

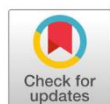
Comercial yeast samples DNA extraction using universal kit

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Abstract

Yeast is a microorganism that is relatively difficult to obtain its DNA mass. This is caused by the content of cell walls with a thick and rigid composition, such as chitin. This study aims to provide information on the best concentration and purity of the DNA extraction results of various types of commercial yeast in different samples weight. The method used is based on Universal Kit procedure with modification. This study indicates that differences in concentration or sample weight do not affect the DNA concentration and purity ratio value of each yeast sample. Each best result obtained from Jago (*Rhizopus oligosporus*), Raprima (*Rhizopus oligosporus*) and Fermipan (*Saccharomyces cerevisiae*) in 50 mg sample, NKL (*Saccharomyces cerevisiae*) yeast in 40 mg. Recommendation weight samples to extract DNA of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* isolates are between 40 mg to 50 mg. The contamination factor causes the difference in the value of purity in each sample during DNA extraction.

Keywords: DNA isolation, Purity, Concentration

Introduction

Yeast or yeast is a type of unicellular microscopic fungus. The size of yeast cells varies, with an average length ranging from 5-20 m and a width ranging from 1-10 m. The shape of yeast cells is also diverse, such as cocci, bacilli, cylindrical, and apiculate. Yeast will grow well in environmental conditions with a sufficient water supply¹.

According to their metabolic properties, yeasts can be divided into two groups, namely oxidative and fermentative yeasts. Yeast groups with fermentative metabolic properties will ferment ethanol by breaking down glucose compounds into ethanol and gas. This type of yeast is often used in food products as a fermentation agent². Meanwhile, yeasts with strong oxidative metabolic properties cannot carry alcoholic fermentation³.

Yeast can be used as a developer, foam degrading agent, enzymes, carotenes, and vitamins. In addition, yeast can also be used as a renewable energy source such as bioethanol or biofuel. Bioethanol can be one way out of the energy crisis that will occur in the future⁴.

Yeast can also be helpful as a biofertilizer used to improve the agricultural sector. This biofertilizer is used as a solvent for phosphate and organic matter degrading. In addition, yeast is also



used in soil pollution recultivation, bioremediation of heavy metals, and as sensors of microbiology⁵. In molecular biology, yeasts function as eukaryotic microbes that can be inserted with other genes. In agriculture, yeast is helpful for improving livestock health⁶.

As living organisms, yeasts have DNA as their genetic material. DNA is located on the chromosomes of the cell nucleus. In addition to the cell nucleus, DNA can also be found in other cell organelles, such as mitochondria and chloroplasts. DNA in the cell nucleus is also referred to as chromosomal DNA. In contrast, DNA in different cell organelles outside the nucleus is called extrachromosomal DNA, which consists of mitochondrial DNA, plasmid DNA, and chloroplast DNA⁷.

DNA isolation is one of the basic techniques that are often used in molecular biology and biotechnology. DNA isolation separates DNA from other particle elements such as proteins, lipids, polysaccharides, and other substances. The results of DNA isolation are usually used in molecular analysis and genetic engineerings, such as transformation, genome editing, and PCR⁸.

Many molecular studies using yeast as the subject. This of course requires important basic information regarding the proper extraction method to obtain pure DNA mass in high concentrations. Baker's yeast and brewer's *Saccharomyces* sp. are the most studied and scientifically known fungi because they are eukaryotic cells that can be easily manipulated as an excellent model for studying various important problems in eukaryotic cell biology⁹. In Indonesia, tempeh yeast is also one of the most studied types of yeast. Therefore, this study will discuss the optimization of DNA extraction methods on species of baker's yeast and tempeh yeast using the Universal Kit. This study determine the effect of variations in the weight of the sample used on the purity value of the spectrophotometric results.

Materials and methods

Study Area

The research was conducted at the Genetics and Tissue Culture Laboratory, Integrated Laboratory of UIN Sunan Ampel Surabaya in April 26th 2021.

Tools and materials

The ingredients used are four kinds of yeast obtained from tempe yeast products *Jago* and *Raprima*, *NKL* tape yeast, and *Fermipan* baker's yeast. Other ingredients are EDTA, isopropanol, 70% ethanol, DNA Rehydration Solution DRS. Tools used include 1.5 mL microtube, micropipette size 1µl - 1000µl, vortex, centrifuge, bio drop, tissue, aluminum foil.

Sample preparation

Powdered samples were measured by analytical balance in 50 mg; 40 mg; 30 mg; 20 mg; and 10 mg. Then all samples were placed in each 1.5 ml microtube that had been marked.

DNA extraction

DNA extraction procedure is according to the protocol of Promega Wizard Universal kit. Carried out with the following modification details:

Cell lysis process

Added yeast sample placed in the microtube with distilled water up to the 1 ml then vortexed. The microtubes were centrifuged at 15,000 x g in room temperature (25°C) for 2 minutes. The supernatant contained in the sample was discarded, and the pellet was left at the bottom of the microtube. Then, all the microtubes were added with 295 µl of 50 mM EDTA solution using a micropipette and vortexed.

The samples were incubated at 37°C for 30 minutes, after which they were cooled at room temperature. Then centrifuged again at 15,000 x g in room temperature (25°C) for 2 minutes. The supernatant on the microtube is removed, and the pellet will remain. Added 300 µl of Nuclei Lysis Solution and vortexed.

Precipitation process

Added 100 µl of Protein Precipitation Solution (PPS) and vortexed. The homogeneous sample was put in the refrigerator for 5 minutes.

Centrifuged at 15,000 x g at 25°C for 3 minutes. The supernatant in the microtube was transferred to a microtube containing 300 µl isopropanol, and the remaining pellet was left. Then after being transferred, it was vortexed and centrifuged again at 15,000 x g at room temperature for 2 minutes. The supernatant was discarded and air-dried by inverting the microtube for several minutes.

Rehydration process

300µl of 70% ethanol was added to the sample and centrifuged at 15,000 x g at room temperature for 2 minutes, and then the supernatant was removed and air-dried for 15 minutes. After that, 50 µl of DNA Rehydration Solution (DRS) added 1.5 µl of Rnase Solution, then vortexed. Incubate with two repetitions, namely incubation at 37°C for 15 minutes and incubation at 65°C for 1 hour for DNA rehydration. DNA samples were stored at a temperature of 2-8°C.

DNA Concentration and Purity Testing

Prepare a BioDrop Spectrophotometer tool that will used to determine the concentration and purity of DNA. Pedestal cleaned with tissue. Dropped DNA Rehydration Solution as much as 1 µl blank on the pedestal. Pressing the “Blank” section on the bio dropScreen to measure the blank, then the pedestal is cleaned again with a tissue. Place 1 µl of the sample DNA isolate solution on the pedestal. Select the “Measure” button on the screen, and it will generate a spectrum display and the amount of concentration to be calculated.

Results

Measurement of concentration and purity in DNA samples using a spectrophotometer (BioDrop). The results of the spectrophotometry on each sample were tempeh yeast (*Jago*), tempeh yeast (*Raprima*), tape yeast (*NKL*), and baker's yeast (*Fermipan*). Each yeast sample contained five weight variations, namely 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg.

Table 1. Spectrophotometric test results of tempeh yeast samples (*Jago*)

Sample	Weight	Concentration	Purity	
			A260/A280	A260/A230
1	10 mg	0.167 µg/ml	2.494	-0.125
2	20 mg	0.376 µg/ml	1.362	1.141
3	30 mg	0.641 µg/ml	1.639	0.198
4	40 mg	0.594 µg/ml	1.727	0.229
5	50 mg	1.229 µg/ml	1.686	0.387

Table 2. Spectrophotometric test results of tempe yeast samples (*Raprima*)

Sample	Weight	Concentration	Purity	
			A260/A280	A260/A230
1	10 mg	0.815 µg/ml	1.963	1.442

2	20 mg	1.731 $\mu\text{g/ml}$	2.083	2.216
3	30 mg	1.736 $\mu\text{g/ml}$	1.854	5.162
4	40 mg	5.145 $\mu\text{g/ml}$	1.983	2.104
5	50 mg	6.361 $\mu\text{g/ml}$	2.078	2.263

Table 3. Spectrophotometric test results of tape yeast samples

Sample	Weight	Concentration	Purity	
			A260/A280	A260/A230
1	10 mg	0.116 $\mu\text{g/ml}$	0.098	0.065
2	20 mg	1.186 $\mu\text{g/ml}$	1.931	1.064
3	30 mg	2.728 $\mu\text{g/ml}$	11.96	3.294
4	40 mg	1.101 $\mu\text{g/ml}$	2.007	2.762
5	50 mg	0.785 $\mu\text{g/ml}$	0.964	0.555

Table 4. Spectrophotometric test results for baker's yeast (*Fermipan*) samples

Sample	Weight	Concentration	Purity	
			A260/A280	A260/A230
1	10 mg	8.316 $\mu\text{g/ml}$	1.802	1.406
2	20 mg	17.16 $\mu\text{g/ml}$	1.970	1.741
3	30 mg	20.37 $\mu\text{g/ml}$	2.013	1.768
4	40 mg	30.70 $\mu\text{g/ml}$	2.013	1.611
5	50 mg	32.34 $\mu\text{g/ml}$	2.016	1.703

Discussion

The results of the spectrophotometry of the tempeh (*Jago*) yeast samples produced various concentrations. The increase in sample weight was in line with the increase in the amount of DNA concentration. The variation of the 50 mg sample weight has the largest concentration compared to the others, namely 1.229 $\mu\text{g/ml}$, and the lowest concentration is 10 mg sample weight, which is 0.167 $\mu\text{g/ml}$. Calculation of purity ratio results can see the table with A260/A280. Found the highest DNA purity ratio in the sample weight of 10 mg, and the smallest was 20 mg. The purity is respectively 2,494 and 1,362. Best sample in *Jago* is 50 mg.

The highest concentration in the tempeh yeast sample (*Raprima*) was 50 mg, 6.361 $\mu\text{g/ml}$, and the lowest concentration was 10 mg, with a value of 0.815 $\mu\text{g/ml}$. The purity values produced by the highest and lowest tempe yeast (*Raprima*) were 2,083 and 1,854 with variations in sample weight, respectively, 20 mg and 30 mg. Best sample in *Raprima* is 50 mg.

The highest concentration of the spectrophotometric results of tape yeast was 2,728 $\mu\text{g/ml}$ with A sample weight of 30 mg, and the lowest concentration was 0.116 $\mu\text{g/ml}$ with a sample weight of 10 mg. The tape yeast sample has a purity value that is different from other samples, and there is a negative value in its purity. The highest purity value is found in 11.96, with a sample weight of 30 mg. The result of the best purity values are 1.931 and 2.007, with a sample weight of 20 mg and 40 mg. However, if we compare the absorbance ratio value of 260/230, we can determine that the best sample weight used is 40 mg.

The results of a spectrophotometric test of baker's yeast (*Fermipan*) samples for concentration have a fairly large value compared to other samples. The largest concentration was 32.34 $\mu\text{g/ml}$, and the smallest was 8.316 $\mu\text{g/ml}$, with sample weights of 50 mg and 10 mg. The value of purity is also relatively not much different. The highest purity is found in the sample weight of 50 mg, which is 2,016, and the

smallest purity is found in the sample weight of 10 mg, which is 1,802. From the concentration amount and purity ratio, we can determine that the best sample weight used is 50 mg.

Determining the purity value in the sample can be measured using a comparison between the absorbance values at a wavelength of 260 nm and 280 nm. The results of the spectrophotometry utilized to see the value of purity are found in A260/A280. The purity value of each yeast sample can be categorized as good and not good. The purity of the sample can be classified as good if it has a value of about 1.8 - 2.0 with a ratio of A260/A280¹⁰. Samples of *Jago* tempeh yeast and tape yeast have poor purity values because the average purity value is below 1.7. As for the *Raprima* tempe yeast and *Fermipan* baker's yeast samples, the purity values were still quite good because they were between 1.8 and 2.0. Only a few values exceed slightly above 2.0, making the purity value not of good quality.

Based on the concentration results produced from each sample with several variations in weight, the highest concentration values in 3 types of samples, namely tempeh yeast (*Jago*), tempeh yeast (*Raprima*), and baker's yeast (*Fermipan*), were found in samples with the highest weight with a weight of 50 mg.

The spectrophotometry results on four different yeast samples had the highest purity values with various sample weights. The tempeh yeast sample (*Jago*) had the highest purity value, 2,494, found in a weight variation of 10 mg. In contrast to tempeh yeast (*Raprima*), found the highest sample purity value in a weight variation of 20 mg with a value of 2,083. The following different purification value was tape yeast which showed the highest yield of 11.96 at a weight variation of 30 mg. The last sample of baker's yeast (*Fermipan*) has the highest purity value, namely 2,016, with the highest weight variation of 50 mg.

The explanation of each purity value in each of the yeast samples can show that can say the value of the purity of the sample that it is not directly proportional to the weight variation of each sample, namely with variations of 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg. The highest variation in sample weight does not necessarily have a large concentration and purity value.

The factor that influences the difference in purity values in each yeast DNA sample is that the DNA isolate is not pure due to contamination from the solvent, which causes the purity value of A260/A280 to be below 1.8. While the sample can also be said to be impure if it is contaminated by protein or other organic materials, causing the purity value to be more than 2.0¹⁰. The difference in the value of purity in each sample is not the effect of differences in the concentration of variations in the weight of the samples used in each yeast sample, namely 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg. However, the factor of the different contamination of yeast samples can cause different purity values, and it can detect that the purity is good or not.

Conclusions

From the results obtained, it can be concluded that there is no effect between the difference in the weight of the yeast sample on the value of DNA purity and concentration. The best result obtained from *Jago* (*Rhizopus oligosporus*), *Raprima* (*Rhizopus oligosporus*) and *Fermipan* (*Saccharomyces cerevisiae*) in 50 mg sample, *NKL* (*Saccharomyces cerevisiae*) yeast in 40 mg. Recommendation weight samples to extract DNA of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* isolates are between 40 mg to 50 mg. The contamination factor causes the difference in the value of purity in each sample during DNA extraction.

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Conflicts of Interest

There are not potential conflicts of interest.

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