death roughly equal [15]. At 72 hours, 1C, 2B, and 4A isolates were still present in the stationary phase, while 2D isolate appeared to move down of the growth curve. At 96 h, 1C and 4A isolates still appeared in the stationary phase, while 2B and 2D isolates were in the death phase. The results of this study were also related to the previous study that amylolytic bacteria had an adaptation phase at 0-10 h incubation, the lag phase at 15-40 hours incubation, and the stationary phase at 45-70 hours incubation [16, 17]. Based on this study, it could be identified that 2B, 2D, and 4A isolates had the highest amylase activity at 72 h while 1C isolate was at 96 h. So, the highest of amylase activity was from 2B isolate. Nevertheless, all of the isolates had the highest activity in the stationary phase.

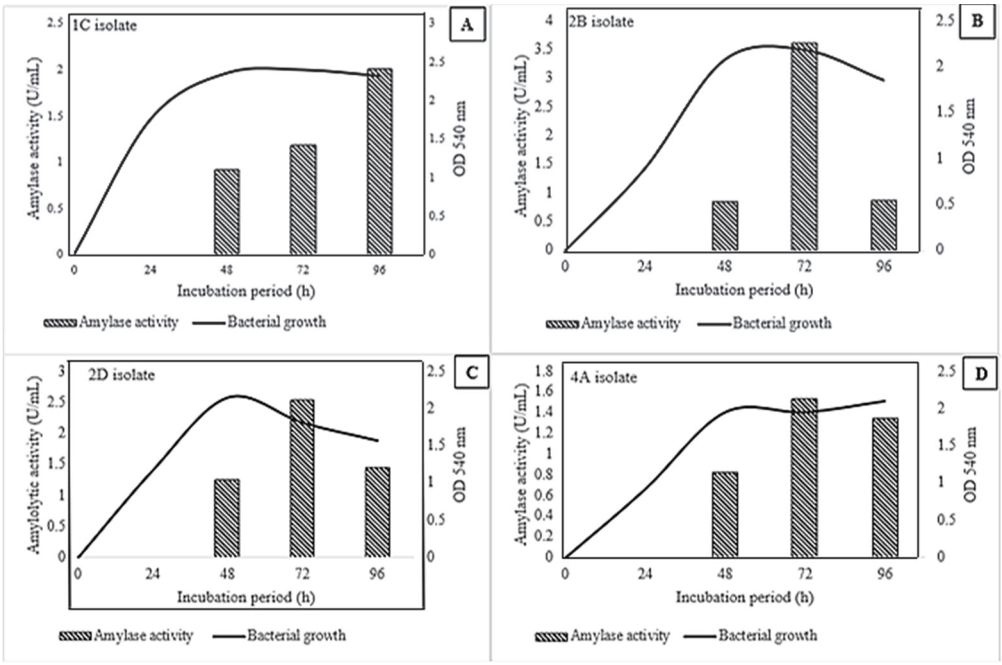

FIGURE 2. Amylase production and growth of potential amylolytic bacteria from sago pulp. 1C isolate (A), 2B isolate (B), 2D isolate (C) and 4A isolate (D) in medium contain amylum.

From this research, it can be found that 2B isolate had high potency to produce an amylase enzyme. At this incubation hour, 2B isolate was in the stationary phase. During the stationary phase, cells switched to a survival mode of metabolism. As growth slows, so too does the synthesis of peptidoglycans, proteins, and nucleic acids. Secondary metabolites, such as antibiotics are synthesized in the stationary phase [17]. Amylase production was growth independence as maximum enzyme production was achieved during the stationary phase [18].

The observations showed that isolate 1C had the highest enzyme activity at 96 h (2.01 U/mL), the other isolates, 4A (1.54 U/mL), 2B (3.62 U/mL), and 2D (2.55 U/mL) showed the highest amylase enzyme activity at 72 h and decreased at 96 h. The activity of amylase enzymes produced by bacteria increased since the first day of culture, peaks on the second day, and the activity decrease on the third and fourth days [10]. The activity of these enzymes was determined by the concentration of enzymes, conformation of enzymes, sequence of amino acids forming enzymes, and kinds of amino acids forming enzymes [18]. Isolate 2B owned the highest activity with amylase activity of 3.62 U/mL at the 72nd h incubation time. Amylase activity of *Chryseobacterium* sp. and *Bacillus* sp. at different incubation periods has also beenreported at range of 3.30-3.40 U/mL [19]. In another case, various species of microorganism can produce a range of enzyme specific activities (from 0.84 to 1.35 U/mg) [20]. *Bacillus* sp. BKL20 strain isolated from natural can reach up to seven U/mg of enzyme specific activity [21].

SUMMARY

Isolation of amylolytic bacteria obtained nine isolates with four highest potentials of producing amylase enzyme with the amylolytic index for each isolate, namely 2B (9.00 mm), 2D (7.28 mm), 1C (5.75 mm), and 4A (4.67 mm). Bacteria isolate 2B had the highest ability to produce amylase enzyme (3.62 U/mL). This result showed that amylolytic bacteria isolated from sago pulp have the potential to produce amylase enzyme.

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