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Optimization of Concentration and Staining Duration of Methyl Green in The Examination of *Escherichia coli* DNA Bands Using Agarose Gel Electrophoresis

Rifky Adrian Prasetya[1], Fusvita Merdekawati[1], Asep Iin Nur Indra[1], Ai Djuminar[1*]

[1] Department of Medical Laboratory Technologist, Bandung Ministry of Health Polytechnic, Cimahi City, Indonesia
*Corresponding author: Rifkyap12@gmail.com

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ABSTRACT

Background & Objective: Nowadays, many alternative dyes are used for staining DNA during electrophoresis, one of which is Methyl Green. Methyl Green has several advantages, including being cheaper than EtBr, having low toxicity, being non-carcinogenic, and as a cationic dye, Methyl Green is resistant to photobleaching. This study aims to determine the optimal concentration and staining duration of Methyl Green as a DNA dye for examining *Escherichia coli* DNA bands using agarose gel electrophoresis.
Method: The research method used is experimental. Amplified *Escherichia coli* 16S rRNA gene DNA, sized 584 bp, which has undergone electrophoresis, was stained with Methyl Green dye at concentrations of 0.10%, 0.15%, 0.20%, 0.25%, 0.00015%, 0.00020%, 0.00025%, and 0.00030% with varying immersion times of 10, 15, 20, and 25 minutes.
Result: The resulting DNA bands were analyzed or measured for surface area using ImageJ software. The mean value for each experimental group was calculated. The highest mean value was used as the basis for determining the most optimal condition.
Conclusion: This study concludes that the optimal concentration and staining duration of Methyl Green, based on the highest mean value of 19,844,845, is 0.00030% Methyl Green with a staining duration of 25 minutes.

Keywords: Electrophoresis; Concentration and Stanning Time; Methyl Green

Introduction

Analysis of amplification results is conducted using electrophoresis with agarose gel, where agarose gel acts as a conductive medium to separate DNA fragments based on their size. Electrophoresis through agarose gel is a technique used to separate, analyze, identify, and purify DNA fragments (Green & Sambrook, 2019). The bands formed by the electrophoresis method on the gel need to be stained with a DNA dye to be visualized under ultraviolet light (Artati, 2013). The position of DNA bands in the gel can be observed after staining with a low-concentration fluorescent intercalating dye (Green & Sambrook, 2019).

Piereto et al. (2015) reported that Methyl Green has the ability to stain DNA by producing fluorescence and can absorb ultraviolet light when exposed to ultraviolet radiation. Methyl Green is capable of detecting at least 4.8 ng of 1.5 kb DNA fragments (Daniel et al., 2015). The color intensity of DNA stained with Methyl Green is comparable to other fluorescent dyes such as EtBr. Additionally, Methyl Green has several advantages, such as its non-toxic nature and resistance to photobleaching when bound to DNA. Therefore, Methyl Green is an effective and cost-efficient dye for DNA staining in agarose gels (Murgai et al., 2018).

Objective

Previous research has highlighted several limitations in using Methyl Green for DNA staining, such as the visibility of DNA bands that appear with EtBr but are not detectable with Methyl Green, the presence of faint DNA bands, and undefined band clusters at the edges or distal ends of the agarose gel. These issues may be related to the absence of established optimal concentration and staining time parameters for using Methyl Green as a DNA stain in agarose gel electrophoresis.

Method

The type of research conducted is experimental. The research design involved staining DNA with Methyl Green at concentrations of 0.10%, 0.15%, 0.20%, 0.25%, 0.00015%, 0.00020%, 0.00025%, and 0.00030%, with staining durations of 10, 15, 20, and 25 minutes. The resulting DNA bands were analyzed by measuring the band area using ImageJ software, and the results were compared to a control, which consisted of DNA bands in agarose gel stained with 0.01% EtBr and incubated for 10 minutes.

The tools used in this study include 1.5 ml Eppendorf tubes, VB columns, collection tubes, micropipettes (10, 200, and 1000 µL), Erlenmeyer flasks, tips, a vortex, a PCR thermal cycler, volumetric flasks, graduated cylinders, Erlenmeyer flasks, an electric stove, electrophoresis set (chamber, comb, gel mold, power supply), immersion trays, a UV transilluminator, and ImageJ software. The materials used include E. coli cultures, 1.5% agarose gel, 1x TAE buffer, 100 bp DNA ladder III plus, 0.01% ethidium bromide, and 2 g of Methyl Green powder.

Isolation was performed using the spin column method, followed by PCR amplification and electrophoresis, using agarose gel at a concentration of 1.5%, with a voltage of 150 watts for 30 minutes. The study proceeded with DNA staining, but prior to that, the Methyl Green stain needed to be purified.

The stock solution of Methyl Green stain with a concentration of 2% was obtained from the purification process of Methyl Green, based on a previous study by Daniel Pietro in 2015. The process involved weighing 0.4 g of Methyl Green and dissolving it in 10 ml of distilled water. Once dissolved, 20 ml of

chloroform was added to separate the crystal violet from the Methyl Green. The solution was centrifuged for 1 minute at 2000 x g. After the two phases separated, the supernatant, which contained 2% Methyl Green, was collected. The final results were analyzed using ImageJ software to obtain accurate readings.

Results

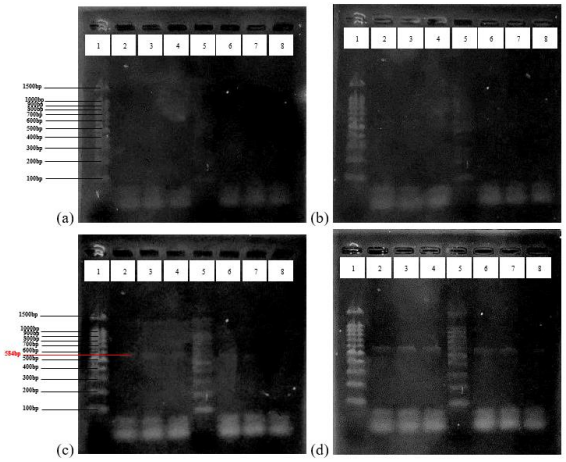

FIGURE 1. Visualization of E. coli DNA bands stained using 0.00025% Methyl Green (2.5 ppm) with staining durations of (a) 10 minutes, (b) 15 minutes, (c) 20 minutes, and (d) 25 minutes. Wells 1 and 5: 100 bp marker, wells 2, 3, 4, 6, 7, and 8: samples

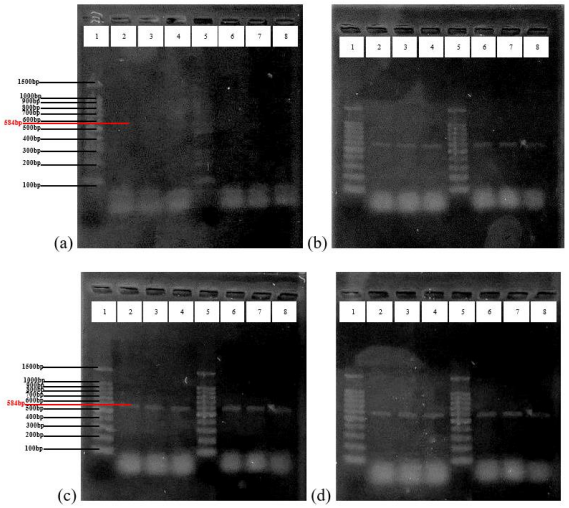

FIGURE 2. Visualization of *E. coli* DNA bands stained using 0.00030% Methyl Green (3 ppm) with staining durations of (a) 10 minutes, (b) 15 minutes, (c) 20 minutes, and (d) 25 minutes. Wells 1 and 5: 100 bp marker, wells 2, 3, 4, 6, 7, and 8: samples.

TABLE 1. Measurement Results of DNA Band Surface Area using Image Software

Stain	Staining Time	Concentration	Area Value
Methyl Green	10 minute	0,00025%	0
		0,00030%	7.122.803
	15 minute	0,00025%	0
		0,00030%	11.206.079
	20 minute	0,00025%	7.273.383
		0,00030%	15.219.377
	25 minute	0,00025%	15.302.400
		0,00030%	19.844.485
Ethidium Bromide	10 minute	0,01%	21.152.500

Discussion

Figure 1 shows the results of DNA staining using Methyl Green at a concentration of 0.00025% (2 ppm). The DNA bands on the gel stained with Methyl Green are visible but appear somewhat faint compared to the clearly visible marker bands. At this concentration, the marker is stained by Methyl Green after 10 minutes of staining, but the E. coli DNA bands are not yet visible. In contrast, Figure 2 illustrates the results of DNA staining with Methyl Green at a concentration of 0.00030% (3 ppm). The DNA bands on the gel stained with Methyl Green are clearly visible. At this concentration, both the marker and the DNA bands are stained by Methyl Green after 10 minutes of staining. This indicates that Methyl Green can bind to DNA bands within the gel.

From table 1, it can be observed that the highest average value was achieved with agarose gel staining using Methyl Green solution at a concentration of 0.00030% with

a staining duration of 25 minutes. This indicates that the optimal conditions for Methyl Green to stain E. coli DNA are a concentration of 0.00030% (3 ppm) and a staining duration of 25 minutes.

In the research using Methyl Green concentrations of 0.10%, 0.15%, 0.20%, and 0.25%, no DNA bands were observed after staining with Methyl Green. The likely reason for the invisibility of the DNA bands could be that the high concentrations of Methyl Green caused difficulties in visualization under UV light. Alternatively, this issue might be due to the suboptimal reading method. Daniel Pietro's 2015 study indicated that Methyl Green is maximally excited by red light at a wavelength of 633 nm and emits light at a wavelength of 677 nm. However, in this study, the reading was conducted using UV light with a wavelength of approximately 300 nm. Therefore, the reading method used in this research may have been inadequate.

The purity of Methyl Green is a critical factor, making the preparation of highly pure Methyl Green solution essential for its effective use. Precision in purifying the solution is important because contamination with crystal violet can interfere with its fluorescent properties. Crystal violet is another dye commonly used in laboratories, but if present in Methyl Green solution, it can disrupt the DNA binding of Methyl Green.

The presence of crystal violet in Methyl Green can occur due to the similarity in their chemical structures and their nature as triphenylmethane cationic dyes (Chen et al., 2010). Crystal violet can interfere with DNA binding to Methyl Green by binding to the same DNA regions as Methyl Green (Fox et al., 1992).

Conclusion

The optimal concentration for staining *Escherichia coli* DNA bands using Methyl Green in agarose gel electrophoresis is 0.00030%. Additionally, the most effective staining duration for visualizing these DNA bands is 25 minutes.

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Conflict of Interest

This research was conducted without any dedicated financial support from funding agencies in the public, commercial, or non-profit sectors.

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