Utility of *Saccharomyces cerevisiae* As Probiotics to Induce Protease Production For Worms Feed Improvement

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Abstract. Saccharomyces cerevisiae is a potential yeast used as probiotic in animal feeds, due to it has several enzymes such as protease that needed to degrade protein material in feed to small molecule in order to easy adsorbed by cells. The research was constructed to determine the protease expression from the *S. cerevisiae* yeast that presented in tofu dregs as feed for Lumbricus rubellus worm, the concentration optimum and fermentation time of probiotic to degrade the residual protein in tofu dregs, and the effect of tofu dregs fermented by *S. cerevisiae* to support the growth of Lumbricus rubellus worm. The results showed that *S. cerevisiae* significantly expressed protease in medium containing tofu dregs with activty of 59.93 U/ml. The addition of *S. cerevisiae* 10% (v/w) in various optical density in tofu dregs medium showed the present of probiotic with OD600 0.6 for 12 hours of fermentation time exhibited the optimum degradation of residual protein in medium. The tofu dregs feed fermented by yeast probiotic for 15 days could increase the weight growth of Lumbricus rubellus as 51.32% (w/w) compared to control without probiotic that only 14% (w/w). Also it increased pulp resistance from the decay process compared to pulp without probiotic.

Keyword: Probiotic, *Saccharomyces cerevisiae*, protease, *Lumbricus rubellus*

1. Introduction

The yeast of *Saccharomyces cerevisae* has a potential used in many sector for food, beverage, and chemical industry. The other application of yeast has also been developed as probiotic animal for cattle and chickens [1]. The effect of yeast on fermentation of rumen fluid in vitro has been reported that it could increase the quality of rumen in the animal feed [2]. The idea of using yeast as an animal probiotic emerged because it has several enzymes that have important functions, protease and zymase.

Protease is a hydrolase group enzyme that will break down proteins into simpler molecules, such as being short oligopeptides or amino acids [3]. Protease can be used to improve the quality of animal feed, because it has a role to degrade the protein content in animal feed to simple molecule, so it can be absorbed easily by the animal cells.

Earthworms have long been known as animals that play an important role as decomposers of organic matter, increase soil fertility, and soil aeration [4]. In Indonesia, the effort to commercialize earthworm cultivation commercially as a profitable entrepreneurial opportunity is also increasingly socialized, both on a household and large scale [5]. The earthworms livestock needs feed source which has a good quality to increase its growth. The feed improvement of earthworms livestock is needed to increase its productivity.

The earthworme feed can be provided from many source, one of them is from the tofu wasfe (dregs). The chemical composition of dregs from tofu factory has been reported containing 17.4% protein and 67.5% carbohydrates [6]. The residual protein content in tofu waste is high enough so that it has the potential to be used as processed feed ingredients for animals that have a selling value. Protein in tofu waste tends to be complex and requires several treatments to optimize its use. Protease expressed by probiotic is important developed to degrade the protein content in the tofu wasfe to be simple. This paper reported the effect of probiotics *Saccharomyces cerevisiae* that added in tofu dregs for feed on worms growth. It also explained the proteases activity expressed by the

yeast probiotic in tofu dregs as earthworm feed.

2. Experimental Method

2.1. Culturing of Saccharomyces cerevisiae

The *Saccharomyces cerevisiae* was cultured in a *Potato Dextrose Broth* (PDB) medium which made from a potato extract 200gr/L , dextrose 2gr/L, 1L aquades then it is sterilized by autoclave at a pressure of 1 atm, temperature of 121^{o}C for 15-20 minutes. Growth curves are made together with the protease production process. *Saccharomyces cerevisiae* isolates were grown on PDB media containing 1,5%/L of tofu dregs, then incubated with shaking at a speed of 130 rpm at room temperature. Then the measurement of optical density (OD) of cell growth with spectrophotometer (UV-1700 Shimadzu)was measured at λ = 600 nm and checked at 2 hour intervals for 24 hours. The extracellular protease extract obtained by separating the medium (tofu pulp) and yeast from the supernatant was carried out by centrifugation of 12000 g. Supernatants obtained were tested for protease activity [7] and protein content [8].

2.2. Measurement of protease activity

The assay of enzyme actvity was carried out based on the previous method [7]. A total of 0.25 mL of enzyme solution was added with 0.25 mL of phosphate buffer solution pH 7 and preincubated at 37°C for 5 minutes. After preincubation, 0.25 mL of the substrate was added (2% casein in a pH 7 phosphate buffer), the mixture was placed at 37°C for 10 minutes. The reaction was stopped by adding 0.5 mL 0.4 M trichloroacetic acid (TCA), which was then centrifuged to take the supernatant. A total of 100 µl of supernatant was added with 5 ml of Bradford reagent, then homogenized and incubated for 5 minutes at 37^{0}C and then absorbance wasmeasured by spectrophotometer at λ 595 nm.

2.3. Measurement of protein content

The protein content was determined based on previous method [8]. Protein concentration was measured using the Bovine Serum Albumin (BSA) protein standard with a concentration of 0.1 to 1 mg / ml. A total of 100 µl of sample was added with 5 ml of Bradford reagent, then homogenized and incubated for 5 minutes at 37^{o}C and then absorbed by spectrophotometer at 595 nm.

2.4. Fermentation of tofu dregs on various probiotic concentration and time fermentation

The medium of Potato Dextrose Brooth was fortificated by 10% (v/w) culture of *Saccharomyces cerevisiae* then cultured by shaking at 130 rpm at room temperature to reach the optical density λ600 nm, at 0.2, 0.4, 0.6, 0.8 and 1.0. The yeast respectively added to erlenmeyer containing 100 gr of tofu dregs, then fermented for 48 hours at room temperature. The initial and final protein content were tested, and also observed the physical properties of the pulp. Each work is repeated 3 times. To search the optimum fermentation time, it was done fermentation of tofu dregs containing probiotic on various time at 4, 8, 12, 16, 20, 24 hours. Each work is repeated 3 times.

*2.5. Determininin*g *of probiotic effect in tofu dregs feed toward earthworms weight growth*

The tofu dregs was fermented with yeast probiotic at optimum concntration and fermentation time then given as feed for earthworms everyday until 15 days. The earthearthworm weigt before and after the treatment was meassured and compared with the control without probiotic.

3. Results and Discussion

Saccharomyces cerevisiae as potential probiotic can be easly cultured in medium potato dextrose brooth. The yeast cell could growth very quick and increase its mass sigficanly on short time, only 2 hours is required to pass a phase where the cell need to adapt on the new environtment. After they adapt the cell will growing fastly until it reach a phase where the cell stop growing and starting to die, the rate process was shown (fig 1). Figure 1 shows the growing curve of *Saccharomyces cerevisiae* which shown a lag, log, stationer, and dead phase. Each phase have a different meaning where lag phase is where microorganisms adjust to new media, so that growth has not occurred in this phase, after *Saccharomyces cerevisiae* adapt they will begin to reproduce or multiply themselves and this condition is called the log phase, stationer phase is where the cell growth and cell death is

the same, lastly deap phase is where the cell begin to die because there is no more nutrient for food. In this study the culture used is at the end phase of the log towards the beginning of stationary phase, because in that phase the number of microorganism cells is the most and abundant so that it is expected to secrete more extracellular protease enzymes which causes the feed fermentation process to take less time but with optimal results.

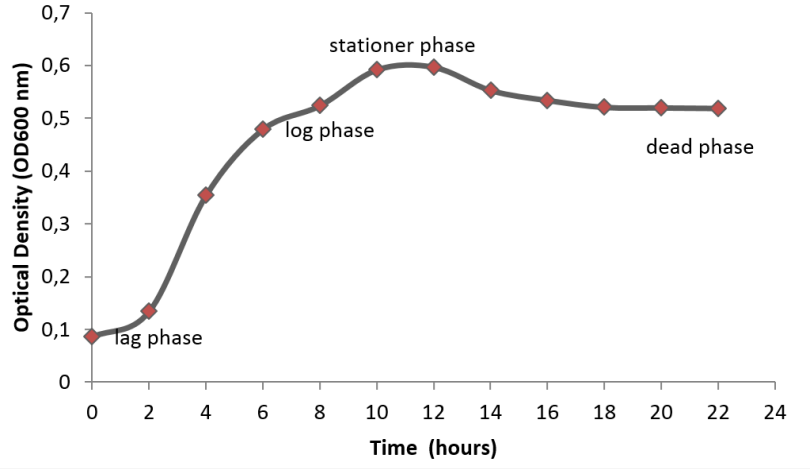

Figure 1. The growing curve of *Saccharomyces cerevisiae*

The presence of protease production in yeast can be proven by determining the hydrolyzed protein in a medium. To find out the amount of hydrolyzed protein, the initial protein content of the growing medium was calculated by increasing the protein content of the media after incubation. The difference in protein content from the analysis is the amount of hydrolyzed protein. To determine the amount of protein contained in a medium (substrate), a standard bolvine serum albumin (BSA) curve consisting of concentrations of 0.2, 0.4, 0.6, 0.8, and 1 µg / ml was used. Bradford test and measured its absorbance at λ 595 (**Figure 2**).

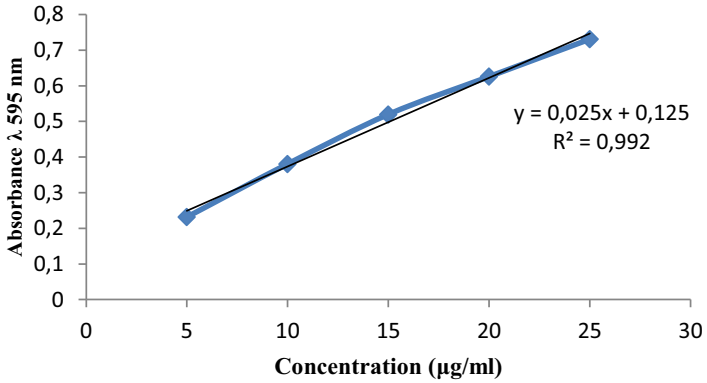

Figure 2. The standart curve of Bolvine Serum Albumin

From a standard curve BSA (Bolvine Serum Albumin) can be calculated the levels of all other substrate proteins used in this study. To determine the protein content of a substrate the first step is to do the bradford test on the desired sample then look for the absorbance value, after the absorbance value is obtained through the formula obtained from the BSA standard curve y = 0.025x + 0.125 protein content can be calculated by changing the value y becomes the absorbance

of the substance and looks for the value of x as the protein content (µg/ml).

Protease production in *Saccharomyces cerevisiae* can be induced by add several protein compounds in the growth media. The addition of inducer can make yeast secrete protease enzymes which are used to hydrolyze protein compounds so that they become simpler compound. Proteins that have become monomers are more easily absorbed by *Saccharomyces cerevisiae*. The difference in protein content from the analysis is the amount of protein hydrolyzed (Table 1).

Table 1. The ability of S.cerevisiae on hydrolyzing of protein in medium containing inducer of BSA and tofu dregs

Medium	Residual Protein content (µg/ml) in medium		Hydrolyzed protein(µg/ml)
	hour 0	hour 18	
+ BSA	6,56	5,16	1,4
+ Tofu dreg	7,84	6,16	1,68

From the calculation analysis results, it can be seen that *Saccharomyces cerevisiae* culture on inducer media has a hydrolysis reaction of 1.4 (µg / ml) on the BSA (Bolvine Serum Albumin) inducer and 1.68 (µg / ml) on the tofu dreg inducer.The Hydrolyzed protein data shown that there is an activity of protease from *Saccharomyces cerevisiae* crude extract, then next we test the crude extract hidrolyzed activity with casein. Measurements of protease enzyme activity were carried out based on the method by Enggel et al., (2004). Some of the supernatants obtained are added with pH 7 phosphate buffer so that the atmosphere is not acidic or basic, the presence of pH7 from the buffer will keep the enzyme from being denatured or damaged. After that a protein substrate which is previously known (casein) is included as a measure of the hydrolysis reaction by the protease enzyme produced by Saccharomyces cerevisiae. Each mixing of supernatant and substrate was incubated for 3-5 minutes to give the protease enzyme hydrolysis time of the protein compound. After that, trichloroacetic acid (TCA) is added, which functions as a stop reaction to the protease enzyme in hydrolyzing proteins. The substrate was centrifuged to be taken by the supernatant and then the bradford test was carried out to determine the protein content. (Table 2).

Table 2. Hydrolyzed protein on substrate BSA and tofu dregs reacted with crude extract supernatant from *Saccharomyces cerevisiae*

No	Medium	Protein substrate (casein) (µg/ml)	Residual protein left (µg/ml)	Hydrolyzed protein (µg/ml)	Protease activity (U/ml enzym)
1.	+BSA	39,4	3,12	36,28	60,46
2.	+ Tofu dreg	39,4	3,44	35,96	59,93

The results of the calculation showed that the protease activity of *Saccharomyces cerevisiae* was quite high at 60.46 U / ml of enzyme on the BSA inducer medium and 59.93 U / ml of enzyme on the tofu feed inducer medium. The activity unit is a measure of the rate at which the reaction takes place, U/ml enzyme has a definition of the amount of µg of protein that is hydrolyzed in 1 minute. The amount of protease activity causes the protein in casein to be hydrolyzed.

After we determine that *Saccharomyces cerevisiae* could secrete protease enzym proven by hydrolyzed protein data the next step is to do an optimizaztion for the enzyme to find the best hydrolysis results for tofu dreg as feed for catlle earthworm **Figure 3**.

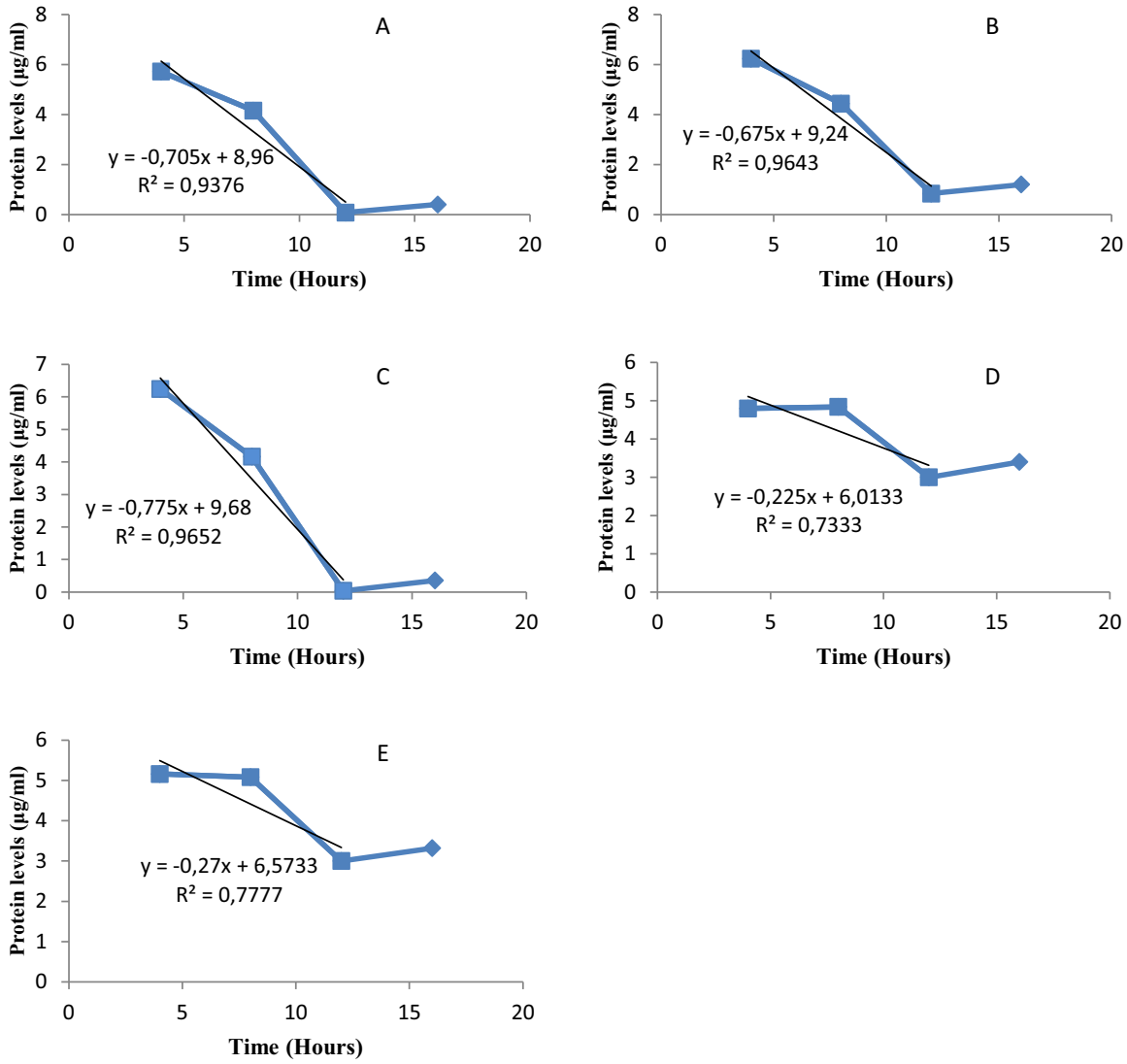

Figure 3. The residual protein degadation in tofu dregs medium on various probiotic concentrations and fermentation time. The optical density of yeast probiotic that used were respectively 0.2 (A); 0.4 (B); 0.6 (C); 0.8 (D); and 1 (E).

Data on protein content obtained from the absorbance analysis showed that at 12 o'clock was the optimal fermentation time. At 0,6 OD concentration is the optimum concentration of *Saccharomyces cerevisiae* microorganisms. In OD 06, the data analysis shows the best slope and the lowest protein content is -0.775 slope, 0.04 protein levels compared to other ODs.

The next observation was the physical condition of tofu waste which was carried out by using several samples of tofu dregs feed with *Saccharomyces cerevisiae* probiotics and the control without microbial or just a normal tofu dreg. The treatment was equated to the sample and the control, the observations were carried out from day zero until it began to notice changes in the tofu feed and began to emerge odor from those given probiotics with those not given probiotics. **Figure 4** shows the physical condition of tofu waste.

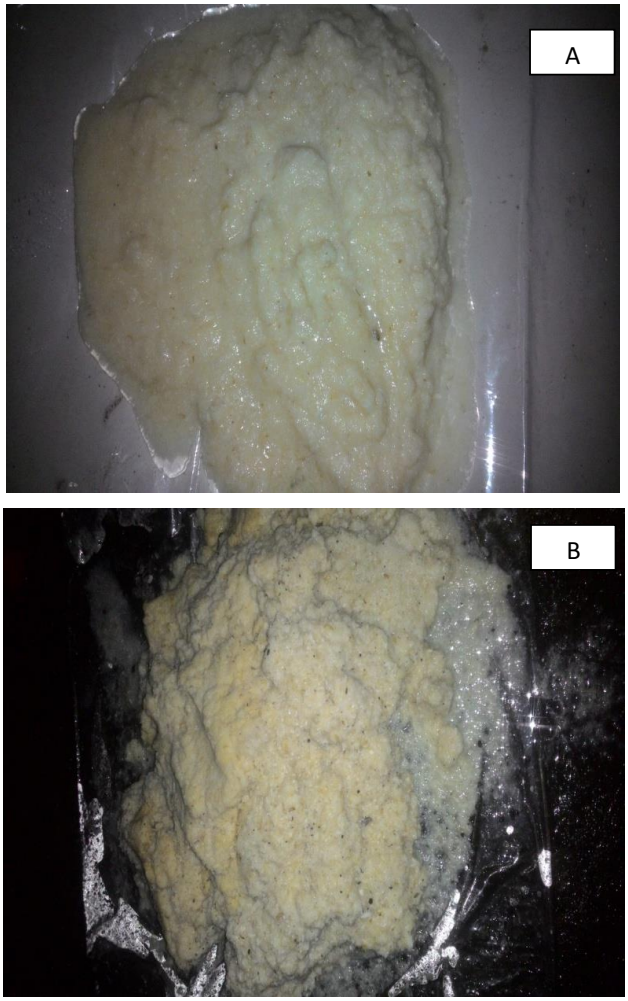

Figure 4. The physical properties of tofu dreg with and without S.cerevisiae probiotic after keeped for one day. (A) with probiotics and (B) without probiotics

Tofu dregs that are not given probiotics after being left for 1 day begin to harden, slimy, bad odor, and there are many small animals such as fleas and maggots. Whereas in the tofu waste plus probiotics has a soft texture that does not smell and almost no disturbing animals.

The last experiment in this study was to determine the efect of probiotic *Saccharomyces cerevisiae* to improve the earthworm feed. Tofu waste is fermented with the composition of probiotics according to the results of the optimization of fermentation levels and time. The food is then given to the worm, the test is carried out up to 15 days and then we weighed the worm, the control variable used is a sample of other worms that are fed tofu waste without fermentation with the same time interval measuring the worm's weight then differentiated the results of the two earthworm samples (Table 3).

Table 3. Weight increase in Lumbricus rubellus worm growth with and without the presence of probiotic on their daily tofu dregs feed.

Time (days)	Weight of worms with treatment (gr)	
	Tofu dregs feed	Tofu dregs feed + probiotic
0	250	250
15	285	378,3
Δ Weight gain (gr)	35	128,3

The growth of worms in the samples that were given tofu dregs alone showed a very large difference when compared to the growth of worms fed tofu waste + probiotics SC. Worms fed tofu in 15 days only grew by 14% from the initial seedlings, while worms fed with tofu waste + SC probiotics grew by 51.32%. This growth proves that giving probiotics is very influential on the growth of worms.

4. Conclusions

Saccharomyces cerevisiae can be used as probiotics in catlle earthearthworm because their ability to secrete protease enzymes in the environment that contain tofu waste. It was proved by the amount of protease activity of 59.93 U/ml of enzyme on the substrate.The optimum concentration and time of *Saccharomyces cerevisiae* probiotics in the fermentation process is at 0.6 optical density (OD) and 12 hours of fermentation time. Probiotic *Saccharomyces cerevisiae* mixed with tofu dregs, gave growth results on earthearthworm by 51.32% while only 14% without probiotics, this result proves that *Saccharomyces cerevisiae* probiotics are able to affect and improve earthearthworm feed proven by incresing in weight growth from the catlle worm.

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