POTENTIAL USE OF SEAWEED AGAR AS A SUBSTITUTE FOR AGAROSE GEL IN DNA ELECTROPHORESIS

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ABSTRACT
Polymerase Chain Reaction (PCR) has become the gold standard test in molecular diagnostics owing to its high sensitivity. The results of DNA amplification from PCR were visualised using gel agarose electrophoresis. However, agarose is expensive and creates an obstacle for molecular examination in Indonesia. This means that not all health facilities in Indonesia can always have a ready stock of agarose, so a replacement for agarose with a good enough result for visualisation with a cheaper one is needed. This study aimed to evaluate the potential use of seaweed agar as a substitute for agarose, and to optimise the optimal conditions for seaweed agar in DNA electrophoresis. This study used a true-experimental research method with a post-test-only control group design. We optimised several parameters of seaweed agar (Swallow brand), including seaweed agar concentration, TAE buffer concentration, voltage variations, and amplicon volume. All electrophoresis results were compared with those of the control. This study shows that seaweed agar can be used as a substitute for commercial agarose gel in DNA electrophoresis under the best conditions, such as using a 2% concentration of seaweed agar, 1x concentration of TAE buffer, 80 volts for 1 h, and 12 µL of DNA amplicons. The limitation of using seaweed agar is that the size of the DNA that can be detected is less than 700 bp.
Keywords*: agarose; electrophoresis; Swallow brand plain agar; seaweed agar*

ABSTRAK
PCR (Polymerase Chain Reaction) telah menjadi standar baku emas dalam diagnostik molekuler karena sensitivitasnya yang tinggi. Hasil amplifikasi DNA dari PCR harus divisualisasikan menggunakan elektroforesis gel agarose, namun harga agarose cukup mahal dan menjadi kendala dalam pemeriksaan molekuler di Indonesia. Hal ini menyebabkan tidak semua fasilitas kesehatan di Indonesia selalu memiliki agarose ini, sehingga diperlukan media pengganti agarosa dengan hasil yang cukup baik untuk visualisasi DNA dengan harga yang lebih murah. Tujuan dari penelitian ini adalah untuk mengevaluasi potensi penggunaan agar rumput laut sebagai pengganti agarosa komersial dan mencari kondisi optimal agar rumput laut dapat digunakan untuk elektroforesis DNA. Penelitian ini merupakan metode penelitian eksperimental dengan desain post-test only control group design. Kami melakukan optimasi agar rumput laut (merek Swallow) pada beberapa parameter seperti: konsentrasi agar rumput laut, konsentrasi buffer TAE, variasi tegangan, dan volume amplikon DNA. Semua visualisasi hasil akan dibandingkan dengan kontrol. Penelitian ini menunjukkan bahwa agar rumput laut dapat digunakan sebagai pengganti gel agarosa dalam elektroforesis DNA dalam kondisi optimal pada: konsentrasi agar rumput laut sebesar 2%, menggunakan konsentrasi buffer TAE 1X, menggunakan tegangan 80 Volt selama 1 jam, dan menggunakan 12 µL amplikon DNA. Keterbatasan penggunaan agar tanpa rasa merek Swallow adalah ukuran amplikon DNA yang dapat dideteksi hanya terbatas pada DNA di bawah 700

pasang basa (bp).
Kata Kunci: agarose, elektroforesis; agar tanpa rasa merek Swallow, agar rumput laut

INTRODUCTION

PCR (Polymerase Chain Reaction) has become a gold standard test in molecular diagnostics due to high sensitivity of result (Khehra, Padda and J. Swift, 2023). There are several types of PCR, including conventional, real-time, and droplet digital PCR. Among several types of PCR, the cost of testing using conventional PCR is the most affordable. However, the results of conventional PCR must be visualized using electrophoresis. In general, DNA electrophoresis uses agarose gel medium. However, agarose is expensive and creates an obstacle for molecular examination in Indonesia (Djankpa et al., 2021). This means that not all health facilities in Indonesia can always have a ready stock of agarose, so a replacement media for agarose with good result visualisation with a cheaper one is needed. Agar from seaweed has a structure similar to that of an agarose gel.

Agar is a mixture of polysaccharides extracted from the cell walls of red algae (Rhodophyta), particularly from the genera Gracilaria and Gelidium (Septi Mauli, 2018). Agar is a polysaccharide consisting of two fractions: agarose and agaropectin (Yuliani, Maulinda, and Sutamihardja, 2017). Agar contains agarose, a neutral (uncharged) polysaccharide, and agaropectin, a sulfate-charged polysaccharide (Rizky Adrin, 2017). Usually, the agarose content in seaweed agar has a percentage of about 55-56% (Yuliani, Maulinda, and Sutamihardja, 2017).

Agarose from seaweed extracts is a suitable medium for DNA electrophoresis. Agarose is preferred for DNA electrophoresis because of its lower High Electroendosmosis (EEO) value, lower sulfate concentration, and fewer charged groups, resulting in better separation and clearer banding patterns. In contrast, agaropectin has a high sulfate content, suggesting that agar containing a high agaropectin content is less effective for use in gel electrophoresis (Zhang et al., 2019). The use of plain powder commercial seaweed agar is the best alternative to agarose because it has a texture and colour (clear) that is close to the shape of the agarose gel, although it definitely contains a mixture of agaropectin.

Based on this background, we examined the potential use of plain powder commercial seaweed agar (abbreviated as seaweed agar) as a substitute for agarose and optimised its optimal conditions for use in DNA electrophoresis.

METHOD

This study used a true-experimental research method with a post-test-only control group design. Researchers optimised seaweed agar from Swallow brand plain agar on several parameters, such as variations in seaweed agar concentration, TAE buffer concentration, voltage variations, and amplicon volume variations, whose results will be compared with the control. A control sample was prepared using agarose.

Agarose and seaweed agar were prepared by dissolving agar powder of a certain weight in TAE buffer in an oven. The homogenised solution was left to warm, poured into an electrophoresis mould, and allowed to solidify. The weight of the agar powder used depends on the concentration of the gel required. For example, a 2% gel was prepared by dissolving agar powder (0.5 g) in 25 mL of TAE buffer. The buffer concentration used to soak the gel in the electrophoresis chamber was adjusted to be the same as that used when preparing the gel. Next, the DNA amplicon was supplemented with a loading dye reagent and inserted into the gel well at a specific volume composition. The electrophoresis gel medium was subjected to electric current at a certain voltage and time.

The quality of the separated DNA was measured visually using UV light. Good DNA quality is indicated by thick DNA bands that appear as few or no smears (Hikmatyar and Royani, 2015). We used a 513 bp DNA amplicon derived from a dengue species to evaluate the potential use of seaweed agar as a substitute for commercial agarose.

RESULTS AND DISCUSSION

Optimisation was carried out to determine the optimum conditions of seaweed agar for use in DNA electrophoresis. The first was seaweed agar concentration optimisation, as shown in Figure 1.

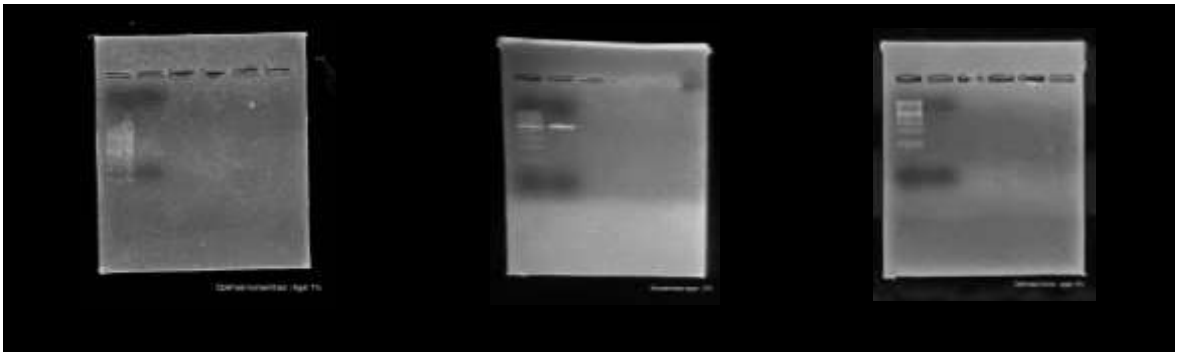

Figure 1. Visualization results of electrophoresis with 1% seaweed agar concentration (left), 2% seaweed agar concentration (middle), and 4% seaweed agar concentration (right)

Figure 1 shows that 2% seaweed agar concentration provided the best DNA visualisation results, with an intact single DNA band of 513 bp. In addition, the concentration of 2% seaweed agar was able to visualise a clear DNA Ladder separation and can be used to determine the exact size of the amplicon. At a concentration of 1%, the DNA band was not visible, and the DNA ladder did not separate well. At a concentration of 4%, the DNA band appeared very thin, making it difficult to identify.

The agar concentration is related to the density of the agar pores. The higher the agar concentration, the denser the pores of the DNA migration medium in electrophoresis, and vice versa. A lower agar concentration causes the pores to be too loose, causing the loss of DNA from the agar, and the results cannot be concluded (Sinaga, Agustina P. Putri and Kata Bangun, 2017). An agar concentration that is too high cannot visualise the DNA bands well, because the DNA amplicons do not migrate completely. Therefore, it can be concluded that 2% is the optimum concentration for DNA electrophoresis on seaweed agar.

TAE buffer concentration optimisation was performed by varying the buffer concentrations, namely, 1x, 2x, and 5x. Ethidium Bromide (EtBr) was dissolved in each buffer concentration (25 µL/liter buffer) as an intercalated dye that functions to bind DNA so that it can fluorescent under UV light, so it is called TAE-EtBr. EtBr binds by inserting between the base bonds in the DNA double strand (Sinaga, Agustina P. Putri and Kata Bangun, 2017).

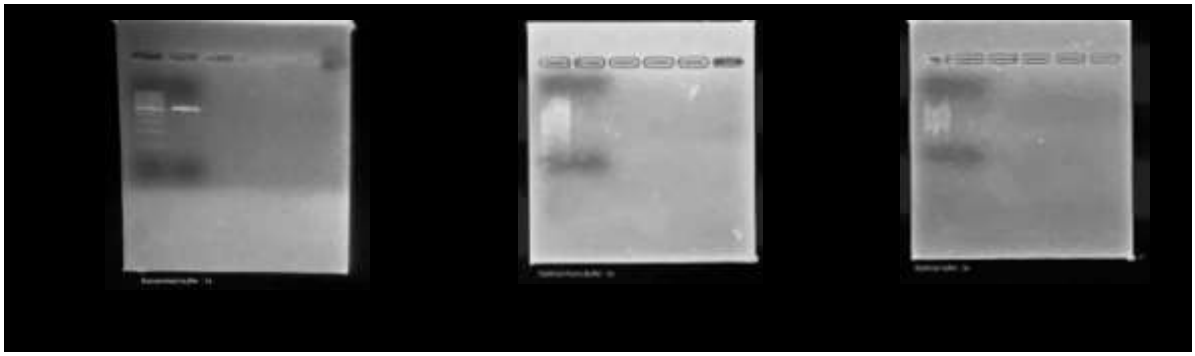

Figure 2. Electrophoresis visualization results with 1x buffer concentration (left), 2x concentration (middle), and 5x concentration (right)
Source: (personal documentation)

Based on the optimisation results using variations in the concentration of the TAE-EtBr buffer, the best results were obtained at a concentration of 1x which was indicated by the visualisation of the well-separated DNA ladder and non-smearing DNA band of 513 bp. This is inversely proportional to the results of electrophoretic visualisation in buffers with 2x and 5x concentrations, which visualise the DNA ladder poorly, and no corresponding DNA bands are found.

The buffer concentration is related to the ionic concentration in the buffer. A higher buffer concentration causes a decrease in ion resistance, such that the buffer has a high melting point. This makes it difficult for agarose to melt and homogenise in the buffer. Conversely, a low ion concentration in the buffer results in a higher resistance in the electrical circuit, which causes heating of the buffer (Sasagawa, 2021). A buffer concentration exceeding 1x shows the result of not separating the DNA ladder due to the too-high melting point of the buffer, which hinders the transmigration of DNA in the agar. Thus, it can be concluded that 1x buffer concentration is the optimum concentration of TAE-EtBr buffer for DNA gel electrophoresis using seaweed agar.

The amplicon volume was optimised by varying the ratio of the amplicon volume to the loading dye to 7:3, 10:3, and 12:3. Seaweed agar with 2% concentration and 1x TAE-EtBr concentration was used in this step.

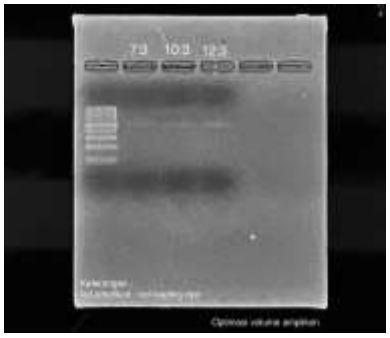

Figure 3. Electrophoresis visualization results of variation in amplicon volume
Source: (personal documentation)

Description:
column 1: 100 bp DNA Ladder
column 2: Sample with amplicons loading dye volume ratio 7:3
column 3: Sample with amplicons loading dye volume ratio 10:3
column 4: Sample with amplicons loading dye volume ratio 12:3

Figure 3 shows the clearest DNA bands obtained at a 12:3 amplicon-loading dye volume ratio. The higher the number of amplicons in DNA electrophoresis, the clearer the DNA band. Therefore, an amplicon volume of 12 µL was determined to be the optimum amount of amplicons to support DNA electrophoresis on seaweed agar.

Electrophoresis voltage optimisation was performed by varying the voltage to 70, 80, or 100 volts for 1 h. Seaweed agar at 2% concentration, 1x TAE-EtBr concentration was used in this step.

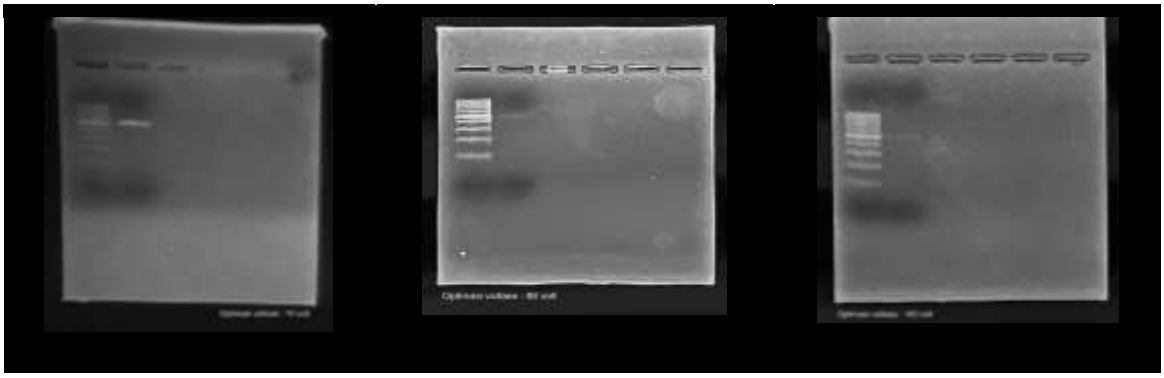

Figure 4. Visualization results of electrophoresis with voltage variations, namely 70 volts (left), 80 volts (middle), and 100 volts (right)
Source: (personal documentation)

The electrophoresis voltage is related to the rate of DNA migration in agar. The rate of DNA migration is proportional to the potential gradient (electrical voltage) and inversely proportional to the resistance (Sonargya and Dholariya, 2024). The greater the voltage, the faster the migration of DNA in the electrophoresis medium. Based on the test results, the best DNA ladder band separation was obtained at 80 V. This voltage shows a clear separation of the DNA ladder,

making it easier for readers to visually see the size of the DNA (in the picture above, the size of the DNA band was 513 bp). Therefore, it can be concluded that the best voltage used is 80 volts.

Based on the results above, electrophoresis was conducted again at the optimum conditions for seaweed agar: 2% agar concentration, 1x TAE-EtBr Buffer, 12:3 volume ratio, 80 volts for 1 h, and using a 100 bp DNA ladder. The amplicons used were those that were diluted to 50x and undiluted.

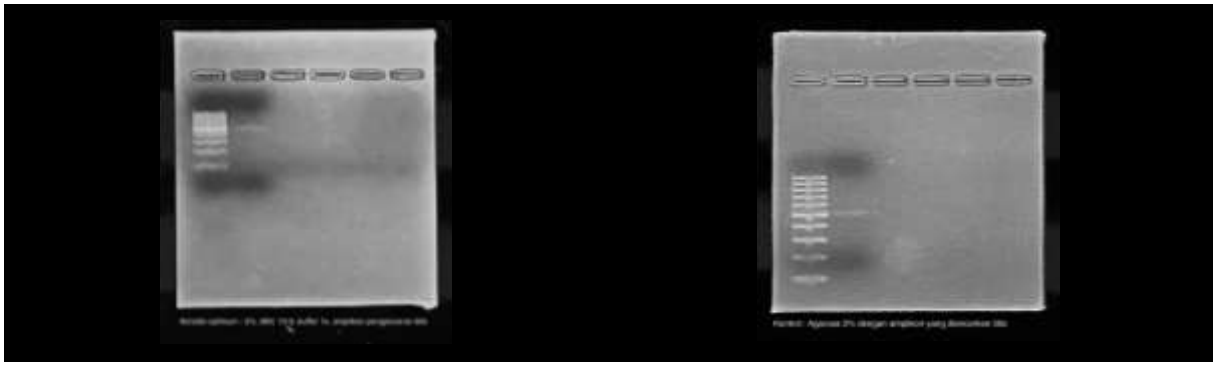

Figure 5. Electrophoresis visualisation results of optimum conditions using amplicon diluted 50x using seaweed agar (left) and agarose (right).
Source: (personal documentation)

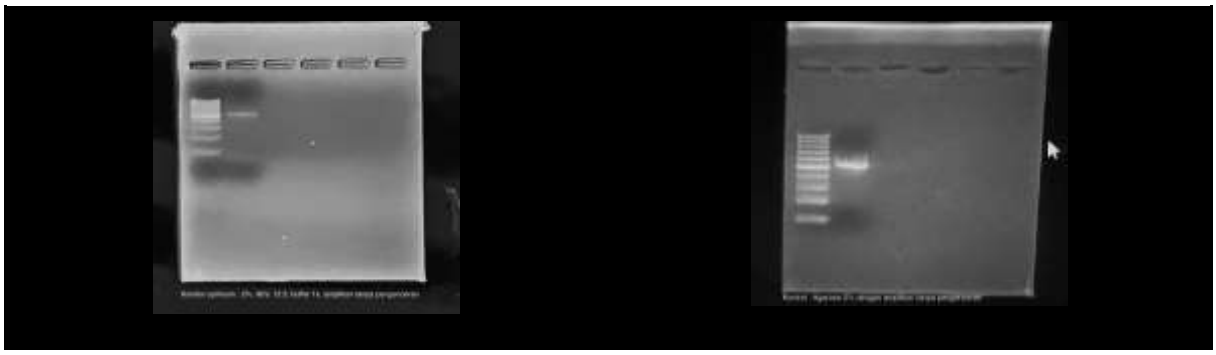

Figure 6. Electrophoresis visualisation results under optimum conditions using undiluted amplicons on seaweed agar (left) and agarose (right).
Source: (personal documentation)

Figure 6 shows that the amplicons were diluted or not diluted, giving DNA bands of 513 bp that were intact, clear, and did not smear. This shows that seaweed agar provides good visualisation results, even though there are only a few amplicons. The disadvantage of using seaweed agar for DNA electrophoresis is that the visualisation results displayed by seaweed agar are still less bright than those of agarose electrophoresis. However, seaweed agar can still be used as a substitute for agarose in DNA electrophoresis, at a limited cost.

Electrophoresis separates DNA based on its molecular size (Hikmatyar and Royani, 2015). To determine the size of DNA, the DNA Ladder was used as a comparison to determine the approximate size of DNA. The DNA ladder is important because good separation of DNA ladder bands can maximise the interpretation of DNA size results (Ziraldo et al., 2019). Therefore, electrophoresis of the DNA ladder was carried out using optimal conditions for seaweed agar to determine the detection limit of the DNA size. DNA ladders of 50 bp (meridian brand), 100 bp (Thermo Scientific brand), and 1 kb (Thermo Scientific brand) were used on seaweed agar under optimum conditions of 2% agar concentration, 1x TAE-EtBr buffer, 12:3 volume ratio, and 80 volts for 1 h.

Figure 4.7. Electrophoresis visualisation results using 100 bp, 50 bp, and 1 kb DNA Ladder using seaweed agar (left) and agarose (right).
Source: (personal documentation)

Electrophoresis results showed the separation of different DNA ladder band patterns. The DNA ladder band patterns were compared for each kit.

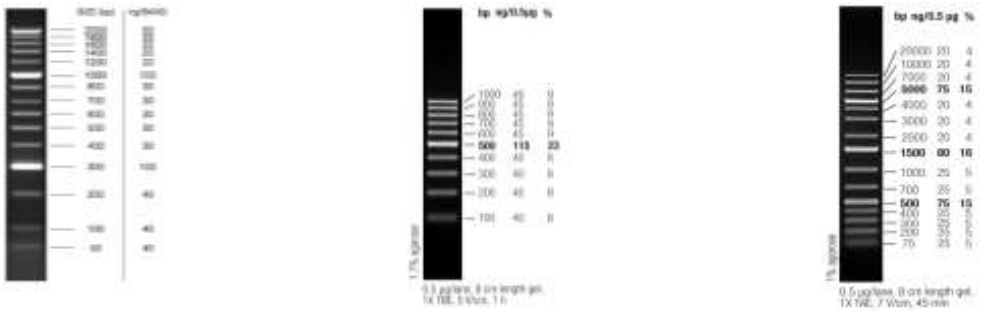

Figure 4.8. The band sizes on the DNA ladder were 50 bp (left), 100 bp (centre), and 1 kb (right) based on the kit.
Source: (Thermo Fisher Scientific Baltics UAB, 2016, 2019; Meridian Life Science Inc, 2024)

Based on the results of electrophoresis using seaweed agar, on a DNA ladder measuring 50 bp, the band was clearly separated to a size of approximately 500 bp. While the DNA ladder measured 100 bp, clearly separated bands up to a size of 600 bp, and at the 1 kb DNA ladder, at a size of 700 bp. This suggests that the use of seaweed agar as an electrophoresis medium is limited to DNA sizes below 700 bp.

Molecular-based examinations can be applied to the diagnosis of diseases caused by pathogenic infections as well as diseases caused by genetic disorders. Diseases caused by infections such as dengue haemorrhagic fever and genetic disorders such as thalassaemia. In the practice of medical laboratory examinations, the speed and accuracy of the examination results are important elements that must be met. Molecular-based examinations using PCR and electrophoresis could be a solution. Although the cost is high, the need for fast and accurate examination results is one of the reasons for the application of this method. The use of seaweed agar as a substitute for electrophoresis media is one of the efforts that aims to reduce operational costs for molecular-based examinations using conventional PCR methods, so that its application can be expanded in healthcare facilities.

CONCLUSION

It was concluded that seaweed agar can be used as a substitute for agarose gel in DNA electrophoresis under optimum conditions, such as using 2% concentration of seaweed agar (Swallow brand plain agar), using 1x of TAE-EtBr buffer, using 80 volts for 1 h, and using 12 µl amplicons to be loaded in the agar well. The size of DNA that can be detected using seaweed agar as an electrophoresis medium is limited to DNA sizes below 700 bp.

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