The growth rate of inulinolytic yeast isolates from yum (Pachyrhizus erosus L.) and their inulinase production capability

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Abstract. Inulinase (EC. 3.2.1.7) is a hydrolytic enzyme that hydrolyze inulin into fructose. This enzyme was very important in the high fructose syrup industry. Microbes producing inulinase can be isolated from various sources, among others from various types of tuber that contain inulin. The isolation of inulinolytic yeast from yum (Pachyrhizus erosus L.) obtained two potential isolates. Research on the growth rate of the two potential yeast isolates in producing inulinase is necessary to understand the pattern of the yeast growth and its ability to produce inulinase. The parameters observed in this study were specific growth rate (µ), generation time (g) and inulinase activity of two selected yeast isolates B2 and B3. The results showed that the specific growth rate (µ) of B2 isolate was 0.11 hours with generation time (g) 6.12 hours, whereas for B3 isolates the specific growth rate (µ) was 0.21 hour and generation time 3.29 hours. The inulinase activity from yeast isolates B2 and B3 were 0.294 IU and 0.235 IU, respectively.

1. Introduction

Individual growth can be interpreted as an increase in the volume of cells and other parts as well as in the quantity of content in the cell. Population growth is the result of individual growth. Microbial growth is the most important factor in determining several aspects of physiology. This is because the growth characteristics reflect the physiological events of a microbe. One of the important types of microbes is yeast (khamir). The yeast used in this study was the result of isolation from yum (Pachyrhizus erosus L.). The yum plant has been well known by the Indonesian people. The yum plant contains pachyrhizone, rotenon, vitamin B1, and vitamin C. Besides that, the yum tuber contains inulin which is beneficial for health and often is used in functional foods [9].

Inulin consists of a straight-chain of 25 - 35 fructose units with ß-2,1-fructofructanosidic bonds with 1 terminal glucose unit [1]. These carbohydrates are produced by Compositae types of plants such as dahlia tubers (Dahlia sp. L), Jerusalem artichoke tubers (Helianthus tuberosus), Chicory (Chicoryum intybus L), dandelions (Taraxacum officinale Weber), yacon tubers (Smallanthus sanchifolius), and small amounts found in onions, garlic, asparagus, bananas, and wheat. Polyfructose with a degree of polymerization of 30 and above is called inulin [3]. Inulin can not dissolve in cold water, but at 500°C temperature can dissolve 50% inulin. This molecule can precipitate in the ethanol-water mixture [4]. Enzymes capable of breaking down inulin into fructose molecules are inulinase (EC. 3.2.1.7), while the yeast is called inulinolytic yeast. The purpose of this study was to determine the specific growth rate in the form of kinetics (µ), generation time (g), and inulinase activity produced by selected isolates.
2. Material and methods

2.1. Microorganisms

The source of microbes used in this study was pure culture isolates from the fruit of bengkoang (Pachyrhizus erosus L.)

2.2. Cultivation of medium and production of inulinase

Media for culture cultivation and inulinase production [5] (g/L): inulin-30; NH₄NO₃-2.3; (NH₄)₂HPO₄-3.7; K₂HPO₄-1; MgSO₄.7H₂O-0.5; yeast extract-1.5 and pH 5. The media was autoclaved at 121°C for 20 minutes. The media was autoclaved at 121°C for 20 minutes. After inoculated with 10% of 24 hours yeast starter, the flasks were incubated at 28°C for twenty-four hours on a rotary shaker at 150 rpm.

2.3. Enzyme assay

An amount of 0.1 ml of the enzyme solution was mixed with 0.1% inulin substrate in a sodium acetate buffer solution pH 5.0 and incubated for 30 minutes at 50°C. The determination of inulinase activity was carried out based on a number of enzymes that are able to break down 1 mol of substrate per minute under certain conditions. The reducing sugar formed was determined by the DNS method [6] [7].

Meanwhile, the measurement of specific growth rate (µ) and generation time (g) was measured using a spectrophotometer at 520 nm. The specific growth rate (µ) is determined according to the formula [8]

\[
\mu = \frac{(\ln X_2 - \ln X_1)}{t_2 - t_1}
\]

and the generation time (hours) was determined by the formula

\[
g = \frac{\ln 2}{\mu} = 0.693\mu
\]

3. Results and discussion

The yeast culture used in this study was derived from yam tuber (Pachyrhizus erosus L), which are known containing inulin. [9]. The inulin content in the various plant parts like tubers and seeds can be use as a carbon source for microbes, and can be found in onions (garlic), daffodils (onions), wheat (wheat bran) as well as in Jerusalem artichokes. [10] [11]. The inulin content in these tubers can be used by inulinolytic microbes to support their growth.

The isolation from yam tuber was obtained 2 yeast isolates which is have the capable in producing inulinase, namely B2 isolate (Figure 1) and B3 isolate (Figure 2). The screening of the inulinolytic yeast isolates were done on ISM medium which is a selective medium for inulinase producing microbes, due to the inulin content as the only carbon source [12]. The characteristics of inulinolytic yeast isolates were showed in Table 1.

| Table 1. The characteristics of yeast isolates from yam tuber |
|-----------------|-----------------|-----------------|
| Characteristics  | B2 isolate      | B3 isolate      |
| Colony color    | yellowish white | white           |
| Colony surface  | flat dull       | convex, dull    |
| Colony edge     | entire          | entire          |
| Cell shape      | oval            | round           |
The examination of the two inulinolytic yeast isolates growth showed that these isolates have a normal growth curve, consisting of lag phase, accelerated growth phase, exponential phase, slowed growth phase, stationary phase and death phase. Microbial growth is a very dynamic process, as is growth kinetics. This kinetics can be used to analyze a product or cell biomass [13]. Based on the data obtained, the growth of B2 isolate is presented in Figure 3.

**Figure 1.** Yeast isolate B2  
**Figure 2.** Yeast isolate B3

**Figure 3.** The growth curve of the B2 yeast isolate

**Figure 4.** The growth curve of B3 yeast isolate
Based on the growth pattern of B2 yeast isolate, the log phase occurred between 12 and 20 hours of age. In this phase all life activities take place optimally, cells divide rapidly because the nutritional needs are well fulfilled. In this phase the cells divide rapidly, where the increase in number follows a logarithmic curve [14].

In this condition, it turns out that the addition of ZnSO4 of 0.25 mM (C2) can shorten the generation time (g), which is 6.12 hours. Meanwhile, the concentrations of C1 (control), C3 (0.5 mM) and C4 (1 mM) were respectively 8.86, 8.55 and 7.30 hours. Meanwhile, the specific activity (µ) was 0.13, the inulinase activity was 0.294 IU. In the growth pattern of isolate B3, it had a growth pattern as shown in Figure 4. In this phase, the log phase growth occurred at the age of 8-20 hours. From that age, the shortest generation time (g) was 3.28 hours, the specific growth rate (µ) was 0.219 and the inulinase activity was 0.235 IU at a concentration of ZnSO4 0 mM (control). Based on the data obtained, isolate B3 was better than isolate B2. This is because B3 isolate has a shorter generation time than isolate 2, and economically - industry is very profitable. This difference in results was due to the different isolates used and of course the different products produced. The inulinase produced from each isolate is directly related to the presence of inulin in jicama. Inulinase enzymes are extracellular and inductive. This means that the enzyme is formed due to the stimulation process. Inulinase enzyme is classified as a primary metabolite because it is produced during the exponential phase. Inulinase is synthesized during yeast growth and reaches a maximum in the stationary phase and is adaptive and is a primary metabolite. The inulinases present in liquid culture are referred to as supernatant inulinases and are thermostolerant [15] [16] [17]. Enzymes can be classified into primary metabolites which are usually formed in the logarithmic growth phase. In this phase, cell growth occurs very rapidly and enzyme production increases [18].

4. Conclusion
The results showed that the specific growth rate (µ) of B2 yeast isolate was 0.11 hours with a generation time (g) of 6.12 hours, while the B3 yeast isolate specific growth rate (µ) was 0.21 hours and a generation time of 3.29 hours. The inulinase activity of B2 yeast isolates and B3 were 0.294 IU and 0.235 IU, respectively.

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