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MANNOPROTEIN PRODUCTION FROM *CANDIDA APICOLA* IN THE FORMULATED BEAN SPROUT EXTRACT MEDIA

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Abstract

"Candida apicola" is an osmotolerant ascomycetes yeast. Yeast in general produces secondary metabolic substances in the form of bioactive substances that can be utilized in biotechnology field, besides that yeast also has antimicrobial properties. "Candida apicola" has a cell wall that contains mannoprotein, a bioactive substance that can be used as an emulsifier in the process of making food. Beansprout extract is used as the media formula for the growth of "Candida apicola" so secure for food production. The media formula used for the growth of "Candida apicola" is derived from bean sprout extract so it is safer for food production. This study aims to determine the growth curve of "Candida apicola" on media formulated and the value of the emulsification activity of mannoprotein obtained from the extraction process. The research begin by looking at the growth curve of "Candida apicola" with an incubation time of 0 to 70th hours and observing several parameters such as optical density, pH and biomass cells. The optimal incubation time in the growth of "Candida apicola" in obtaining mannoprotein occurred at 70th hours with absorbance value of 1.5817, pH 5.69 and biomass cell 0.0271 gr/ml. The incubation time of 70th hours in 500 ml of the formulated media produced a mannoprotein of 1.5982 gr and had a 50% of emulsification activity.

Key words**:** *yeast, "Candida apicola", formulated media, mannoprotein, emulsifier*

INTRODUCTION

Indonesia is a tropical country with a wide diversity of microorganisms that includes land, water, and air consisting of protists, monera, and fungi include yeast (Kanti, 2007) [15]. Yeast genus *Cryptococcus, Candida* and *Debaryomyces* is a group of yeast that is often found in soil ecosystems (Kanti, 2005) [14]. Yeasts have important enzymes including phosphatase, lipase, zymase and proteinase which function in the decomposition of organic compounds and are used in industrial purposes (Spencer and Spencer, 1997) [22].

Utilization of yeast caused by the bioactive component existence such as proteins and antimicrobial compounds. Protein produced have proteolytic activity with a molecular weight of 97 kD (Balia, *et al.*, 2011) [2]. Some species of yeast such as *Candida* have proteolytic abilities (Roostita and Fleet, 1996) [19] and can produce extracellular proteases (Fleet, 1990) [11]. *Candida apicola* can generate biosurfactants, fatty acid membranes and several enzymes such as proteases, glucanases, and proteinases (Vega *et al.*, 2015) [26].

Yeast generally has cell walls with dry weight ranging from 23-32% of cells (Saeed *et al*. 2017) [20] consist of polysaccharides in the form of beta-glucans and mannan sugar polymers 20-60%, proteins 15-30%, fat 5-20% and slightly of chitin. Proteins contained in cell walls are mostly bind to mannan-oligosaccharides (MOS) called mannoproteins (Eurasyp, 2011) [8]. Other references state that the content of mannoprotein in cell walls is 40% of the dry weight of cells (Klis *et al*., 2002; Uscanga and François, 2003) [16, 25].

Mannoprotein constitutes of bioemulsifier of the glycoprotein groups that can be used in stabilizing oil in water in food products (Farahnejad *et al.*, 2004) [10]. Several studies have been carried and stated that mannoprotein can be utilized as an emulsifying agent in the manufacture of foods such as salad dressing and mayonnaise. Mannoprotein obtained when the cell undergoes an autolysis process in the cell wall with the help of heat extraction (FAO, 2017)

[9]. The process of autolysis, glucanase and proteinase enzymes will degrade cell walls containing mannoproteins so that they can be released from the surrounding medium (Alexandre and Gulloux-Benatier, 2006; Martinez, et al., 2016) [1, 17]. The number of cell walls influenced by the growth curve of yeast, which is a young yeast has thin cell walls whereas an aged yeast has thick cell walls (Balia, et al., 2017) [3, 4].

Media for yeast growth can use ingredients that have high nutritional value and support yeast growth by utilizing bean sprout extract. Bean sprout extracts have macronutrient and micronutrient elements as well as several other materials in media formulas such as vegemite, sugar, and antibiotics that can supply yeast needs and inhibit the growth of bacteria. This research aims to determine the growth curve of *Candida apicola* on media formulas by observing optical density, pH and cell biomass so that obtained the optimal time to produce mannoprotein and determine the emulsification activity value of the obtained mannoprotein.

MATERIALS AND METHODS

Materials

This research was conducted using several instruments including Petri dish, ose, plastic wrap, parafilm, incubator, beaker glass, micropipette (1,000 µl), blue pipette, bunsen, laminar, falcon tube, Erlenmeyer, schoot, pan, Raypa Sterilclav-75 autoclave, Termo Scientific hot plater stirrer, stirring rod, cuvvet, spectrophotometer, oven, 18,000M N-Biotek centrifuge, Eutech pH 700 pH meter, Sartorius TE2145 scale and Raypa Mixtube vortex.

The materials used in this research are bean sprouts, vegemite, antibiotics and sugar as ingredients for making media. Yeast *Candida apicola* isolate was obtained from Prof. Roostita L. Balia's collection. The media used in the rejuvenation process of *Candida apicola* were Oxoid CM0920 Yeast and Mould Agar (YMA) and Himedia M255-500G Malt Extract Broth Base (MEB). Furthermore, other materials are used in the testing process such as buffer solution, blank solution, and oil. Mannoprotein extraction process is carried out using materials namely aquades, 0.1M potassium citrate, and 90% chilled ethanol.

Methods

Yeast strain

Candida apicola isolates were obtained in the form of yeast and mold agar (YMA), so it was necessary to isolate cultivation in malt extract broth (MEB) or liquid media by collecting 2 ose of *Candida apicola* in YMA, afterward transferring them to beaker glass containing 5 ml of MEB. The beaker glass is closed tightly, then stored in an incubator at 25°C for 48 hours (Ukit, 2013 - modification) [24].

Media Formula

The main ingredient in making media formulas is bean sprout extract. Heats 3 liters of distilled water that has been added by 1 kilogram of bean sprouts as fas as 1 liter of water volume. Afterward, sugar, vegemite, and antibiotics were added. The mixture of ingredients sterilized at 121°C for 15 minutes. The media formula is ready to use (Balia *et al.*, 2018) [5].

Growth of Yeast in Media Formula

Candida apicola that has planted on MEB is taken as much as 1% (50 µl) then transferred to 5 ml of the media formula, incubated at 25°C with incubation time 0^{th} to 70^{th} hours, carried out testing of optical density. pH measurement and cell biomass in every 5 hours the incubation time.

The Growth Curve of *Candida apicola*

The growth curve testing consists of optical density, pH and biomass. The testing of optical density was performed by using a 600nm spectrophotometer. The measurement of pH was carried out on *Candida apicola* isolates using a pH meter and biomass testing was carried out by drying at 40°C for 12-16 hours (Garcia and Casas, 1999 - modification) [12].

Extraction of Mannoprotein

Extraction of mannoprotein was started by separating of precipitate using a centrifuge, the outcome of precipitate was added 0.1 M potassium citrate then sterilized by autoclaving at 121°C for 15 minutes to 2 hours. Furthermore, the isolate was centrifuged at 6,000 rpm for 15 minutes at

4°C, the supernatant was added with 90% chilled ethanol and stored at 4°C for 12-16 hours until the precipitation process completed. The results of precipitation obtained were centrifuged at 6,000 rpm for 15 minutes at 4°C followed by washed twice with chilled ethanol (Torabizadeh *et al*., 1996) [23].

Measurement of Emulsification Activity

Measurement of emulsification activity started with taking mannoprotein that had diluted with 1 ml of distilled water and oil into the Eppendorf tube, then conducted agitation using vortex for 3 minutes and stored for 24 hours to measure the Emulsification Index by following formula (Cooper and Goldenberg, 1987) [6]:

Emulsification Index

$$= \frac{\text{Total height of the emulsified layer}}{\text{Total height of the liquid layer}} \; x \; 100\ \%$$

RESULTS AND DISCUSSIONS

The Growth Curve of *Candida apicola*

The result of *Candida apicola's* growth shows that the lag phase occurs at 0^{th} to 5^{th} hours. The lag phase is the adaptation time of cell in growth media so that nutrient in media affects the growth of yeast. The lag phase in *Candida apicola* has a pretty short time because it is caused by media formula, which is originated from bean sprouts extract with nutrient content such as protein, fiber, vitamin C, K, thiamin, riboflavin, and folate. These nutrient contents are the main component, which should be filled in yeast media as carbon source and nitrogen. Carbon sources can be dextrose, raffinose, and glycerol. This can be supplied by the presence of vegemite added into bean sprouts extract. Besides, there is an antibiotic with the type of penicillin as the inhibitor of bacteria growth in media. The exponential phase occurs in $10t^{h}$-$45t^{h}$ hours, where the growth curve happens to increase. According to Gonzales (2010) [13] explains that the exponential phase is at 12^{th}-36^{th} hours. After experiencing the maximum growth by utilizing nutrients in the media, the cell will occur in the stationary phase. The stationary phase occurs at 50th-60th hours and is followed by the death phase at 65th -70th hours with an absorbance value of 1.5267 to 1.5817. The death phase is marked by decreasing the amount of cell, observed from its absorbance. According to Wahono et al. (2011) [27], the time of death phase is ranging from 48th to 78th hours. The death phase causes mannoprotein degradation in the cell wall by enzymes such as glucanase and proteinase so that the incubation time at 65th -70th hours is the best in yielding mannoprotein.

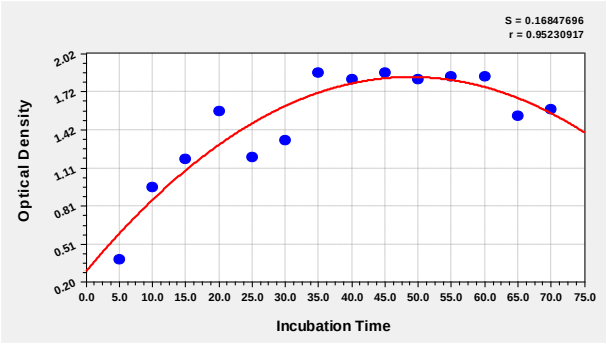

Fig. 1. The growth curve of *Candida apicola*
Source: Own results in the laboratory.

The Growth Curve of *Candida apicola*'s pH

pH decreases at 0^{th} to 55^{th} hours and increases at 60^{th}-70^{th} hours. The decrease of pH becomes more acid because of the high nutrient content in growth media so that it happens the degradation of glucose and arises the activity of the fermentation process to produce amino acids.

The incubation time of 0-15 hours possesses pH 6 so that it causes the growth of yeast and is also followed by decreasing pH until 5.5 at 55th hours. At the incubation time of 60^{th}-70^{th} hours, pH increases to 5.7. It is due to nutrients at growth media decreasing so that there is no fermentation activity, which is led to increasing pH.

The death phase of the cell happens at the incubation time of 70 hours and can be used as the harvest time of *Candida apicola* to extract mannoprotein.

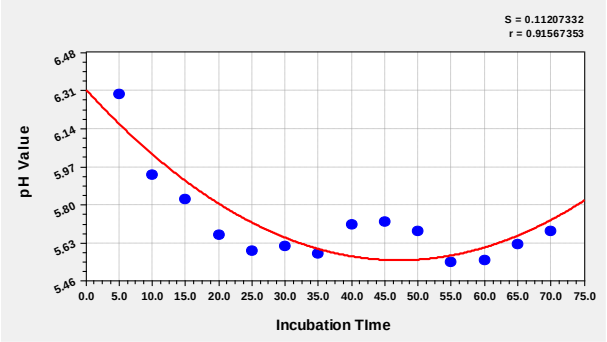

Fig. 2. The growth curve of pH from *Candida apicola*
Source: Own results in the laboratory.

The Growth Curve of *Candida apicola* Biomass

Biomass from *Candida apicola* is obtained by drying at 40oC for 12-16 hours. The growth phase of yeast can be determined by biomass concentration. The stage of lag phase to exponential phase will lead to increasing biomass concentration and will experience a deceleration stage until it reaches a maximum biomass concentration point in the stationary phase of up to 48 hours (Nur Utami and Dwi, 2016) [18].

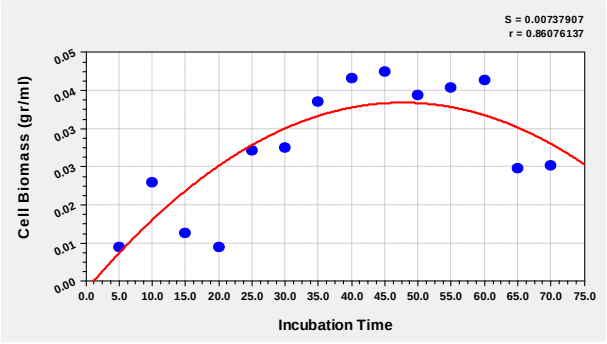

Fig. 3. The Growth Curve of Biomass From *Candida apicola*
Source: Own results in the laboratory.

Based on the measurement of biomass in Figure 3 shows that at 0^{th} – 20^{th} hours, the growth of biomass is pretty low. This reflected that the growth of the cell has not increased or is still in the lag phase. The growth at the lag phase tends to be slow because of the adaptation period in the growth media. The exponential phase from *Candida apicola* happens at 25^{th} to 60^{th} hours where *Candida apicola* has utilized nutrients contained at growth media for its growth. This can be seen from the amount of dry biomass which is around 0.03 to 0.04 gr/ml. The increase of biomass cell is caused by the presence of substrate at growth media so that it happens cell division and cell growth. After 65^{th} and 70^{th} hours, cell biomass decreased to 0.02 gr/ml. The decrease of dry biomass can be caused by the low content of glucose at growth media for growing cell so that yeast can not convert carbon and decreases biomass (Setyati, et al., 2015) [21].

Mannoprotein Extraction of *Candida apicola*

Mannoprotein extraction can be done to *Candida apicola*, which has experienced the incubation process for 70 hours. This is due to the measurement of the growth curve has shown that the death phase occurs at 70th hours. Extraction with lye is carried out by using potassium citrate, which can express protein and polysaccharides from the yeast wall and continue with the heating process. The death phase can generate the secondary metabolism that is expressing mannoprotein at the cell wall so that degradation by glucanase enzyme and proteinase. The expressing of mannoprotein from the cell wall can be done by extracting heat-treatment by lye.

Extraction with lye is carried out by using potassium citrate, which can release protein and polysaccharide from the yeast wall. Mannoprotein deposits are produced including 1.5982 gr/500ml, 0.07202gr / 1.5ml, and 0.7507gr/500ml. Mannoprotein from *Candida apicola* can be used as an emulsifier at food products. Balia et al (2017) [4] have stated that mannoprotein from yeast cell walls can be used as an emulsifier, which safe for food, is non-toxic, and environmentally friendly.

Emulsification Activity of Mannoprotein

The emulsification activity is done to know the quality of biosurfactant by measuring the ability of mannoprotein in degrading the oil. Emulsifier property from mannoprotein is obtained from mannose, which binds with protein. Protein has a function in activating emulsification as the amphiphilic structure used as a surface-active agent (Amaral *et al*., 2008) [2], and mannose, which is a monosaccharide, has a function as hydrophilic polymer (Dikit *et al*., 2010) [7]. The result shows that emulsification activity for 1 hour as much as 50% (EA) and 24 hours as much as 50% (EA24). The emulsification activity of

50% shows that mannoprotein extracted from *Candida apicola* can emulsify the oil with good enough. The prior study from Dikit *et al.* (2010) [7] generates the emulsification activity of 41%.

The utilization of mannoprotein as emulsifier obtained from yeast extract has been carried out, one of that is the utilization of mannoprotein at salad dressing by using *Saccharomyces cerevisiae*. The utilization of mannoprotein from yeast can be utilized in the food making process and non-toxic (Dikit *et al*., 2010) [7]. According to Amaral *et al.* (2008) [2], *Candida utilis* can be used as an emulsifier in salad dressing and gives good results in addition to mannoprotein extracted from *Saccharomyces cerevisiae* in making mayonnaise.

CONCLUSIONS

Mannoprotein can be obtained from the extraction process of *Candida apicola* on the media formula with an incubation time of 70th hours that produces a precipitate weight of 1.5982 gr/500ml with an emulsification activity value of 50%, and an optical density value of 1.5817, pH 5.69 and cell biomass 0f 0.0271 gr/ml.

Mannoprotein than has been obtained from the extraction of *Candida apicola* is necessary further testing of the characteristics and levels of the protein contained.

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