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The Potency of Amylase Producing Bacteria in the Liquid Waste of Tapioca Factory

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ABSTRACT

The need of amylase in a number of industries, especially food, beverage, textile, leather, and paper industry, is increasing. It is urgent to explore the potential of amylase from various sources including the liquid waste of tapioca factory. The aim of the research is to isolate the amylase producing bacteria from the liquid waste of tapioca factory. The research was started by collecting and screening sample and subsequently purifying the bacteria. The sample collection was done in disposal, grinding, and controlling basin. The sample was screened by using the NB medium and then spread on the NA medium by using spread plate method and incubated for 48 hours at 30 ^{o}C temperature to obtain a single colony of bacteria. The bacteria were purified by using the selective amylolytic starch agar (SA) medium. The result of the research was 15 single colonies of bacteria, which 7 of them that performed amylase activity with isolate code of HCB6, CCG6, DCG2, ACB, ECG6, ICG2, JCK6. The bacteria with potency to produce amylase was the bacteria with isolate code HCB6, whose amylase index was 3.78.

Key words: activity, amylase, bacteria, liquid waste tapioca, single colony, starch agar.

INTRODUCTION

Amylase enzyme is utilized to hydrolyze the starch into a simpler molecule namely maltose and glucose enzyme, which constitute the enzyme group that is vital in industry, with a market share approximately 25% of world's enzyme market (de Carvalho et al., 2008). The amylase enzyme has been widely used in industries, including bread industry, syrup, sweetener, oligosaccharide mixture, dextrin, textile industry, ethanol producer, examination of liquid waste which contains starch, detergent industry, pharmacy, and enzyme supplement (Kahraman, 2007). The enzyme needed in industry is extracted from various cells of living organisms.

In the last decade, there have been numerous researches on amylase in animals (Ueda et al., 2008), plants (Fendri et al., 2013), microorganism (Liu et al., 2011). However, there are more enzymes extracted from various microorganisms, since microorganisms produce enzyme which can be used by human in large number and varieties, in addition to the fact that microorganisms can be cultured to obtain the enzyme.

A number of microorganisms like bacteria, yeast, and fungi are known to produce amylase in its growth stage in starch subtract, which provides starch for cells. In the research, isolation of amylase from tapioca liquid waste was done. The liquid waste being used are as follows; the

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water used to wash unskinned cassava; water used to wash and soak skinned cassava; water used to wash wet tapioca starch; and water used to precipitate tapioca starch (first precipitation). The volume of the liquid waste reached 12-15 times of the volume of the cassava that was being processed. The liquid waste still contained a considerable amount of starch. The isolation of microorganisms from the habitat which still contains nutrients required for growth was expected to be obtained from qualified microorganism. The purpose of the research was to find out the potency of the bacteria being isolated by the liquid waste of tapioca factory as producer of amylase.

MATERIALS AND METHODS

2.1 Materials and Methods

The research was carried out in the Laboratory of Biochemistry of Department of Chemistry, Faculty of Mathematics and Natural Sciences and the Integrated Laboratory of Universitas Jenderal Soedirman Purwokerto. The material being used in the research was the sample of tapioca liquid waste obtained from the tapioca factory in Cipawon Village, sub-district of Bukateja district of Purbalingga. Nutrient Broth (NB) liquid medium with composition as follows, yeast extract 0.5%, peptone 0.5%, and NaCl 0.5%; Nutrient Agar (NA) solid medium with compositions as follows: kamir extract 0.5%, peptone 0.5%, NaCl 0.5%, agar 1.7%, selective amylase medium (yeast extract 0.2 %, peptone 0.5 %, NaCl 0.05 %, $MgSO_4$ 0.05%, $CaCl_2$ 0.015%, agar 2%, and soluble starch 1%). Agar starch medium (SA) is composed of all components of selective medium and contains 8% agar, iodine solution, aluminum foil, pH meter paper, wrapping. The tools being used in this research consist of measuring pipette, measuring cup, reaction tube, Erlenmeyer, pH meter, thermometer, inoculation loop, Bunsen burner, petri disk, cup, stir bar, micro pipette (soccorex), incubator, autoclave, oven, and hot plate stirrer.

2.2 Procedures

2.2.1 Collecting of Samples

The sample of tapioca liquid waste was taken through group random sampling method, while pH and temperature at the sample location were measured. As much as 40 mL of the liquid waste sample was inserted with aseptic method into Erlenmeyer 250 mL containing 10 mL NB five x concentrated medium which has been sterilized and labelled. The sample was poured so that NB became 1 x concentrated medium. The sample of tapioca liquid waste was incubated by using shaker bath in similar temperature and pH as those in its origin habitat for 2x24 hours. Isolation was initiated by dilution until the degree of 10^{-6}. As much as 0.1 mL of culture was spread in the NA medium in petri disk by using spread plate method and incubated for 48 hours at the temperature of 30 ^{o}C.

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The colony that grew was isolated and purified by re-scraping in quadrangle way on the NA medium and it was re-incubated for 48 hours at the temperature of 30 ^{o}C to obtain the single colony.

2.2.2 *Bacteria Purification* (Tiwati et al., 2007)

For bacteria purification, each bacteria colony that grew differently in the previous culture was taken as much as one inoculation loop and scraped on every other petri disk which contains amylolytic selective agar medium, incubated for 48 hours at the temperature of 30 ^{o}C. Every pure isolate which could grow was assumed to be capable to use the medium which contains that starch. To ensure this, iodine test was done by dripping iodine on the source of isolate containing agar, and if transparent zone is found on the media, it indicated that amylase enzyme has been produced by the isolate. The diameter of the transparent zone and the diameter of bacteria were measured and the relative amylase activity was calculated.

RESULTS AND DISCUSSION

The research was initiated by sample collection, where a sample of tapioca liquid waste was collected in Cipawon village, Kecamatan Bukateja, Kabupaten Purbalingga. The location of sample collection was the grinding, disposal and controlling basin. As much as 40 mL of water sample was put into nutrient broth (NB) 5x concentrated 10 mL medium so it became NB 1x concentrated medium. The sample was incubated by using shaker incubator for 48 hours with room temperature. The NB medium which was transparent shows that the existing bacteria growth in the medium (Figure 1).

Figure 1. The growth of bacteria in NB medium.

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The next step was staging dilution to obtain the single colony. The degree of dilution was 10^{-2}, 10^{-4}, and 10^{-6}. The sample of diluted water was grown in NA (Nutrient Agar) medium with spread plate method and then incubated for 48 hours at the temperature of 30 ^{o}C. The bacteria colony that was obtained was re-grown in the new NA medium with quadrant streak method so that single colony was obtained (Figure 2).

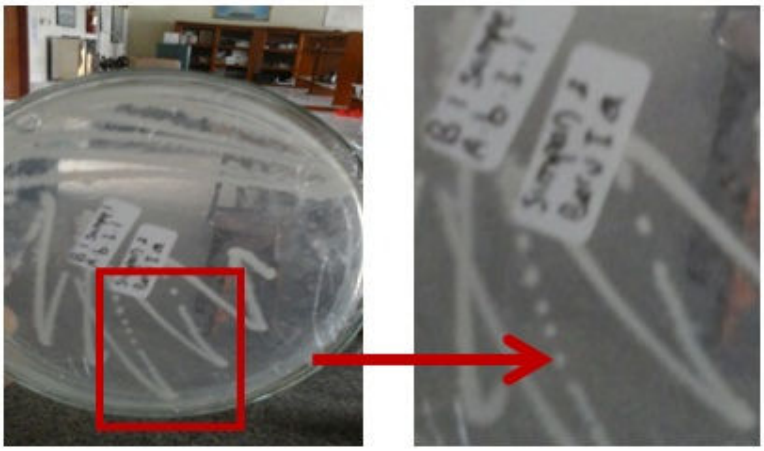

Figure 2. Colony of bacteria that was grown in NA medium.

Next, the capability of amylase activity was examined by using a starch agar (SA) medium. The bacteria colony was moved into the SA medium and incubated for 48 hours and then iodine solution was added. The iodine coloring was one of the methods to detect the amylase activity and it functioned further as reagent to detect the existence of amylum. Amylum that reacts with iodine will result in dark-blue color, this color is formed when the molecule of iodine entered into the empty space in the spiral shaped amylum molecule. The color resulted depends on the average size of the molecule (Page, 1997). The bacteria colony with amylase activity will form a transparent zone. The transparent zone around the bacteria colony shows the hydrolysis of amylum into simple sugar due to the amylase activity resulted by the bacteria. The simple sugar will produce transparent color when reacted with iodine because there was no spiral shape of the amylum. From the bacteria isolate was found 15 bacteria colonies and only 7 of them that produced transparent zone, they are bacteria colonies with isolate code HCB6, CCG6, DCG2, ACB, ECG6, ICG2, JCK6. Qualitative test of several isolates can be seen in the Figure 3. The diameter of the transparent zone can be seen in the Table 1.

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Figure 3. Qualitative test of bacteria isolate from tapioca liquid waste.

Table 1. The result of qualitative amylase test

Code of Isolate	Diameter of the Transparent Zone (mm)	Diameter of Bacteria (mm)	Amylase Index
ACB	1.05	0.45	1.32
CCG6	1.50	1.23	0.22
DCG2	1.29	0.88	0.47
ECG6	0.84	0.49	0.71
HCB6	0.87	0.18	3.78
ICG2	1.08	0.84	0.29
JCK6	0.86	0.36	1.38

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The diameter of the transparent zone was divided by the diameter of the colony, demonstrating the relative activity. From the relative activity test it was found that HCB6 has a relatively high amylase activity, which was 3.78. Vaseekaran et al. (2010) obtained 3 chosen isolates, which was BS1, FS1, and GS1 with amylase index consecutively was 4; 3.6.; 3.4. Amri et al. (2010) has 4 chosen isolates, which were A1, A3, A5, and B1 with amylase index consecutively were 1.67; 1.25; 0.6; and 1.33. The existence of structural gen which regulates the protein synthesis in the bacteria cell will determine the capability of bacteria in producing amylase. Besides, environment will also influence the expression of structural gen which codifies the production of amylase. For example, the existence of glucose in a medium in certain concentration will undermine amylase.

CONCLUSIONS

The isolation of bacteria of tapioca liquid waste resulted in 7 isolate that were potentials to produce amylase. The isolate which activities were relatively high was isolate coded HCB6 with amylase index was 3.78.

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