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Potential amylase-producing yeast isolated from indigenous fermented beverages originating from Bali, Indonesia

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Abstract. Indonesia has many fermented beverages, and yeast become one of the agents in fermented process. Yeasts has a role to transform carbohydrate complex into simple compounds with release secondary metabolism to environment like amylase enzyme. This study aims to get the isolate of yeast that can potentially produce amylase enzyme. This research conducted in October 2018 until March 2019 in Microbiology Laboratory of Universitas Negeri Jakarta. The screening test of potential isolate producing amylase enzyme was performed on yeast isolate from eight source of indigenous fermented beverages that can grow in YMA medium with pH 2. Screening was carried out on YPSA medium with diffusion agar method. From 50 Isolates, 16 isolates with the codes IB4, IB15, IB20, IB21, IB26, IB36, IL78, IL80, IL81, IL86, IL88, IL97, IL113, IL136, IL146, and IL150, were able to form clear zone after 1-day incubation in room temperature. The highest amylolytic index was produced by IL86 (1,019 mm). Forming the clear zone is proof that yeast can transform starch become simpler sugar like maltose as iodine-starch reaction is resulting amylose helix and iodine become I_3^- that filled main core helix. In addition to this, iodine forms complexes with starch molecules showed a dark purple colour.

1. Introduction

Indonesia is a country that has various kinds of local foods and drinks, one of them is fermented food. Local fermented food is made in a home industry that has not set a standard for the production process

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so that it will produce a variety of yeast [1-3]. Yeast is one of the microorganisms that has an important role in the food fermentation process. In Balinese brem fermented foods, the dominant yeast grown in the fermentation process is *Saccharomyces cerevisiae*, *Candida glabrata*, *Pichia anomala* and *Orientalist Issatchenkia* [4]. In addition, yeast can also have the potential to produce amylolytic activity, which can degrade starch into sugar with the help of amylase enzymes [5].

Amylase belongs to a group of hydrolytic enzymes that have the ability to break the bonds of glycosides in starch [6]. Based on the classification of enzymes regulated by International (IUB) starting in 1961 the amylase enzyme belongs to a group of hydrolase enzymes that catalyze biological hydrolysis reactions or hydrolytic solutions of CC, CO, CN, PO, with the help of water (H2O) [7].

This enzyme works to break the bonds of α-1,4-glycosides in starch randomly, especially in long chains so as to produce maltotriosa and maltose from amylose polymers in starch and produce glucose and a little dextrin from amylopectin polymer making up starch. Because it can break the glycoside bonds randomly, this enzyme works faster than other amylases such as β-amylase [8].

Amylase enzymes are needed in the textile industry, starch hydrolysis, food manufacturing (beer, bread, syrup, artificial sweeteners, animal feed industry), ethanol production, detergents, drugs, and enzyme supplements. In the process of work, the amylase enzyme has the ability to break the bonds of glycosides found in starch. The results of the hydrolysis are simpler molecules such as glucose, maltose, dextrin, and other organic acids [9].

This study aims to determine yeast isolates from fermented drinks from Bali and Lombok that produce amylase enzymes by looking at the clear zone tested with lugol droplets. A total of 50 isolates from the isolation in the previous study were tested in this study in order to obtain isolates with the potential to produce amylase enzymes.

2. Methods

2.1. Yeast isolation

50 yeasts representative isolated was obtained from fermented food Tuak and Bream Lombok. Direct and Serial dilution method were used to isolate the yeast followed the method [10]. All yeasts were cultivated in a YMB medium containing 1% (w/v) glucose, 0.3% (w/v) malt extract, 0.3% (w/v) yeast extract and 0.5% (w/v) peptone. The inoculated petri plates were incubated at 28 ^{0}C for 2 days. The isolates were picked up and further inoculated on sterile potato dextrose agar plates by point inoculation and incubated at 28°C for 48 h in order to obtain pure yeast plates.

2.2. Screening of amylase-producing yeast

Selection was done by inoculating yeast on starch agar peptone yeast media (YPSA) using agar diffusion method by making wells based on [11]. YPSA consist of 10 g *soluble starch*; 5 g peptone; 2 g yeast extract; 0,1 g $MgSO_4.7H_2O$; 0,1 g $CaCl_2.7H_2O$; 0,5 g KH_2PO4, 20 g for 1000 ml medium. Making wells using sterile straws was carried out on YPSA media which had been divided into four quadrants. Amylase activity was measured using the iodine-starch method [12]. A total of ± 20 µL of yeast suspension was inoculated into the media. Media composition i.e. yeast suspension was made by adding 5 mL of sterile distilled water to the working culture of yeast isolates and then the suspension was homogenized using vortex. Incubation was carried out at 30 ° C for 72 hours. Amylase activity is seen by the appearance of a clear zone around the colony after being poured with iodine [13]. The results between the diameter of the clear zone and the diameter of the colonies are expressed as relative enzyme activity [14].

2.3. Calculation of amylase index

Yeast isolates that have the potential to produce amylase were tested by measuring the amylolytic index. Each sample measuring amylase enzyme was repeated three times [15]. Determination of the amylolytic index value is based on Goldbeck, with the formula: IA = 2.6 Observation of yeast morphology [16].

Yeast isolates that have the potential to produce amylase enzymes are observed maximally. Macroscopic observation of yeast morphology is the texture, color, surface, profile and edge of the colony [17].

3. Result and discussions

3.1. Qualitative amylolytic activity test

50 yeast isolates from Bali and Lombok palm wine fermented inoculated into YPSA's selective medium. Yeast inoculated from isolates that have been tested to grow at 37° C and low pH 1-2. Qualitative testing was carried out by iodine administration and observed the formation of clear zones (figure 1).

Amylase activity test using starch substrate. Starch is one type of carbohydrate that requires the amylase enzyme to digest it. Yeast isolates that produce amylase can be seen from the formation of clear zones around the yeast colonies. To clarify the presence of a clear zone, a dense starch medium overgrown with bacteria was dripped with a solution of lugol's iodine. The area outside the clear zone will be purplish blue after being given the solution, because the solution of lugol's iodine will react with non-hydrolyzed starch. Clear zones are not stained because the starch found in the zone has been hydrolyzed into simpler compounds such as disaccharides or monosaccharides.

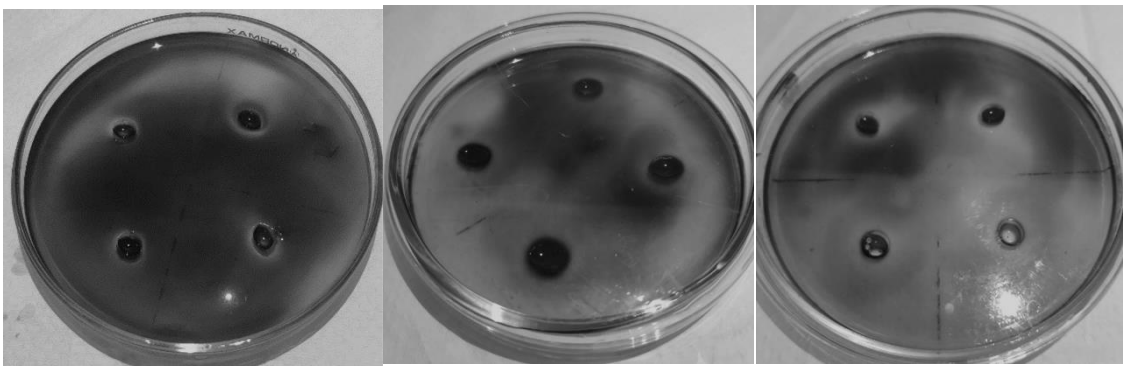

Figure 1. Clear zone formed around yeast colonies in YPSA medium.

Qualitative test results showed that 16 isolates had amylolytic activity (Figure 1) which are IB4, IB15, IB20, IB21, IB26, IB36, IL78, IL80, IL81, IL86, IL88, IL97, IL113, IL136, IL146 isolate codes and IL150. The clear zone formed around the yeast colonies shows that the isolate is able to hydrolyze starch and has the potential to produce amylase enzymes. While as many as 34 yeast isolates do not form clear zones because they cannot degrade starch. This is due to the need for different carbon sources for each isolate. The presence of clear zones in yeast has the potential to produce amylase enzymes because hydrolyzed starch is a simpler compound such as disaccharides and monosaccharides [18]. The absence of clear zones around the colonies indicates a reaction between iodine reagents and non-hydrolyzed starches in the YPSA medium [19].

3.2. Result of amylolytic index

There are differences in the Amylolytic Index values produced by 16 isolates (table 1). This indicates that each isolate has a difference in hydrolyzing starch in YPSA media. The highest amylolytic index value was found in IL86 isolates, which was 1,019 mm, followed by IB20 isolates with amylolytic index value of 0.9 mm while isolates with the lowest amylolytic index were IL150 isolates with a value of 0.093 mm.

Determination of the potential amylolytic index of yeast isolates was carried out using agar (well) diffusion method based on Fossi et al. [20]. The diffusion method was conducted by performing wells to determine the ability of yeast isolates to hydrolyze compounds in the medium after iodine reagents. The positive result will show the presence of clear zone around the wells [21]. This method is an effective method to see the clear zone formed so far. According to Listari, the advantage of the well

method is that it has clear zone measurements since yeast isolates that grow not only on the surface of the media but also below the growth media [22]. The testing of potential amylase yeast isolates based on amylase index was carried out by growing yeast isolates on YPSA medium (starch yeast pepton agar) and this test using yeast suspension.

The clear zone formed around the yeast colonies shows that the isolate was able to hydrolyze starch [23]. Starch will form a deep blue complex with iodine reagent (Fig. 1). The iodine-starch reaction is caused by the presence of helical amylose and iodine in forming I_3^- which fills the helical nucleus. A hydrolyzed starch will cause the starch-iodine complex to breakdown to form a clear zone [11,24]. The absence of clear zones around the colonies indicates a reaction between iodine reagents and non-hydrolyzed starches in the YPSA medium [18]. Schoch el al., reported that the ability or power to produce an amylase enzyme in a microbe was characterized by the formation of a clear zone in a medium containing starch [8].

Table 1. Amylolytic index value (IA) of the yeast isolates from the YPSA medium.

Isolates	**Diameter (mm)**
	Clear Zone
IL81	0,351
IB4	0,722
IB15	0,255
IB36	0,675
IB80	0,639
IB20	0,9
IL88	0,544
IL86	1,019
IB26	0,278
IL97	0,146
IL78	0,206
IL113	0,155
IB21	0,161
IL136	0,243
1L150	0,093
IL146	0,148

3.3. Result of macroscopic identification

Macroscopic testing was carried out by growing all isolates in the petri dish with YMA media for 24 hours. Based on the results of macroscopic identification, there are differences in edges, elevations, surfaces, and textures of the 16 isolates that exist (Table 2; Figure 2). Molecular testing is needed to determine the type of each isolate.

The results showed that the yeast obtained was 100% non-pigmented yeast. Research by Nasreen et al. also obtained the same result with the isolation from apples, oranges and bananas and showed 100% non-pigmented yeasts [25]. Pigments can be produced by yeast under environmental stress conditions such as direct exposure to sunlight. Gmoser et al. explains that pigmentation appears to be caused by the protection mechanism released by yeast on oxidative stress and light [26]. Dirnawan et al. managed to isolate yeast from leaf surfaces and produce pigmented dominant yeast isolates of 57% [23]. Tan et al. states that leaf surfaces exposed to light and changing temperatures are dominated by microorganisms that have pigments [27].

The nutrients available in fruits are sufficient enough to support yeasts growth so that there is not stress condition which leads the yeasts to form pigments. According to the study conducted Medeiros which said that sugar content such as fructose and glucose in fruit can be used by yeasts [15]. Crieger

stated that yeast can use various carbon substrates but mainly uses sugars such as glucose, fructose, sucrose, and maltose [28]. There are some isolates which resulted no clear zones around the wells. It is thought to be because not all yeasts found in the fruit have amylolytic ability, which is to produce amylase enzymes. *Saccharomyces cerevisiae*, for example, is one of the yeast samples commonly found in fruit but does not have the ability to produce amylase. Research by Cappucino et al. confirmed this statement that *S. cerevisiae* was known to be non-amylolytic yeast, but efficient in the process of glucose fermentation to ethanol [29].

Table 2. Result of macroscopic identification.

Isolate Code	Colour	Elevation	Margin	Surface	Texture
IB4	White	Flat	Undulate (Wavy)	Striated	Butyrous
IB15	White	Convex	Entire (Even)	Smooth	Mucoid
IB20	White	Convex	Entire (Even)	Smooth	Mucoid
IB21	White	Flat	Undulate (Wavy)	Striated	Butyrous
IB26	White	Convex	Entire (Even)	Smooth	Mucoid
IB36	White	Convex	Entire (Even)	Smooth	Mucoid
IL78	White	Flat	Undulate (Wavy)	Striated	Butyrous
IL80	White	Convex	Entire (Even)	Smooth	Mucoid
IL81	White	Convex	Undulate (Wavy)	Smooth	Mucoid
IL86	White	Convex	Undulate (Wavy)	Smooth	Mucoid
IL88	White	Convex	Undulate (Wavy)	Smooth	Mucoid
IL97	White	Convex	Entire (Even)	Smooth	Mucoid
IL113	White	Convex	Entire (Even)	Smooth	Mucoid
IL136	White	Filamentous	Filamentous	Filamentous	Butyrous
IL146	White	Flat	Lobate (Lobes)	Rough	Butyrous
IL150	White	Flat	Lobate (Lobes)	Rough	Butyrous

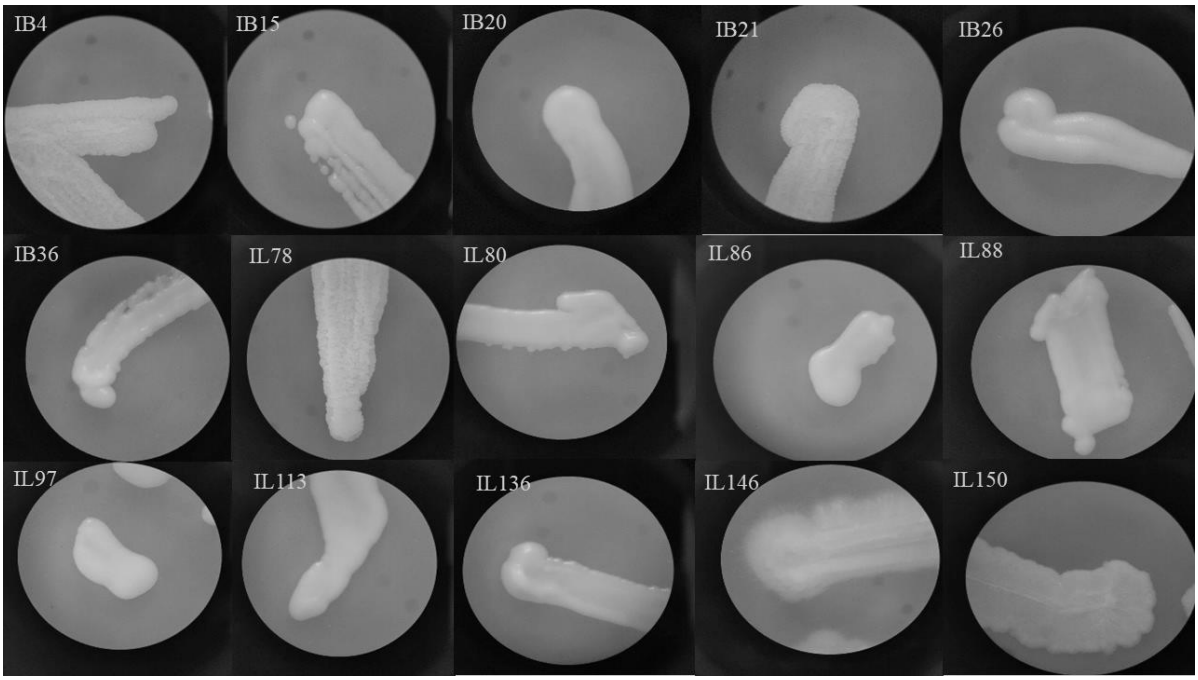

Figure 2. Macroscopic identification with a stereo microscope. Line 1 (from left to right) IB4, IB15, IB20, IB21, IB 26, Line 2 IB36, IL78, IL80, IL81, IL86, Line 3 IL88, IL97, IL113, IL136, IL146, Line 4 IL150.

4. Conclusion

There were 16 yeast isolates which showed amylolytic activity from Balinese and Lombok palm wine fermented drinks with the codes IB4, IB15, IB20, IB21, IB26, IB36, IL78, IL80, IL81, IL86, IL88, IL97, IL113, IL136, IL146, and IL150. The highest amylase index was found by IL86 isolate with a value of 1,019 mm and the isolate with the lowest index was IL150 with a value of 0.093 mm. Further research is needed to determine the activity of amylase enzymes in food products or animal feed, how the number of colonies are formed and their role in degrading the starch. In addition, pathogenicity tests need to be carried out to ensure that existing isolates are safe for use in food. Molecular identification is also needed to be done to ascertain the types of amylolytic yeast isolates above.

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