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Optimalization of NaOH concentration in alkaline lysis method on quality and quantity of *Candida albicans* DNA

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Abstract

Background: The concentration of NaOH in the alkaline lysis method needs to be optimized, especially when isolating DNA for its application in PCR which has not been maximized using the Candida albicans fungus which has a complex cell wall and is difficult to break. Thus, this optimization is expected to increase the efficiency of the alkaline lysis DNA isolation method in fungal species. *Objective:* The purpose of this studied was to determine the effect of optimizing the concentration of NaOH in the alkaline lysis method on the quality and quantity of *Candida albicans* DNA. Materials and *Methods:* This researched is experimental by performed DNA isolation used alkaline lysis method used NaOH concentrations of 1.5 N, 1.75 N, and 2.0 N and controlled (NaOH 0.2 N). Calculation of DNA quality and quantity using a nanodrop spectrophotometer. *Results:* The results showed the ordered of obtained the high to low DNA quality (A260/280) was NaOH concentration 2.0 N>1.75 N>1.5 N>0.2 N (1.86±0.44-0.95±0.18) while for DNA quantity was NaOH concentration 2.0 N>1.75 N>1.5 N>0.2 N (187.7±58.3-9.6±3.5 ng/µl). *Conclusion:* There is an effect of optimizing NaOH concentration on the lysis of fungal cell walls in the alkaline lysis method, namely increased the purity valued and concentration of sample DNA isolation results gradually. The use of 2.0 N NaOH concentration produced the best quality and quantity of DNA, namely 1.86±0.44 for DNA quality and 187.7±58.3 for DNA quantity.

Keywords

Alkaline lysis, *Candida albicans*, DNA Isolation, NaOH concentration.

1. Introduction

The majority of infectious disease-related deaths globally are caused by fungi. The most frequent fungi causing invasive mycotic diseases are *Candida* species, with *Candida albicans* as the main cause of invasive candidiasis (Lee et al., 2020). Prompt diagnosis is necessary due to the increasing incidence of mortality among individuals with invasive candidiasis each year. Histopathology and fungal culture analysis are the gold standard for diagnosing candidiasis (Pitarch et al., 2018). Rapid and accurate diagnosis is necessary as culture methods take two to five days to provide correct findings and lack sensitivity (Sidharta et al., 2021).

A rapid diagnostic technique with good sensitivity and specificity is the Polymerase Chain Reaction (PCR) technique. This technique has been widely used to find diseases caused by harmful bacteria, viruses and fungi. PCR has a sensitivity and specificity of about 85% for identifying fungal infections. For the amplification reaction, the PCR technique requires DNA samples with adequate amount and quality of DNA (Gupta, 2019).

An important step in molecular analysis is the isolation of deoxyribonucleic acid (DNA). A fundamental requirement in molecular analysis is that sufficient and high-quality DNA must be collected in an isolation so that subsequent analysis procedures can run smoothly. Cell wall lysing, separation of DNA from solids such as cellulose and proteins and DNA purification are the three main processes in DNA isolation (Syafaruddin & Santoso, 2020).

One of the conventional methods that can be used to isolate DNA from bacteria or fungi is the alkaline lysis method. When compared to the filter-based kit method, the alkaline lysis method is simpler, cheaper, and easier to use (Delaney et al., 2018). However, there are things that can be done to optimize the success of this method in isolating DNA, especially for species such as *Candida albicans*, which has a complex cell wall that is difficult to break. The concentration of NaOH used in the alkaline lysis method is one of the factors that affect the results of DNA isolation (Hardianto et al., 2015).

Research on DNA isolation by alkaline lysis method has been conducted since the last few decades. Rodriguez et.al. (2017) conducted research on *Saccharomyces cerevisiae* fungi with a NaOH concentration of 0.2-0.3 N and obtained low DNA quantity results. Another study conducted by Faradisa et. al. (2021) found that the alkaline lysis method with a NaOH concentration of 0.2 N resulted in poor DNA quantity and quality in *Candida albicans* DNA isolation. Low DNA quantity values were also obtained in the research of Barqly et.al. (2021) using 0.2 N NaOH concentration for *Aspergillus niger* DNA isolation.

This study aims is to optimize the concentration of NaOH used during the cell lysis process in the alkaline lysis method in *Candida albicans* DNA isolation. The parameters to be compared consist of the quality (purity) of DNA and the quantity (concentration) of DNA, so as to obtain the results of *Candida albicans* DNA isolation with the highest quality and quantity of DNA.

2. Materials and Methods

2.1. Types of research

This type of research uses experimental methods by performing DNA isolation using alkaline lysis method using different concentrations of NaOH to determine the quality and quantity of Candida albicans DNA. This research was conducted at the Molecular Biology Laboratory of Nahdlatul Ulama University Surabaya (UNUSA), Jl. Raya Jemursari No. 51-57, Jemur Wonosari, Kec. Wonocolo, Surabaya City, East Java. Subsection

2.2. Research methods

The sample in this study was a pure culture of *Candida albicans* ATCC 10231 obtained from the Balai Besar Labortorium Surabaya (BBLK), Jl. Karangmenjangan No. 18 Surabaya. Materials and tools used included sterile distilled water, buffer solution 1 (Glucose 1 M; Tris-Cl 1 M, pH 8; EDTA 0.5 M pH 8.0), buffer solution II (NaOH*, SDS 1% (w/v)), buffer solution III (Potassium acetate 5 M; Glacial acetic acid; pH 5. 4); chloroform; 70% ethanol; TE buffer (Tris EDTA 50 mM, pH 0.8); 0.8% agarose; TAE 1X buffer (Tris 40 mM; Acetic acid 20 mM; EDTA 1 mM); RedSafe, loading buffer/dye; EtBr (Ethidium Bromide). The NaOH* solutions used were concentrations of 0.2 N (control); 1.5 N; 1.75 N; and 2.0 N (w/v), nanodrop spectrophotometry (*Thermo sientific*), UV-Transilluminator (*PacificImage Electronics*), and gel electrophoresis (*Mupid-eXu*).

This study begins with the rejuvenation of *Candida albicans* fungi obtained from BBLK on Sabouraud Dextrose Agar (SDA) slant media, then macroscopic observations are made with Gram staining and germ tube tests to ensure the purity of the sample. The germ tube test was carried out by taking one round ose of *Candida albicans* colony and inserted into a serology tube containing 0.5 mL of serum which was then incubated for 1-2 hours in an incubator. Then 1 drop of colony was taken and dripped on a glass object and then observed on a microscope with 10x and 40x objective lens magnification. The germ tube test is said to be positive for *Candida albicans* if a cell shape is found that germinates like a racket (Sophia et al., 2021).

The alkaline lysis method is the method of Faradisa et. al. (2021). DNA isolation begins with the preparation of a fungal suspension from a pure culture of *Candida albicans* grown in 10 mL of Sabouraud Dextrose Broth (SDB) media. Next, the fungus is incubated at 37°C for 3-5 days. Furthermore, DNA isolation with alkaline lysis method that has been optimized. Optimization of the method with NaOH concentration in buffer II solution, namely 1.5 N; 1.75 N; and 2.0 N. As a research control, 0.5 N NaOH concentration was used. DNA isolation begins with a 1.5 mL microtube is filled with 1 mL of the Candida albicans fungal culture, which is then centrifuged at 15,000 rpm for 2 minutes to create a pellet. After adding 150 µl of buffer II solution, the pellet was once again suspended in 150 µl of buffer I solution. After that, the sample was well combined and allowed to dissolve for 30 seconds. Subsequently, 150 µL of buffer III solution was added and mixed thoroughly. After adding two drops of chloroform, the mixture was centrifuged for two minutes at room temperature (25°C). 400 µL of supernatant was poured into a fresh microtube, along with 1 mL of ethanol. The mixture was then combined and centrifuged for 10 minutes at 4°C at 15,000 rpm. To assess the quality and quantity of DNA, the pellet was dried and then dissolved in 50 µL of DNA TE (Faradisa et al., 2021).

Using a nanodrop spectrophotometer, the quality (purity) and quantity (concentration) of the DNA isolation findings were measured at wavelengths of 260 and 280 nm (Å260/Å280). The procedure involved preparing a sample of DNA isolation findings, up to 1 µL per treatment, then dripping it onto a nanodrop spectrophotometer. Next, readings of the DNA purity and concentration graph were made at 260 and 280 nm wavelengths. If the DNA purity falls between 1.8 and 2.0 and the concentration is greater than 100 ng/µL, the DNA quantity values are considered acceptable (Faradisa et al., 2021).

Utilizing SPSS version 21.0, additional statistical analysis was performed by contrasting the outcomes of qualitative and quantitative DNA testing. After confirming that the data were homogeneous and normal, the One Way Analysis of Variance (ANOVA) test and Post Hoc test employing Least Significant Difference (LSD) were performed. If the data does not meet the requirements, the Kruskal Wallis test is carried out and continued with the Mann Whitney test. Data is declared significant or has a difference if $p<0.05$.

3. Results and Discussion

3.1. Result

The results of *Candida albicans* fungal rejuvenation were observed microscopically by Gram staining and germ tube test. Figure 1 presents observations of *Candida albicans* fungi in Gram staining and Figure 2 presents observations of *Candida albicans* fungi in the germ tube test.

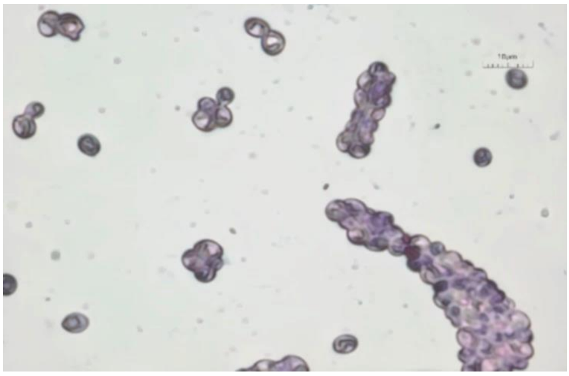

Figure 1. The results of Gram staining on the sample, namely oval-shaped yeast cells and are gram positive, purple in color (1000x microscope magnification).

The results of Gram staining in Figure 1 are in accordance with the research of Indrayati & Sari, (2018) which shows that in Gram staining the *Candida albicans* fungus is Gram positive in the form of an oval with a diameter of ± 5 μm.

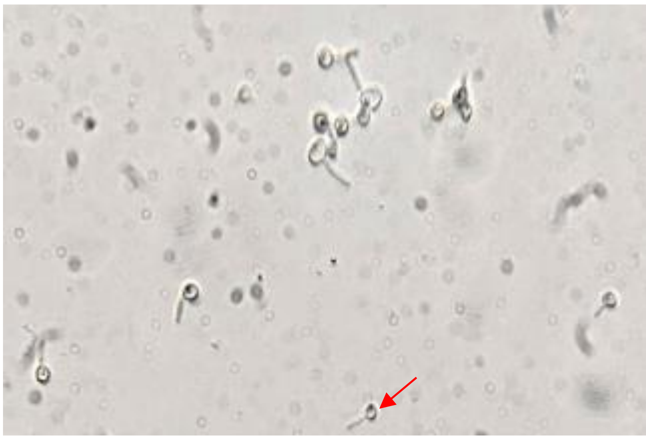

Figure 2. The results of *Candida albicans* the germ tube test a the presence of yeast cells in the form of racket-like sprouts (400x microscope magnification).

The results of the germ tube test in Figure 2 in accordance with the research of Sophia et al., (2021) that it is said to be positive for *Candida albicans* if a cell shape that germinates like a racket is found. *Candida albicans* fungus that has been rejuvenated and identified, then made a suspension and isolated its DNA using the alkaline lysis method which has optimized the concentration of NaOH in buffer II solution. Each NaOH concentration treatment was carried out 8 times. The quality and quantity test results of DNA isolation with different NaOH concentration treatments can be seen in Figure 3 and Figure 4.

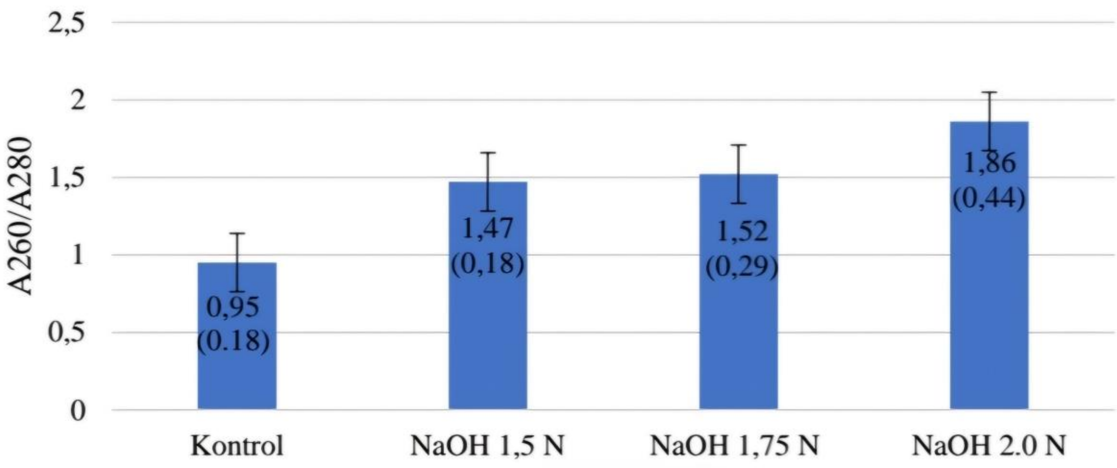

Figure 3. Quality Chart of DNA Isolation Results

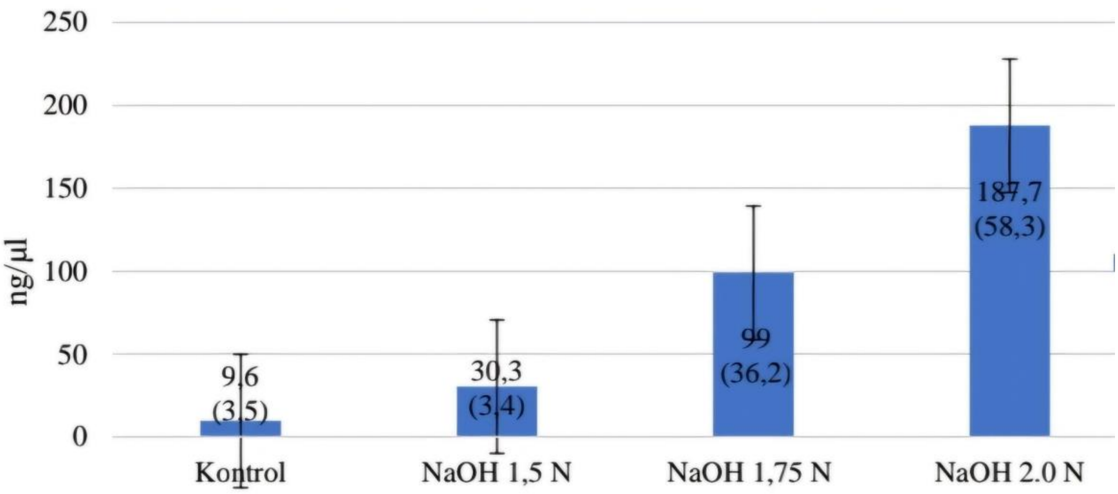

Figure 4. Quantity Chart of DNA Isolation Results

Figure 3. shows the DNA quality results in mean values (SD), n=8. DNA quality of purity values for control (0.95±10.18), concentration of 1.5 N NaOH concentration (1.47±0.18), 1.75 N NaOH concentration (1.52±0.29), concentration of NaOH concentration 2.0 N (1.86±0.44). One Way Annova statistical test and LSD test ($p<0.05$) were applied to the data. Meanwhile, Figure 4. shows the results of DNA quantity in mean values (SD), n=8. DNA quantity of concentration values for control (9.6±3.5); 1.5 N NaOH concentration (30.3±3.4); 1.75 N NaOH concentration (99.0±36.2); 2.0 N NaOH concentration (187.7±58.3). Kruskal wallis statistical test and Mann whitney test ($p<0.05$) were applied to the data. The highest DNA quality and quantity results were found in the 2.0 N NaOH concentration treatment.

3.2. Discussion

Good DNA purity and concentration may be defined as the kind and quantity of DNA needed for the success of later processes like PCR, DNA sequencing, or electrophoresis. The resultant ratio (A260/A280) of 1.8-2.0 is the quality of DNA required in these activities. Meanwhile, DNA concentrations of around 10-20 μg/mL are required for PCR, while DNA concentrations of around 10-50 μg/mL are required for DNA sequencing and electrophoresis (Barqly et al., 2021). Concentration values >100 ng/μL and purity values between 1.8-2.0 indicate good DNA isolation results. High concentration values do not always mean high purity values. This is because the A280 value (contaminant value) has an influence on the purity value. The A280 value indicates contaminants, while the A260 value will have an impact on the DNA concentration value (Iqbal et al., 2016).

The results of quality research on the treatment of NaOH concentration 2.0 N (1.86±0.44) obtained results with good categories when compared to the treatment of NaOH concentration 0.2 N (control) (0.95±0.18); NaOH 1.5 N (1.47±0.18); and NaOH 1.75 N (1.52±0.29). DNA quality is influenced by several factors including the incomplete cell wall lysis process and the less than optimal precipitation process. This is believed to be due to the difficulty of performing the lysis process on complex fungal cell walls, where there are protein, carbohydrate, and organic material contaminants derived from fungal cell walls, such as α-glucan, β-glucan, galactomannan, and chitin cannot be destroyed by alkaline solutions such as NaOH with low concentrations, resulting in low quality DNA (Merck, 2021). In less than optimal DNA precipitation can also affect the results of DNA quality which causes protein or RNA contaminants so that DNA cannot be separated from its contaminants (G-Bioscience, 2016). These findings is in accordance with the research of Rodriguez et al., (2016) on the *Saccharomyces cerevisiae* fungus in the use of isopropanol or potassium acetate cannot produce pure DNA, thus affecting the results of poor DNA quality.

The results of DNA quantity research in the treatment of NaOH concentration 2.0 N (187.7±58.3) obtained results with good categories when compared to the treatment of NaOH concentration 0.2 N (control) (9.6±3.5); NaOH 1.5 N (30.3±3.4); and NaOH 1.75 N (99.0±36.2). One of the variables influencing the amount of DNA is the number of cells collected during the DNA isolation procedure. The structure of the cell wall that protects the fungus determines why the number of cells is less. Fungi are known to have complex and thick cell walls, making it difficult to lyse. The DNA lysis method in an alkaline solution containing NaOH with a concentration of 0.2 N which is unable to lyse the fungal cell wall, is thought to be an influential factor in the low quantity of DNA obtained. This aligns with the study conducted by Faradisa et al. (2021) regarding *Candida albicans* fungal cells using 0.2 N NaOH obtained low DNA quantity results. In the same study, it was also found that the low quantity of *Aspergillus niger* DNA was 1.8 μg/mL (Barqly et al., 2021). Meanwhile, in the research of Zhu & Wu, (2019) using samples of fungi, yeast, plants, and algae containing β-glucan cell walls can be lysed with 2.0 N NaOH and have the highest DNA yield results.

The optimization results of the base lysis method on the NaOH concentration used during the cell lysis process showed good results to be implemented in molecular laboratories. However, there are limitations in this study, namely that researchers did not measure the concentration of the fungal suspension before the DNA isolation stage. Where, if this step is applied the possibility of biased results will be reduced. Fungal DNA isolation is carried out when the fungal suspension sample is in the exponential phase. To obtain good quality DNA, a sample concentration of 5×10^6 cells or an optical density (OD) value of λ660 nm = 1.0 is often used.

4. Conclusions

The conclusion drawn from the research findings is that the impact of optimizing NaOH concentrations of 1.5 N; 1.75 N; and 2.0 N on the lysis process of fungal cell walls in the alkaline lysis method, namely an increase in the value of quality and quantity of sample DNA isolation results gradually. The use of 2.0 N NaOH concentration produced the best quality and quantity of DNA, namely 1.86±0.44 for DNA quality and 187.7±58.3 for DNA quantity.

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Conflicts of Interest: There are no research conflicts

Author Contributions: Nasya Desinta Putri: Designing research, Performing laboratory work, Analyzing data, Obtaining funding. Endah Prayekti: Research supervisor.

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