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Original Research Paper

# Quality and quantity of *Aspergillus niger* DNA isolation using filter-based kit and cooling method

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## Abstract
Polymerase Chain Reaction (PCR) is an important technique to improve sensitivity in the detection of fungal infections, such as those caused by *Aspergillus niger*. The availability of pure DNA and DNA isolation techniques are important factors in implementing PCR. This study aims to compare the quality and quantity of DNA isolation of *Aspergillus niger* using the Filter Based Kit method and cooling. The experimental research design was used with a qualitative test of DNA isolate using Agarose Gel Electrophoresis (1.5%) and a quantitative DNA test using a UV-Vis Spectrophotometer (wavelengths of 260 nm and 280 nm). Data analysis compared the qualitative and quantitative results of DNA isolates from both methods. The results showed the presence of DNA bands in both isolation methods, with thicker bands in the Filter Based Kit method. The average concentration of DNA after isolation using the Filter Based Kit (6,478 ng/μl) was higher than that of the cooling method (5,994 ng/μl). The purity of DNA was also higher in the filter-based kit (1.7) than in the cooling method (1.1). The Filter Based Kit method contains chemical components that support the successful isolation of DNA. It can be concluded that the filter-based kit method produces *Aspergillus niger* DNA isolate with better quality and quantity than the cooling method. The implication of these findings is that the Filter Based Kit could be a better option for the isolation of *Aspergillus niger* DNA in laboratory applications.

**Keywords:** *Aspergillus* niger; cooling method; filter-based kit

## 1. Introduction

*Chronic Pulmonary Aspergillosis* (CPA) is a chronic fungal infection of the lungs caused by *Aspergillus* fungus. This infection is a serious condition, especially in individuals with a history of lung diseases such as tuberculosis. CPA can cause progressive, potentially fatal lung damage without proper treatment. This infection is caused by the inhalation of spores, such as *Aspergillus* spp. (Chabi et al., 2015; Despois et al., 2022; Lamoth & Calandra, 2022; Rozaliyani et al., 2020; Setianingrum et al., 2020). One of the species is *Aspergillus* niger (Barac et al., 2023; Denning et al., 2013; Hollingshead et al., 2020; Jakribettu et al., 2019).

An accurate diagnosis of CPA is important because many diseases have a similar appearance but require different treatments. Fungal cultures of the respiratory tract are not sensitive to diagnosing CPA. There are many new tests available, for example serological tests to detect *Aspergillus* IgG (Wilopo et al., 2019). *Polymerase Chain Reaction* (PCR) has been suggested as a complement to serological testing to improve sensitivity, Although it has not been routinely performed for CPA (Larkin et al., 2020). In the implementation of sensitive, specific, and reliable PCR tests, the availability of pure DNA and DNA isolation techniques are very important factors (Kumar & Mugunthan, 2018; Wickes & Wiederhold, 2018). The use of DNA isolation techniques is supposed to result in efficient extraction with good quantity and quality of DNA, which is pure and free from contaminants, such as RNA and proteins. The isolated DNA can be assessed for quality and quantity using gel electrophoresis and spectrophotometry (Gupta, 2019).

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DNA isolation techniques have undergone many developments in various methods, including Cetyltrimethylammonium Bromide (CTAB), SDS-Proteinase K, salting-out, phenol-chloroform, silica column-based, Cellulose-based paper and paper-based filters (Dairawan & Shetty, 2020; Gupta, 2019). Method Filter Based Kit is a commonly used method for isolating DNA samples using reagent kits. Its main advantages are relatively fast workmanship and ease of use because it is already in the form of a commercial kit (Djurhuus et al., 2017; McFall et al., 2016; Shi et al., 2018). However, this method has drawbacks, such as the relatively expensive price of the kit and the time required in the procurement process, as well as the number of samples that can be isolated (Rana et al., 2018; Yahya et al., 2017). Alternatively, the cooling method is an attractive option. This method relies on the process of cell wall filtration at -20°C and has simpler stages, making it more effective and efficient in terms of cost and time (Hermansyah et al., 2018). No studies have explicitly compared these two methods in the context of DNA isolation from *Aspergillus niger* (Barqly et al., 2021; Rana et al., 2018).

This study aims to compare the quality and quantity test results of the filter based kit method and the cooling method in the isolation of *Aspergillus niger* DNA. This concept is based on the need to determine effective DNA isolation methods for pathogenic fungi, specifically *Aspergillus niger*. By comparing the two commonly used methods, this study is expected to provide new insights into the choice of the most effective isolation method for future studies

## 2. Research Methods

This study uses an experimental research design in which variables such as incubation time, chemical type, and temperature are systematically controlled. This study divided the sample into two groups: one group used the Filter Based Kit method and the other group used the cooling method. Each group was repeated five times to ensure consistent results and better generalization.

The tools used in this study include UV-Vis spectrophotometer, electrophoresis, micropipette, gel documentation, vortex, centrifuge, test tube, freezer, stopwatch, tube rack, stirring rod, erlenmeyer, beaker glass, droppipette, thermometer. The ingredients used include: *Aspergillus niger* strain FNCC 6018 mushroom isolate obtained from the Center for Food and Nutrition Studies, Gadjah Mada University (PAU-UGM), Wizard Genomic DNA purification kit (promega), PDA Media (*Potato Dextrose Agar*), aquabidest, Agarose gel, red gel, TE solution, TBE solution, loading dye solution, Lyticase enzyme.

DNA isolation using the filter-based kit method was carried out by weighing *Aspergillus Niger* mushrooms as much as 40 mg and putting them into a small sterile mortar, then grinding and putting them into a 1.5 mL microtube. *Aspergillus Niger* mushrooms that have been grinded, centrifuged at a speed of 13,000 - 16,000 xg for 2 minutes to form pellets, then the supernatant is discarded. The resuspension cells were added 293 µL of EDTA 50M, 7.5 µL of lyticase enzymes were added, then slowly pipetted 4 times for homogenization, and incubated at 37°C for 30 - 60 minutes. Add 300 µL of Nuclei Lysis Water, add another 100 µL of Protein Precipitation Solution, then vortex for 20 seconds. Followed by centrifugation for 2 minutes at a speed of 13,000 - 16,000 xg, after which it is put in the freezer for 5 minutes. Adding 300 µL of room temperature isopropanol to the new Eppendorf tube, the supernatant is transferred to a new Eppendorf tube containing 300 µL of isopropanol slowly until the DNA looks like thread. Centrifuge for 2 minutes at a rate of 13,000 -16,000 xg, then the supernatant is discarded and the pellets are dried on absorbent paper, let the pellets dry for 10 - 15 minutes. Then 50 µL of DNA Rehydration Solution is added, and 1.5 µL of RNase is vortexed for 1 second, followed by centrifugation for 5 seconds. Incubated at 37°C for 15 minutes, then continued with incubation at 65°C for 1 hour in a water bath, DNA Rehydration was stored in the freezer overnight.

DNA isolation using the cooling method was carried out by means that *Aspergillus Niger* that had been weighed weighing 40 mg was put into a small sterile mortar, then grinded and placed into a

microtube measuring 1.5 mL. The suspension is then centrifuged for 3 minutes at 12,000 rpm, then the cell pellets are taken and the supernatant is discarded. A total of 10 µL of pellets were then placed in an Eppendorf tube and mixed with 200 µL of sterile aquadest, then vortexed for 45 seconds. Next, the samples were cooled at -20°C overnight, and repeated cooling and thawing were performed.

Qualitative tests of isolated DNA isolates were carried out with Agarose Gel Electrophoresis using agarose gel at a concentration of 1.5%. The first step involves heating the agarose powder in the TBE solution to a boiling point, followed by pouring the agarose solution into a mold that has been fitted with a well-making comb. After that, the agarose solution is cooled to form a gel at room temperature. The hardened gel is then inserted into the chamber, and the TBE buffer is poured to soak the gel. The DNA and the loading dye solution are mixed in a 1:1 ratio and applied to each well. Next, the electrode is connected to the power supply and turned on for 1 hour. After the electrophoresis process is complete, the tool is turned off, the gel is taken, and placed in a UV-transluminator for observation. DNA quality is analyzed using Gel Doc-imaging and available software.

Meanwhile, the quantitative test of DNA was carried out with a UV-Vis Spectrophotometer. The initial stage involves dilution of DNA isolates using vortex and centrifugation for 15 seconds. An aquabidest of 3980 µL was added to the falcon tube, then 20 µL of DNA isolate was added as a 200x dilution. The mixture is then stirred using a vortex for 30 seconds. DNA absorbance was measured using a UV-Vis Spectrophotometer with wavelengths of 260 nm and 280 nm.

Data analysis was carried out by comparing the results of qualitative and quantitative tests of DNA isolates from both isolation methods. In the qualitative analysis, comparisons were made based on the presence and clarity of the DNA bands read on electrophoresis. Meanwhile, in quantitative analysis, comparisons are made based on the level of DNA purity of each method. Purity is considered good if the value is in the range of 1.8 to 2.0.

## 3. Results and Discussion

### 3.1. Results

The results of the DNA quality test visualization using agarose gel electrophoresis at a concentration of 1.5%. presented in Figure 1.

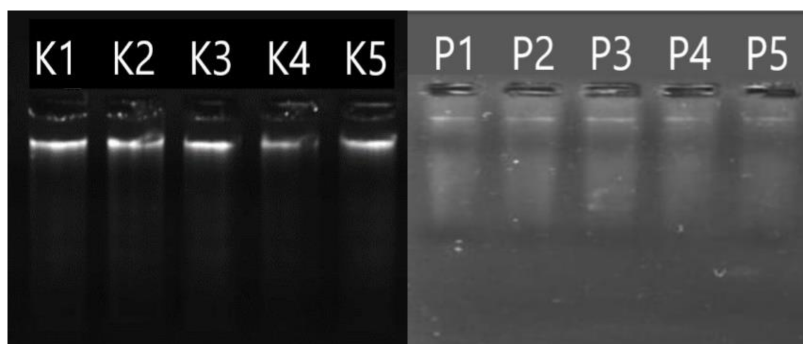


**Figure 1.** The Results of Visualization of DNA Isolates Using Agarose Gel Electrophoresis (Source: primary data, 2023). Description: K1-K5 = DNA Isolation Results of Filter Based Kit Method, P1-P5 = DNA Isolation Results of Cooling Method

The results of electrophoresis showed that DNA bands could be observed on agarose gels from each isolation method. The isolation using the filter based kit shows a thicker DNA band when compared to the DNA band in the cooling method. After the qualitative test was carried out, a quantitative test was carried out on each DNA isolate using UV-Vis spectrophotometry. The results of the quantity test are presented in the form of Table 1.

**Tabel 1.** Results of quantitative test of *Aspergillus* niger DNA isolate

| Sample | Purity Value | | | Concentration |
|---|---|---|---|---|
| | A260 | A280 | A260/A280 | (ng/µL) |
| K1 Sample | 0.901 | 0.466 | 1.9 | 6.757 |
| K2 Sample | 0.958 | 0.522 | 1.8 | 6.322 |
| K3 Sample | 0.843 | 0.507 | 1.6 | 6.150 |
| K4 Sample | 0.867 | 0.554 | 1.5 | 6.502 |
| K5 Sample | 0.888 | 0.568 | 1.5 | 6.660 |
| **Average** | | | **1.7** | **6.478** |
| Sample P1 | 0.753 | 0.752 | 1.0 | 5.647 |
| Sample P2 | 0.740 | 0.0648 | 1.1 | 5.550 |
| Sample P3 | 0.745 | 0.899 | 0.8 | 5.587 |
| Sample P4 | 0.789 | 0.943 | 0.8 | 5.917 |
| Sample P5 | 0.836 | 0.512 | 1.6 | 6.270 |
| **Average** | | | **1.1** | **5.994** |

Source: primary data, 2023
Information:
K1 – K5: DNA isolation results of the filter-based kit method
P1 – P5: DNA isolation results of the cooling method

The quantitative test of DNA isolated by the filter based kit method showed a purity value with a range of 1.5-1.9 and an average purity of 1.7. The DNA concentration of the method showed the lowest value of 6.150 ng/µL, the highest value of 6.757 ng/µL with an average of 6.478 ng/µL. The purity of the DNA isolated by the cooling method ranged from 0.8-1.6 and the average purity was 1.1. The DNA concentration obtained showed the lowest value of 5,550 ng/µL, the highest value of 6,270 ng/µL with an average of 5,994 ng/µL.

## 3.2. Discussion

The success of a DNA isolation process can be confirmed by using the agarose gel electrophoresis method. JIf the isolation is successful, the DNA band in the gel will be visible. However, if the isolation fails then the DNA band will not be clearly visible or even the DNA band will not be visible at all inside the gel (Couto et al., 2013). In this study, DNA bands could be observed inside agarose gels from both isolation methods. This shows that DNA isolation is successful. The visualization results also showed that the DNA band was not fragmented. According to Dwiyitno et al. (2018) Fragmented bands tend to look blurry or undefined, and may sometimes appear as dispersed gel spots rather than regularly sharp, centered bands.

The results of electrophoresis also showed that there were no DNA samples showing significant smears, which indicated the absence of sample degradation. A smear is a term used to describe stains that appear in the DNA movement pathways on agarose gel, which can sometimes be difficult to distinguish clearly. The appearance of smears can be caused by the presence of material other than DNA that is also isolated or DNA that has been degraded. Usually, smears occur when DNA is degraded or contaminated with different-sized DNA fragments. The presence of a smear may indicate that DNA isolation is unsuccessful or that the sample contains DNA that has been degraded. Thus, the presence of a smear in the DNA isolation process can indicate problems related to the quality or integrity of the isolated DNA (Latif & Osman, 2017; Setiani et al., 2021).

The results of DNA concentration measurement showed that the average concentration of isolation using the filter based kit method was 6.478 ng/µL, higher than the cooling isolation which only reached 5.994 ng/µL. According to Sophian et al. (2021), the value of the concentration of DNA is considered good if the isolated DNA has a concentration above 20 ng/µL. Generally, the concentration of DNA

used in the process Polymerase Chain Reaction (PCR) is 100-250 ng for eukaryotic genomic DNA per 50 μL reaction (Donastin et al., 2022). However, according to Nieman et al., (2015) states that in a standard PCR test, the sufficient amount of DNA is between 0.25 to 1.8 nanograms per microliter in a total volume of 200 microliters. Thus, the concentration of DNA obtained from both isolation methods is sufficient to perform PCR. The concentration of DNA measured by spectrometry method corresponds to the results of the evaluation of agarose gel electrophoresis, characterized by a relatively weaker or thinner band on the cooling method. This is in accordance with research Setiani et al. (2021) which states that the visualization of the thickness of the DNA band looks different depending on the amount of concentration of DNA extracted.

The results of DNA purity measurement showed that the average concentration of isolation using the filter based kit method was 1.7 higher compared to the cooling isolation which only reached 1.1. DNA purity is classified as good if it has a purity with a range of 1.8-2. Nucleic acid purity assessment was carried out by measuring the spectrophotometric absorbance ratio of each sample at wavelengths of 260 nm and 280 nm, which is often referred to as the A260/A280 ratio. Measuring the A260/A280 ratio is a commonly used method in assessing the purity of DNA today. A ratio of less than 1.8 for DNA indicates the presence of contamination, which usually comes from proteins (Šarkanj et al., 2018). The main component proteins that can reduce the purity of DNA isolates due to improper preparation, while ratios above 2.0 indicate potential contamination of extraction residues such as RNA, phenols, alcohols, and salts (Dwiyitno et al., 2018). Some researchers use proteinase K to clean protein and phenol contaminants so that a good level of purity is obtained (Donastin et al., 2022; Rachmawati & Khoiriyah, 2018).

In the process of DNA isolation, lysis is a step that plays an important role in producing good concentration and purity of DNA (Sutanta et al., 2021). Fungal DNA isolation has its own challenges when compared to bacteria or mammalian cells. This is due to the complexity and thickness of the fungal cell wall, which inhibits the efficient release of DNA. The high content of polysaccharides in the cell wall, such as manan, β-glucan, and chitin, further complicates the process of DNA isolation from filament fungi. Thus, the importance of selectively disrupting filament fungal cell walls without damaging genomic DNA must also be considered (Donastin et al., 2022; Kumar & Mugunthan, 2018). In this study Used enzyme *Lyticase* as an additive to aid in the cell lysis process. This is in line with Goldschmidt's research et al., (2014) which states that the enzyme *Lyticase* can help the process of cell filtration. Other researchers are using liquid nitrogen to aid in the process of cell filtration (Donastin et al., 2022).

DNA isolation using the filter based kit method showed better results than the cooling method based on DNA quality and quantity assessment. This is due to various chemical components that help the successful DNA isolation process. Components such as cell lysis solution and nucleus lysis solution are used to destroy the cell wall and nuclear membrane of the fungus, allowing access to genetic material. In addition, there is a protein precipitation solution that aims to clump proteins and cell debris after the destruction process. Isopropanol is used to clean the DNA from the remaining cell debris, whereas ethanol helps in the precipitation of DNA and purifies it from the less soluble salts in isopropanol. In addition, there is a DNA rehydration solution that functions to rehydrate DNA after cell lysis (Fadllan et al., 2019; Setiani et al., 2021).

## 4. Conclusion

The filter based kit method produces better quality and quantity of *Aspergillus niger* DNA isolation than the cooling method. These results indicate that the use of filter based kits can improve efficiency in DNA isolation from *Aspergillus niger*. Nonetheless, keep in mind that this study has some limitations, such as the use of only one strain of *Aspergillus niger* and the use of certain DNA isolation techniques.

The implication of these findings is that the filter-based kit method may be a better option for isolation of *Aspergillus niger* DNA in laboratory applications. For further research, it is recommended to expand the sample of the *Aspergillus niger* strain used, use clinical samples as an isolation source, and consider variations of DNA isolation techniques to gain a more comprehensive understanding of the effectiveness of different DNA isolation methods.

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