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THE POTENTIAL OF MANNOPROTEIN EXTRACTED FROM *CANDIDA APICOLA* CELL WALL AS EMULSIFICATION AGENT

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Abstract

Yeast has enormous potential as a biological agent in producing protein, one of that is Candida apicola. The cell wall of yeast Candida apicola generally contains 90% mannoprotein. Mannoprotein is bond between manna with protein. Mannoprotein can be used as an emulsifier at some food products and also has antimicrobial character. This research is aim to obtain the growth curve and to know the emulsification activity, and also the level of produced protein. This research was begun by creating the growth curve, and the parameter used to know the growth curve of yeast cell Candida apicola was optical density (OD), pH and dry biomass. The optimum incubation time was generated at 70th hour marked by the highest absorbance value as much as 1.9044 A, at pH 4.6, and the amount of biomass 0.0177 g/mL. Then, it was followed by measuring the emulsification activity and protein. The emulsification activity, which was generated, was 42% with the protein level of 1.6850 mg/ml.

Key words**:** *Candida apicola, emulsifier, mannoprotein, yeast*

INTRODUCTION

Indonesia has abundant biodiversity, including microorganism, animal, and plant. The microorganism is the most used resource because it has enormous potential in producing protein. Various advantages of microorganism include having a short life cycle, fast growth, high productivity, and facilitate us to do manipulation genetic or manipulation in the fermentation process.

Yeast has the capability of producing protein and its product, including amino acid and peptide [1]. One of the yeast types *Saccharomyces cerevisiae* produces some protein that has an antimicrobial character such as organic acid and protein1. Protein is generated as many as 0.023 U/mg, Optimum pH 5, Optimum temperature 25°C, and the molecule weight 97.4 kD [2]. That organic acid produced from yeast can be used as the food preservative. Besides the biocomponent based on protein, a research report that there is the dissolved mannoprotein in water (hydrophilic) and extracted from the cell wall of yeast *Saccharomyces cerevisiae*. Its molecular weight is as many as 76kDa with the composition of mannoprotein carbohydrate as much as 58% and protein 42% (Dikit et al., 2010). Mannoprotein produced can be used as an emulsifier at various food product while *Candida* has a capability in secreting protease extraceluler [3]The utilization of protease enzyme is to reduce protein fog in the last process of making beer and wine.

Mannoprotein is an important part of yeast cell wall. It is a bond between mannan and protein forming glycoprotein called mannoprotein. Besides carbohydrate, there are also protein (6-25%) and fat (1-7%). Manna generally consists of D-mannose and some components such as D-glucose/D-galactose/D-xylose and phosphate, which bind with protein [9].

Commercialization of local yeast isolate from non-*saccharomyces* strain has not many been explored. Currently, only *Saccharomyces cerevisiae* is considered in the context of application on the industry because *Saccharomyces cerevisiae* has clearer information starting from cell composition and also this species has provided as a yeast industry. Then, the local species of Indonesia

yeast can be a better source in the industry, with the condition that is acceptable and safe in the context of health.

Candida apicola is a type of *ascomycetes* yeast, which has a high osmotolerant and is naturally found in the fermentation of wine [14]. In the prior research, this yeast can be found in a traditional product like shrimp paste. The research about cell wall of *Candida apicola* has not specifically been done yet. Based on other studies, *Candida* cell walls generally contain 90% mannoproteins, which mainly are mannose located in the outermost layer functioning as a structural component. Based on another research, the cell wall of *Candida* generally contains 90% mannoprotein, which comprises of mannose (hydrophilic) and protein (hydrophobic). Candida has potential in its utilization as an emulsifier by extracting mannoprotein contained in the cell. Protein serves as a stabilizer of emulsion oil in the water while mannose is a hydrophilic polymer that can create the amplified structure of mannoprotein [10].

The produced emulsifier is the surface activity that can decrease surface pressure between air and liquid or liquid and liquid, which is there in the emulsion system. To obtain mannoprotein, which can be used as an emulsifier, is created the growth curve that is information about the life phase of yeast including adaptation phase, exponential phase, stationer phase, and death phase. The growth curve is used to know the velocity of yeast cell growth and the environmental impact on yeast growth. The parameter used includes the value of Optical Density (OD), pH, and biomass. The measurement of biomass in yeast is to know the amount of mannoprotein. The growth can be declared as an increase of cell mass. pH can affect a cell growth so that it is needed optimum pH for its growth. Biomass production will increase along with an increase of incubation time. Based on that explanation, the author has the desire to study and to conduct research in analyzing the origin of cell wall *Candida apicola* as emulsifier agent.

MATERIALS AND METHODS

Research Material

This research used several tools such as Petri dish, phalcon tube, beaker glass, Erlenmeyer tube, Schott bottle, micropipette (1,000µL), incubator, spectrophotometer, cuvett, pH meter, analytical scales, centrifuge, autoclave, aluminum foil, plastic wrap, laminar, 2 ml and 1 Eppendorf, 5 ml, pan, stirring rod, oven and parafilm, magnetic stirrer, dropper pipette, centrifuge.

The material, which was used in this research, was the yeast culture of *Candida apicola*, which isolated from shrimp paste by Faculty of Agricultural Industrial, Universitas Padjadjaran. The material, which was used in this research, was the yeast culture of *Candida apicola*, which isolated from shrimp paste by Faculty of Agricultural Industrial, Universitas Padjadjaran. Media used in this research was Yeast Mould Agar (YMA), which was used as growth media, Malt Extract Broth (MEB), which was used as production media, methylated, ammonium sulfate (NH4) 2SO4), phosphate buffer, alkaline EDTA (Na2CO2 10 g/ L, EDTA 1 mmol/ L), benzoic acid, 0.1 M HCl, acetate buffer 0.05 M pH 5, Comasie Briliiant Blue (CBB) G-250, standard solution of BSA (Bovine Serum Albumin), Bradford reagent.

Propagation of *Candida apicola* isolate

Isolate of *Candida apicola* obtained in yeast mold agar (YMA) was taken 2-3 ose, and then, it was moved in Malt Extract Broth (MEB), which had been filled in the test tube as much as 5 ml. After that, the test tube containing isolates was closed and wrapped by plastic wrap to avoid contamination, and then it was stored in an incubator on temperature 25 for 48 hours [13].

The Growth of *Candida apicola*

The testing a growth curve of *Candida apicola* was carried out to see the optimization of growing time from isolates. The planting time was used from 0 hours to 75 hours with the time interval of 5 hours for each isolate. Isolate of *Candida apicola*, which has been planted in Malt Extract Brotch (MEB), was take as much as 50 µl (1%) then

stored into 5 ml media MEB according to the planting time (from 0 hours to 75 hours). After incubation in media, then it was carried out checking of the resulted optical density (OD), pH, and Biomass.

Mannoprotein Extraction

Yeast cell of *Candida apicola* in the form of centrifuged deposits was previously weighed and added potassium citrate 0.1 M 20 gram/ 100 ml then entered to autoclave at 121^{oC} for 2 hours. The results of autoclaved yeast cell were centrifuged on 6,000 rpm for 15 minutes at 4C, and then it was conducted the separating process of supernatant and deposit on yeast *Candida apicola*. The obtained supernatant was added cold ethanol and stored on 4^{o}C until the precipitation process completed for 12-16 hours. After the precipitation process had completed, it was carried out again centrifugation process at 6,000 rpm for 1 minute at 4c, and then it was followed by washing with ethanol 2 times [11].

Measurement of Emulsification Activity

Measurement of emulsifier activity was determined by taking inserted liquid isolate in microtube, which had been carried out mannoprotein extraction and oil substrate within the same amount from each sample. Those two ingredients were inserted into microtube and stirred by using vortex at high speed for 2 minutes. Stabilization of emulsion was calculated on 1st and 2nd hours by calculating EA.

Determination of Protein Level

Determination of protein level was begun by determining the wavelength of standard solution BSA. Determination of maximum wavelength of standard solution BSA was done by making BSA solution 2.00 mg/mL and reacted by Bradford reagent then measured its absorption by using spectrophotometer UV-Vis at a range of wavelength 595

RESULTS AND DISCUSSIONS

The Growth of *Candida apicola*

The growth curve (Fig. 1) gives a description that in the yeast life cycle has 4 phases that is adaptation phase, exponential phase, stationer phase, and death phase [4]. Based on the growth curve can be determined the right incubation time by *Candida apicola* in producing mannoprotein.

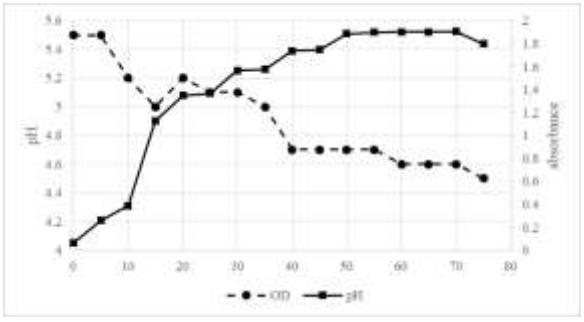

Fig. 1. The growth curve based on the value of OD and pH
Source: Own results.

The growth phase of *Candida apicola* shows that the adaptation time of *Candida apicola* has a relatively short time that grows in 0-10 hours. In the 10th-20th hours occurs exponential phase, marked by significant growth in the growth of the cells. In the exponential phase, the cell begins to divide and enters into the most active period of cell reproduction, and its regeneration time is constant. According to [12] in the stationary phase, the growth rate is slow so that the amount of yeast, which lives and dies, is a balance, and its population is stable. Meanwhile, the death phase happens in the 70th -75th hours in forming a new cell. The reason for the growth yeast stops in this phase because cell happens lack of nutrients. The optimum incubation time in this research is obtained at 70th hours, marked by the highest absorbance value 0.90440 A.

The rate acidity or pH are one of the important factors, which affect the growth of microorganism in media because every microorganism has a range optimum pH on its environment. The measurement of pH directly uses pH meter. The average of pH in this research can be shown in figure 1. In this research pH in the initial media is not regulated. The resulted pH begins from 0 to 75th hours ranging between 5 to 4. Based on the pH curve, the growth of *Candida apicola* yeast shows the decreased value (Figure 1). The longer the incubation time, the lower the

generated pH. This can be seen in the 75th hours in which pH attains 4.5 (acid). This condition is caused by the presence of acids such as lactic acid, acetate, and pyruvate. The rate of acidity will affect the increase of biomass. pH 4 corresponds to [8], which declares that yeast generally can growth in the range of pH 4-6. pH in the *Candida s*p can grow optimally on 4, but it can also grow at pH 3-7. Roostita et al [1] have stated that *Saccharomyces cerevisiae* needs optimum pH in producing some protein, which as an antimicrobial compound. While according to [5] mannoprotein, which is generated from *Saccharomyces cerevisiae* and *Kluyveromyces marxianus*, is stable at pH 3-11. This indicates optimum pH, resulted by *Candida apicola*, is not significantly different from its prior research in producing protein.

The biomass production of yeast cell *Candida apicola* in MEB scale 800 ml. The biomass calculated is the dry biomass weight (g/ml). The result of biomass production can be shown in Fig. 2.

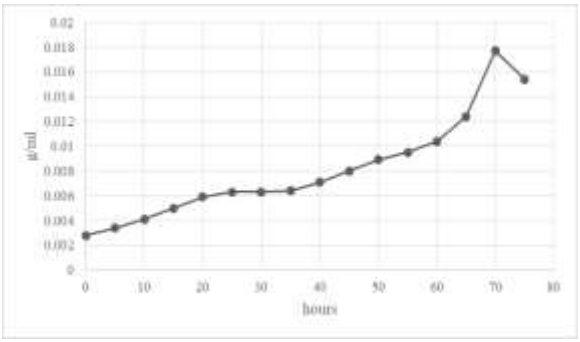

Fig. 2. The growth curve of *Candida apicola* based on dry biomass (g/ml)
Source: Own results.

Biomass production of *Candida apicola* yeast in media MEB as much as 0.10085 g/ml, attained at 5th hours incubation time. At 70th hours, yeast isolate of *Candida apicola* generates the highest biomass as much as 0.104900 g/ml. Moreover, to know the biomass production of *Candida apicola* yeast, the determination of biomass can also be used to know yeast growth. The growth has been defined as the increase of cell mass[15]. The growth of yeast is observed every 5 hours until 75 hours. This is due to the slower growth of yeast compared to bacterial growth. Besides that, the time used generally has a range between 72 hours to 96 hours [7]. Based on the observation result of yeast growth, the optimum incubation time to produce crude extract of mannoprotein is at 70th hours, marked by the highest absorption value as much as 1.9166 A in pH 4-6 with the amount of dry biomass as much as 0.0177 g/mL.

Mannoprotein is an important part of the yeast cell wall [6]. Its protein has bound with a sugar molecule, especially mannose residue ranging from 50-90% (Pablo et al.2018). Mannoprotein can be extracted from yeast wall by adding potassium citrate and sterilized by using an autoclave (121ºC) for 60 minutes at 4ºC [12]. *Candida apicola* cell can be extracted as much as 500 ml, obtained from 70th hours at pH 4. *Candida apicola* yeast can be isolated form shrimp paste. The result of mannoprotein extraction from *Candida apicola* is obtained 3.0086 g/mL.

Emulsification Activity and Protein Level of *Candida apicola* Mannoprotein

The emulsifier is a surface activity, which can decrease surface pressure between air and liquid or liquid and liquid in one emulsion. Emulsion serves to stop a separation between droplet oil and water so that it reduces the strength of repulsion among a different phase on the surface, and it makes both of phase can blend easily. The crude extract of mannoprotein from Candida apicola is tested its emulsifier activity and protein level. Table 1 shows data regarding the value of emulsifier activity and protein level.

Mannoprotein can be used as an emulsifier, caused by its composition consisting of hydrophilic mannose and protein, which can emulsify hydrophobic oil in water. In the prior research, *Saccharomyces cerevisiae* cell well has the dissolved mannoprotein where this mannoprotein can be used as bio emulsifier on some food products.

Table 1. The level protein of *Candida apicola* extract (mg/ml) and emulsification activity

Fraction	Protein (mg/mL)	Emulsification Activity (%)
Crude Extract	1.685	42

Source: Own results.

Table 1 showed that *Candida apicola* generates a protein level of 1.685 mg/mL with the emulsifier activity 42%. The usage of mannoprotein as a bio emulsifier has been done by Dikit et al in 2010 in which it is obtained from *Saccharomyces cerevisiae* in salad dressing by adding some other emulsifiers such as Arab gum and lecithin. The addition 0.6% of mannoprotein generates an emulsion activity 41% so that the usage of mannoprotein commercially in food can allow. The emulsifier in mannoprotein has a non-toxic character and can be used in the food industry [3]. Bioemulsifier character in mannoprotein can be used in food processing and beverage because it can stabilize the coexistence of different phases in a product. The resulted protein is effective as oil stabilization in water. In a wine product, the consumer usually refuses to the wine bottle, which contains a crystal deposit, so it can reduce the commercial value of that wine. The usage of mannoprotein extract from *Saccharomyces cerevisiae* greatly contributes to the chemical stability of wine by preventing crystallization of tartrate salt and protein fog.

CONCLUSIONS

The growth curve is created to determine the right incubation time for producing mannoprotein. The optimum value of OD (Optical Density) is obtained in 70th hours, marked by the high absorption value 1.90440 A, pH 4.6 , with the amount of dry biomass 0.01770 g/mL. The crude extract of mannoprotein *Candida apicola* has emulsification activity 42 % and its protein level 1.6850 mg/mL.

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