International Journal of Health Science and Technology e-ISSN 2685-8673
Vol. 5, No. 2 (2023), pp. (142-148)
https://doi.org/10.31101/ijhst.v5i2.3273

Original Research Paper

Comparison of DNA isolation of Candida albicans with filter-based kit and cooling methods

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Submitted: 6 September 2023 Revised: 17 Oktober 2023 Accepted: 27 November 2023

Abstrak

Candidiasis is a disease caused by the fungus Candida sp., which can infect the skin, hair, mucous membranes, and internal organs. Laboratory diagnosis of Candida albicans can be done molecularly with the aim of identifying fungal DNA through DNA isolation as an initial stage. This study used an experimental research design used to compare two DNA isolation methods, namely filter-based kits and cooling. DNA purity is assessed by measuring absorbance at 260 nm and 280 nm. Qualitative evaluation is carried out through visualization of DNA bands in electrophoresis. After conducting five DNA re-isolation experiments for both filter-based kit and cooling methods, the highest DNA concentrations were observed in samples isolated using filter-based kits, with a result of 6.465 µg/µL. In addition, an adequate level of purity is achieved with this method. In addition, thicker and lighter bands of DNA are observed, which can be attributed to the use of the enzyme Lyticase and other chemicals. The variability of isolation results can be affected by several factors, including extraction speed, sample type, extraction method, and environmental conditions. Technical errors during measurements can also affect the amount of DNA. In comparison, DNA isolation using filter-based kits for Candida albicans resulted in superior quantity and quality of DNA compared to cooling methods.

Keywords : Candida albicans, DNA isolation, Filter based kit and cooling

1. Introduction

Candidiasis is a disease caused by fungal species Candida sp, which is also acute and sub-acute. This disease generally attacks the mouth, vagina, nails, lungs of men and women (Getas, 2014); (Millsop & Fazel, 2016). The most common causative agents of Candidiasis are: Candida albicans, which is a kind of opportunistic pathogen and part of the normal human microflora (Gong et al., 2016); (Volkova et al., 2021). Laboratory diagnosis of fungi Candida albicans involves microscopic and macroscopic observations, culture methods, as well as serological examination to see β-glucan in the cell wall of the fungus (Coronado-Castellote & Jiménez-Soriano, 2013); (Asghar et al., 2019). This procedure can also be done molecularly with the aim of identifying fungal DNA through DNA isolation as an initial stage (Camp et al., 2020); (Eghtedar Nejad et al., 2020).

The principle of DNA isolation is to obtain pure DNA that is not mixed with other cell components such as proteins and carbohydrates. DNA isolation has several stages, namely cell isolation, lysis of cell walls and membranes, extraction of cells in solution, purification and finally precipitation (Gupta, 2019). In general, DNA isolation includes three important things, namely the results of DNA can produce a high level of purity, the DNA must be intact and also the amount must be sufficient. DNA isolation methods have undergone rapid development along with the evolution of the times. Methods that have been developed include: Extraction methods using silica matrice, alkaline, magnetic beads, EtBr-CsCl Gradient Centrifugation, Chelex-100, Chromatography-Based DN, Cetyltrimethylammonium Bromide (CTAB), SDS-Proteinase K, salting-out, phenol-chloroform, silica column-based, Cellulose-based paper and paper-based filters (J Shetty, 2020).

Method Filter based kit which is a commonly used method of isolating DNA samples using reagent kits. This method is widely used because in terms of work it is relatively fast and easy to use because it is already in the form of a commercial kit (McFall et al., 2016);(Djurhuus et al., 2017);(Shi et al., 2018). However, this method has a weakness factor, namely the price of the kit is relatively expensive and also requires a long time in the procurement process, because this chemical is an imported product (Yahya et al., 2017). An alternative method that is expected to provide solutions to these various problems is the cooling method. This method has the principle of the cell wall leaching process at a certain temperature, which is -20oC and has a slightly simpler stage so that it is more effective and efficient in terms of cost and time (Hermansyah et al., 2018).

Candidiasis, as a disease that can affect a variety of organs, shows the complexity of the medical challenges that need to be addressed. In this context, the development of DNA isolation methods is crucial, and this study discusses two approaches, namely Filter Based kits that are commonly used but have financial and time constraints, and cooling methods that are expected to be an efficient alternative. Through comparison of quantitative and qualitative assays, this study is expected to make a significant contribution to our understanding of the effectiveness of DNA isolation methods in the context of the fungus Candida albicans. Thus, improved diagnostic methods can be implemented more effectively.

2. Method

This study used an experimental research design. In this study, systematic control of certain variables was carried out. Variables such as incubation time, type of chemical used, and temperature can be carefully regulated and controlled. In this design, two groups were divided, namely the experimental group using the Filter based kit method and other experimental groups using the cooling method. In each group, 5 repetitions were made to ensure more consistent results and better generalization.

The tools used in this study include: Petri dish, test tube, ultra centrifuge, mini centrifuge UV-Vis spectrofotmeter, microtube, Frezzer, electrophoresis set, micropipette, cryo tube rack, oshe, vortex, Gel Doc-imaging, waterbath, hot strier, blue tip, yellow type, white type. While the materials used include: Candida albicans ATCC 10231 mushroom isolate obtained from the Yogyakarta Health and Calibration Laboratory Center (BLKK), Wizard SV Genomic DNA purification kit, ethanol, Sabouraud Dextrose Broth (SDB) liquid media, agarose gel, TE buffer, Tris borate EDTA (TBE) Buffer, dye loading solution.

At the sample preparation stage, Candida albicans fungus isolate is put into Sabouraud Dextrose Broth (SDB) liquid media then mixed and divortex to become a suspension. Furthermore, DNA isolation was carried out using two different methods. The first method, the Filter Based Kit, starts by transferring 1 ml of Candida albicans mushroom suspension into a 1.5 ml microtube and centrifuged at a rate of 13,000–16,000 × g to form cell pellets. The supernatant was removed, the cells resuspensioned within 293µl 50mM EDTA, and 7.5µl lyticase were added for cell wall digestion at 37°C for 30-60 min. After cooling, centrifugation is performed, and cell pellets are resuspended in 300µl Nuclei Lysis Solution. Protein Precipitation Solution (100µl) was added, vortexed, and the sample cooled on ice for 5 min. Next, centrifugation is carried out for 3 minutes, the supernatant (containing DNA) is transferred into a 1.5 ml microcentrifuge tube containing 300 µl of isopropanol temperature room. DNA strands that look like threads are incubated until a mass is formed, then centrifuged for 2 minutes. The supernatant is discarded, and the DNA pellets are washed with 300µl of room temperature 70% ethanol, followed by drying for 10–15 minutes. DNA Rehydration Solution (50µl) and RNase Solution (1.5µl) were added to pure DNA, vortexed, and incubated at 37°C for 15 min. Alternatively, DNA is rehydrated overnight at room temperature or 4°C. As a result, DNA is stored at a temperature of 2–8°C.

In the DNA isolation process by cooling method, a total of 1.5 ml of Candida albicans fungal suspension in Sabouraud Dextrose Broth (SDB) was taken and inserted into a test tube. A 7.5 µl lyticase enzyme was added, then the tube was centrifuged for 3 minutes at a speed of 12,000 rpm. The cell pellet is taken out and the supernatant is discarded. Next, 10 µl of pellets were taken, and 200 µl of sterile aqueous aqueous was added to the tube which was then vortex for 45 seconds. This process ends with cooling at -20°C for 2 days, with freeze thawing periodically.

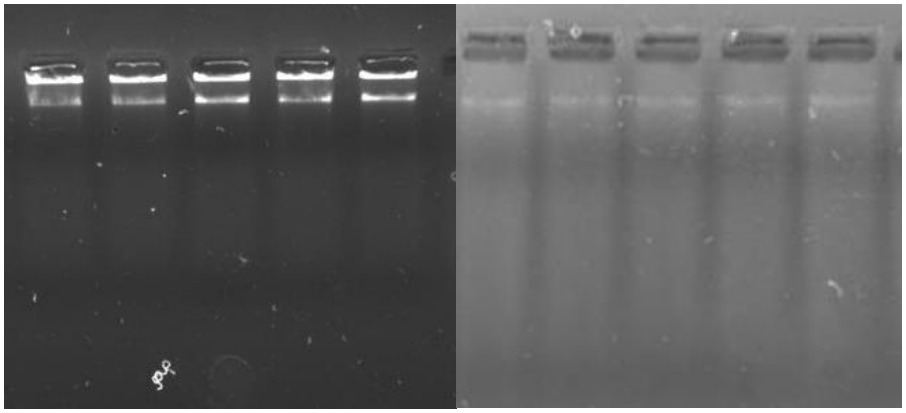
Qualitative testing of DNA isolation results was carried out with Agarose Gel Electrophoresis. The concentration of agarose gel used is 1.5%. In the first stage, agarose powder is heated with TBE to boiling, then agarose solution is poured into a mold that has been fitted with a well-making comb and cooled at room temperature until a gel is formed. After the gel has hardened, the gel and mold are put into the chamber, and the TBE buffer is poured until the gel is submerged. DNA and dye loading solution (1:1) are applied to each well. The electrodes are connected to the power supply and turned on for 1-2 hours. Upon completion, the electrophoresis device is turned off, the gel is removed, and transferred to a UV-transluminator for observation. The quality and quantity of DNA were analyzed using Gel Doc-imaging and readily available software

DNA quantitative testing is performed with a UV-Vis Spectrophotometer. The initial step was dilution of DNA isolates using vortex and spindown for 15 seconds. Aquabidest as much as 3980 µl was pipetted, then added with DNA isolate as much as 20 µl in the falcon tube as a dilution of 200x. Next, stirring with vortex for 30 seconds is carried out. DNA absorbance is measured using a UV-Vis spectrophotometer with wavelengths of 260 nm and 280 nm. DNA isolates taken as much as 20 µl were stored at -20°C for use on the next occasion.

Data analysis was carried out by comparing the results of qualitative and quantitative tests of DNA isolates from each method. The analysis of qualitative test results is carried out by comparing the presence or absence and thickness of DNA band visualization read on electrophoresis. The analysis of quantitative test results is carried out by comparing the level of DNA purity in each method. Purity can be said to be good if the value is 1.8 - 2.0.

3. Result and Discussion

DNA quality test visualization results using agarose gel electrophoresis concentration of 1.5%. It is presented in figure 1 as follows:



Source: primary data, 2023

Figure 1. Repondent characteristics by age

Information:
K1 – K5 : DNA isolation sample Filter Based Kit Method
P1 – P5 : DNA isolation samples Cooling Method

Based on the results obtained as figure 1 it is known that the bands resulting from the DNA isolation method Filter based kit brighter and thicker, while the results of DNA isolation by the DNA band cooling method can be

visualized on electrophoresis but the resulting band appears thin. This is due to the method Filter based kit Has the advantage of using enzymes Lyticase which can help the cell leaching process (Goldschmidt et al., 2014). In addition some other chemicals such as DNA rehydration solution, protein precipitation solution, DNA rehydration solution, RNase solution, and cell lysis solution can help in cell lysis and dissolving cellular proteins. High concentrations can affect the thickness of DNA bands, but DNA isolation methods result Filter based kit still shows the presence of smears that are quite carried away and look thick. Smear What is carried can be caused by the presence of contaminants such as proteins or carried away by the remaining solution in the isolation process.

Samples resulting from DNA isolation by the cooling method obtained DNA fragments of fairly thin bands. this is due to the DNA isolation process of the cooling method by modifying the procedure with the addition of Lyticase enzyme as much as 7.5 ul to the sample without any mixture of other chemicals as done in the Filter based kit method. In the cooling method, although it is not perfectly visualized on electrophoresis, but can produce thin DNA bands, this proves that DNA isolation is successful with the cooling method. This method is quite advantageous in terms of fairly simple processing procedures without requiring a long incubation time as is done in the kit method. The difference in results in each sample can be seen from the large concentration of extracted DNA, the quality of extracted DNA is also shown by the presence of smears on the DNA band, the fewer or no smears show the better the quality of DNA.

Thick, clumped DNA bands show high concentrations and total DNA extracted intact. Meanwhile, DNA bands that are broken during the extraction process can be caused by excessive physical movements that can occur in the pipetting process, when flipped and ependorf, centrifuges, or even because of temperatures that are too high and due to the activity of certain chemicals (Arslan et al., 2021); (Setyawati &; Zubaidah, 2021).

The medium used in electrophoresis is agarose gel with a concentration of 1.5%. The concentration of agarose gel greatly affects the rate of DNA migration in the process of electrophoresis. The concentration of agarose used will determine the size of the pores of the gel that will separate the DNA. The lower the concentration of agarose, the smaller the gel matrix will be and the DNA fragments can be separated further apart based on their size. Agarose gel is the standard method for identifying and purifying fragments Deoxyribo Nucleic Acid (DNA) and Ribose Nucleic Acid. The advantages of this gel are easier, simpler and the rate of separation is faster forming fragments and is not toxic. It's just that this gel has high sensitivity and is easily damaged so it is highly recommended to require a high level of accuracy and caution in the process. Before electrophoresis, a DNA suspension must be added Loading Buffer (dye) first, which serves to increase density, so that DNA will always be at the bottom of the contribution. DNA will always be at the bottom of the well. Loading buffer (dye) also functions as a dye to make it easier to put DNA samples into wells (Widiyanti et al., 2014); (Motohashi, 2019); (Setyawati &; Zubaidah, 2021).

The results of quantitative tests are presented in the following table:

Table 1. Quantitative test results of Candida albicans mushroom samples filter based kit and cooling methods

Sample	A260	A280	Purity Value (A260/A280)	concentration (ng/ µl)
K1 Sample	0,736	0,368	2,0	5,520 ng/ul
K2 Sample	0,799	0,412	1,9	5,992 ng/ul
K3 Sample	0,878	0,498	1,8	6,585 ng/ul
K4 Sample	0,798	0,419	1,9	5,985 ng/ul
K5 Sample	0,862	0,486	1,8	6,465 ng/ul
P1 Sample	0,860	0,598	1,4	6,450 ng/ul
P2 Sample	0,862	0,621	1,4	6,465 ng/ul
P3 Sample	0,858	0,561	1,5	6,435 ng/ul
P4 Sample	0,830	0,604	1,4	6,225 ng/ul
P5 Sample	0,818	0,701	1,2	6,135 ng/ul

Source: primary data, 2023

The results of testing the quantity and quality of DNA showed different results. DNA concentration and purity are quantitatively measured using the spectrophotometer method with a UV-Vis spectrophotometer at 260

nm and 280 nm waves. In samples resulting from DNA isolation methods Filter based kit The spectrophotometer showed a fairly good DNA concentration of 6.465 Ng/ µl And also for the level of purity obtained pure results ranging from 1.8-2.0. The extraction results with a ratio of 1.8 to 2.0 are DNA with high purity and are not contaminated with protein residues. The samples that showed a good level of purity were K1 (2.0), K2 (1.9), K3 (1.8), K4 (1.9), K5 (1.8). Results that show purity values below 1.8-2.0 indicate that there is still protein contamination. Samples showing DNA close to pure is produced from DNA isolation by cooling methods namely P1 (1.4), P2 (1.4), P3 (1.5). P4 (1.4), P5 (1.2). Of all samples calculated for concentration and purity, in general, the concentration of isolated DNA has a relatively large concentration and is not uniform. The quantity of DNA shows that the purity value in each sample is different and the level of DNA concentration obtained varies. The concentration of DNA produced can be influenced by several factors at the time of DNA extraction and sample conditions. The extraction speed factor is one of the influential factors because at the stage of cell lysis and precipitation supernatant collection must be sampled, so that some samples occur DNA deposition. Other factors that can affect the quantity of DNA such as the type of sample used, extraction methods, and less sterile environmental conditions (Nugroho et al., 2017); (Sentani et al., 2017); (Retnaningati, 2021);(Prasetyoningrum et al., 2023).

Measurements using a spectrometer can also be used to determine the purity of DNA from the extraction. The level of purity can be determined by calculating the ratio between the values of 260 nm and 280 nm. The value of 260 nm is the maximum value of DNA can absorb light, the value can be used to estimate the concentration of DNA, while the value of 280 is the maximum value of protein residues can absorb light. The average value of DNA purity obtained from the results of this study is said to be pure if the value of the A260/280 ratio is between 1.8 to 2.0 (Lucena-Aguilar et al., 2016);(Romlah et al., 2018);(Dewanata &; Mushlih, 2021).

Low DNA purity values can be caused by residual Dye Loading contained in the pores of agarose gel. The rest of the material is Ethidium Bromide found in the pores of Agarose gel, so there is Smear thin between the DNA bands. The DNA purification stage can produce DNA that is free of impurities. While at the DNA washing stage with the addition of ethanol can precipitate DNA because nucleic acids will precipitate and are difficult to dissolve in ethanol while impurities can dissolve with ethanol. The difference in quantity results can also be caused from a technical point of view at the time of measurement, homogenization before the spectrophotometer and improper pipetting process causing DNA to break into fragments of fractions. The technical error caused the concentration of DNA in the spectrophotometer results. The technical error caused the concentration of DNA in the spectrophotometer results to be lower (Isbir et al., 2013); (Lucena-Aguilar et al., 2016); (Mardan-Nik et al., 2019).

4. Conclusion

Based on the results of the study, data analysis and discussion in this study, it can be concluded that the results of the DNA isolation filter based kit method show that the DNA band of the fungus Candida albicans is well visualized and has high purity compared to the DNA isolation cooling method. The results of this study provide guidance for researchers and practitioners in choosing a DNA isolation method that suits their specific needs. With a better understanding of the advantages and disadvantages of each method, researchers can make informed and contextual decisions in designing follow-up research or developing more sophisticated DNA isolation techniques. This comparison can also be the basis for further in-depth research in an effort to continue to improve the efficiency and accuracy of DNA isolation techniques in the fungus Candida albicans.

Suggestion

An important suggestion for the next researcher is to further explore certain aspects in an effort to optimize the DNA isolation method of *the Candida albicans fungus* using a filter-based kit and cooling method. It is also important to consider the clinical applications of this DNA isolation as well as compare it with other isolation methods to determine its relative advantages and disadvantages.

Acknowledgments

This research can be carried out well thanks to assistance from various parties. Therefore, researchers are very grateful to the head of the study program, who has made it easier to obtain permits

to conduct research, to supervisors and examiners who have facilitated the running of this research, and to the *Yogyakarta Health and Calibration Laboratory Center* (BLKK) which has provided *Candida albicans mushroom isolates*.

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