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Searching for the best agarose candidate from genus *Gracilaria*, *Eucheuma*, *Gelidium* and local brands

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ABSTRACT

Objective: To explore the potential of local agar of genus *Gracilaria*, *Eucheuma*, *Gelidium* and local brands as an alternative for imported agarose for DNA electrophoresis, and to examine their ability related to separation and migration of DNA fragments in DNA electrophoresis.

Methods: Their performance at various concentrations were compared via an experimental study with a specific brand of imported commercial agarose used in molecular biology research. The measured variables were separation and migration during electrophoresis of a DNA fragment.

Results: The local agar genus *Gracilaria gigas*, *Gelidium*, brand "B" and brand "S" could separate DNA fragments at a concentration between 1% and 2%, with an optimum concentration of 2% w/v, as good as a specific brand of imported commercial agarose.

Conclusions: Their performance were very close to that of commercial agarose and can still be improved by further agar purification as well as by pH and sulfur control.

1. Introduction

Major advances in molecular biology characterization cannot be separated from the availability of polysaccharides from seaweed or algae [1]. Most DNA separation and analysis require agarose gel electrophoresis [2,3]. Problems arising in this method include poor accessibility and high costs, mainly in the case of less developed and developing countries, considering the fact that most of the materials are imported. This problem needs to be considered in the majority of developing countries with regard to confirming diagnoses. One of the imported materials is agarose, a polysaccharide polymer extracted from seaweed that is frequently used in biochemistry for gel electrophoresis [3]. A considerable amount of literature has been published on agarose gel electrophoresis. These studies have shown that agarose gel electrophoresis is the most effective way to separate DNA fragments [3–5].

Indonesia, which is considered the seaweed producer of the world, produces tons of various types of seaweed [6]. The centers of seaweed production spread from the east to the west of Indonesia in order to supply the world demand. For instance, data in 2010 stated that Indonesian seaweed production on genus *Gracilaria* and *Eucheuma cottonii* (*E. cottonii*) was around 3 082 112 tons [7]. These genus have the potential to be used in DNA separation, but have not yet been explored.

The objective of this research was to examine the separation and migration capacity of DNA fragment by using the local agar of genus *Gracilaria*, *Eucheuma*, *Gelidium* and local brands. Later, those properties are compared to a specific brand of imported commercial agarose. This research was aimed at an

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exploration of a new agarose candidate for gel electrophoresis by using the local agar from Indonesian seaweed.

2. Materials and methods

The agarose materials used in this research were: (1) agarose electrophoresis grade, obtained from MP Biomedicals, California, United States of America with catalog No. 820721. It was used as a control. This white agarose powder was ordered from an international supplier, and further information about this product was available at www.mpbio.com; (2) local produced agarose materials, including: a. agar powder, consisting of genus *Gracilaria gigas* (*G. gigas*), *Gelidium*, *Eucheuma spinosum* and *E. cottonii*. The powder used was still under research and development and had not been commercialized as of the time of this writing; b. rods agar, consisting of genus *Gelidium*; c. commercial agar of brands "S, B, C and N"; d. agar sheets, consisting of *Nico nori*, *Nori* and *Seaweed nori*.

Both a and b agars were obtained from a local seaweed factory, located in Surabaya, Indonesia, and the genus was verified by the quality control division of the factory, while c and d agars were obtained from a local market in Surabaya, Indonesia.

Agar powder was purified by heating and centrifugation. The solvents used consisting of a Tris Borate EDTA buffer (TBE 0.5×) prepared by diluting 5× TBE. Each different concentration of agar (0.5%, 1.0%, 1.5% and 2.0%) was heated and then transferred into a centrifuge tube for 5 min at 3 000 r/min. By this way, particles with high molecular weights were expected in the bottom of centrifuge tube. After cooling, the agar was readily formed and was used for the next step. A third-quarter part of the top freeze agar was collected and heated in a microwave for 1 min. About 20 mL of it was later mixed with ethidium bromide of 2 μL and molded into a gel tray. It was ready to use for electrophoresis after gel freezes. This research was conducted at the molecular biology laboratory, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia.

3. Results

Table 1 shows the summary of the performance of the different tested agars. Here, we conducted a visual assessment

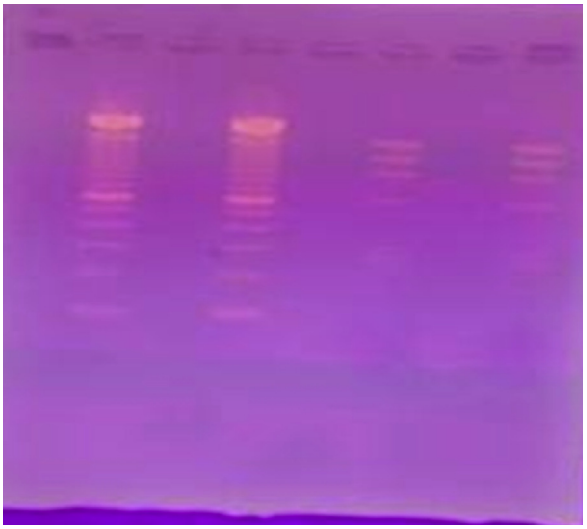

Figure 1. DNA electrophoresis on agar powder from *G. gigas* at 2% w/v with DNA marker 100 bp (sequences 1, 2 from left) and ØX 174-Hae III Digest (sequences 3, 4 from left).

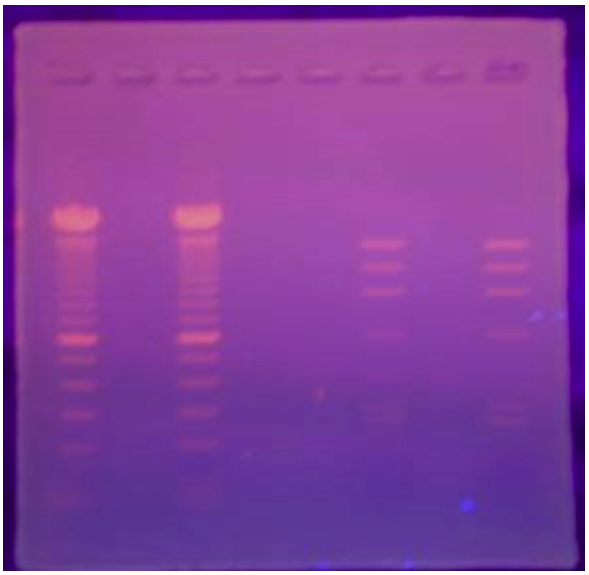

Figure 2. DNA electrophoresis on agar powder from genus *Gelidium* (2% w/v) with DNA marker 100 bp (sequences 1, 2 from left) and ØX 174-Hae III Digest (sequences 3, 4 from left).

Table 1
Gel strength of the tested agars in experiment.

State	Percentage (%)	Agar powder				Rod agar	Commercial agar				Agar sheet		
		G. gigas	*Gelidium*	*Eucheuma spinosum*	*E. cottonii*	*Gelidium*	Brand B	Brand S	Brand C	Brand N	*Nico nori*	*Nori*	*Seaweed nori*
After gelling	0.5	+ +	+ +	−	−	−	+ +	−	± ±	± ±	−	−	−
After ELP run		+ +	+ +				+ +						
After gelling	1.0	+ +	+ +	−	−	−	+ +	+ +	± ±	± ±	−	−	−
After ELP run		+ +	+ +				+ +	+ +					
After gelling	1.5	+ +	+ +	−	± ±	−	+ +	+ +	± ±	± ±	−	−	−
After ELP run		+ +	+ +		−		+ +	+ +					
After gelling	2.0	+ +	+ +	+ +	+ +	± ±	+ +	+ +	± ±	± ±	−	−	−
After ELP run		+ +	+ +	−	−	−	+ +	+ +					

ELP: Electrophoresis; −: Liquid; ±: Gel and liquid; +: Gel.

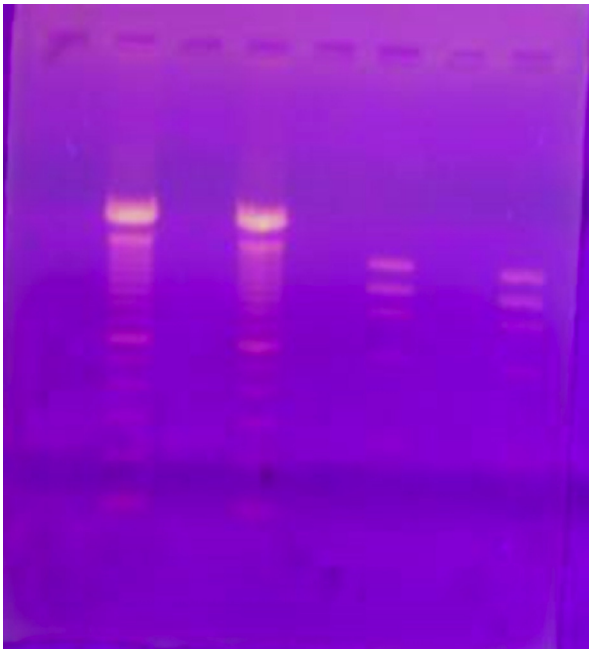

Figure 3. DNA electrophoresis on agar from market brand B (1% w/v) with DNA marker 100 bp (sequences 1, 2 from left) and ØX 174-Hae III Digest (sequences 3, 4 from left).

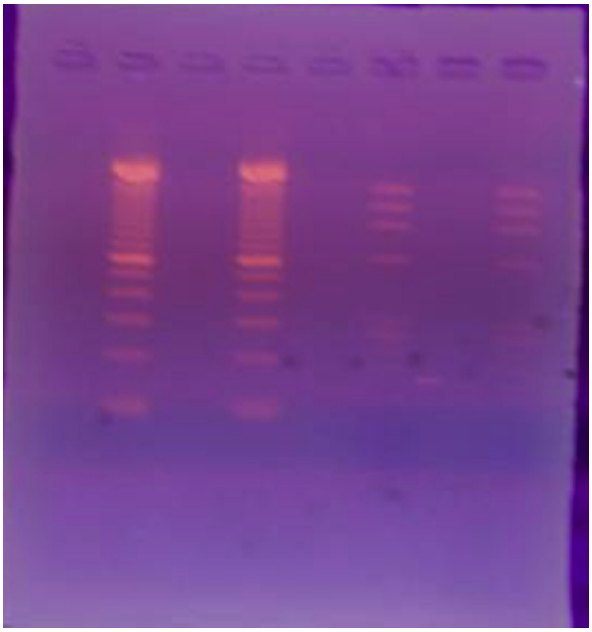

Figure 4. DNA electrophoresis on agar from market brand S (2% w/v) with DNA marker 100 bp (sequences 1, 2 from left) and ØX 174-Hae III Digest (sequences 3, 4 from left).

and expert judgment was provided by a second author and was confirmed by another author and a laboratory assistant. As depicted in Table 1, only agar powder of genus *G. gigas*, *Gelidium* and market agar from brand "S" and brand "B" showed gel strength. In addition, the gel was colorless and could be observed easily whereas, others exhibited less gel strength and the gel was not clear and transparent enough in color, which eventually caused difficulty in regard to observation. The highest quality selected agar was used for electrophoresis and was compared with a specific brand of imported commercial agarose as a control.

3.1. G. gigas

Electrophoresis result was generated from agar powder of genus *G. gigas* by using DNA 100 bp and ØX 174-Hae III Digest markers (Figure 1).

3.2. Gelidium

Electrophoresis result came from agar powder genus of *Gelidium* using DNA 100 bp and ØX 174-Hae III Digest markers (Figure 2).

3.3. Agar brand "B"

Electrophoresis result was generated from agar brand "B" by using DNA 100 bp and ØX 174-Hae III Digest markers (Figure 3).

3.4. Agar brand "S"

Electrophoresis result was generated from agar brand "S" by using DNA 100 bp and ØX 174-Hae III Digest markers (Figure 4).

From the above selected agars, we conducted a proximate analysis. Proximate analysis included analysis of moisture, ash, crude protein, crude lipid, crude fiber, carbohydrates, nitrogen free extract and metabolic energy, as shown in Table 2.

Comparation results in electrophoresis of the imported agarose and local agar based on migration and resolution are shown in Figures 5–8.

Table 2
Results of proximate analysis (%).

Selected agar	Moisture	Ash	Crude protein	Crude lipid	Crude fiber	Carbohydrate	Nitrogen free extract	Metabolic energy (Kcal/kg)
Genus *Gelidium*	89.9965	2.1739	2.1321	0.8837	1.1922	84.8070	83.6146	3213.17
Genus *Gracilaria*	94.9097	1.0945	1.6811	0.8041	0.1558	91.3300	91.1742	3467.99
Agar of brand S	97.9158	1.2966	1.2572	0.8245	0.1325	94.5380	94.4050	3574.12
Agar of brand B	87.4816	1.0000	2.1110	0.7748	0.1204	83.5960	83.4754	3196.94

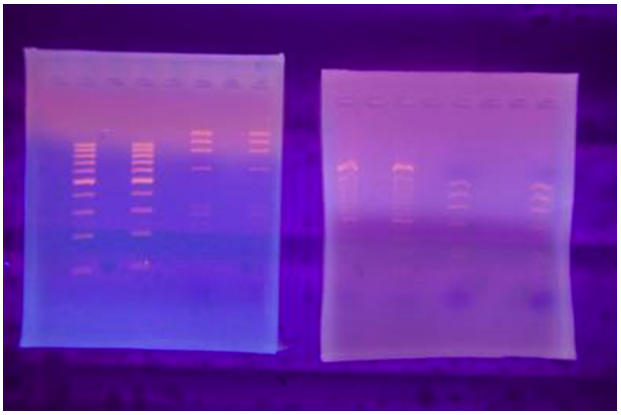

Figure 5. Agar of local genus *G. gigas* (left) and imported agarose (right).

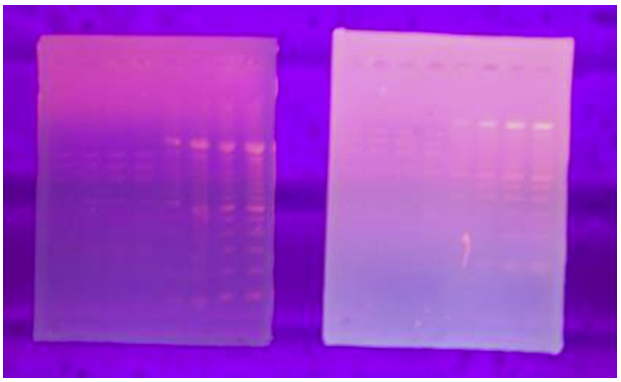

Figure 6. Agar of local genus *Gelidium* (left) and imported agarose (right).

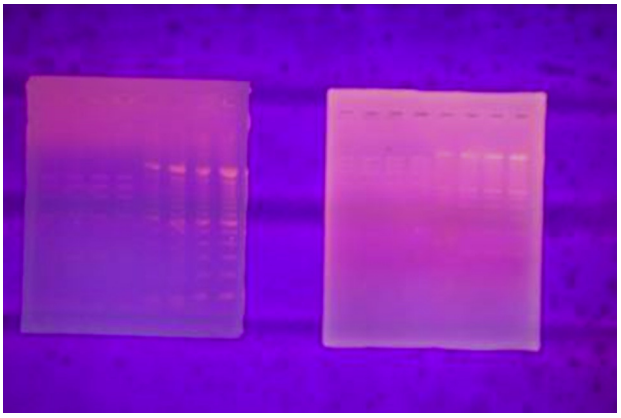

Figure 7. Agar of market brand B (left) and imported agarose (right).

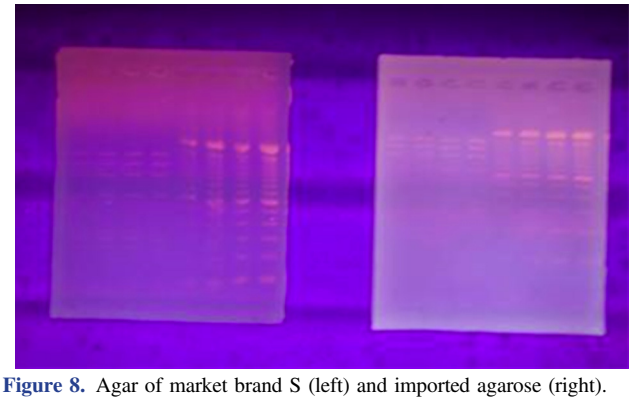

Figure 8. Agar of market brand S (left) and imported agarose (right).

4. Discussion

As shown in Figure 5, both imported and local agar genus *Gracilaria* could separate very well and migrate DNA. However, the resolution of the imported agarose was brighter and sharper when compared with *G. gigas* in regard to both markers (100 bp and ØX 174-Hae III Digest). This condition can also be seen in Figures 6–8. Local agarose separated and migrated the DNA fragment, but the resolution was not very sharp. In general, the results showed that local agar could be a good agarose alternative despite the fact that its separation ability and separation resolution were not very sharp. Within the tested concentration range, local agar could separate the DNA fragments at all concentrations, and the best separation and resolution were obtained at concentrations of 1%–2%. Some of the shortcomings of the market agars mainly lay in the separation of DNA fragments when they were used at a low concentration (0.5%). In general, at a concentration of 2%, the separation of DNA fragments was found to be better than at another three lower concentrations. There were some factors affecting the separation and migration ability of the DNA fragments, which included agarose concentrations, the size of DNA fragment, the voltage applied, the type of agarose, the presence of ethidium bromide and the electrophoresis buffer [4]. The effects of concentrations on the electrophoresis results have been documented in other studies, where it has been commonly shown that the concentration is dependent on the DNA molecular weight, which varies from 0.5% to 1.5% [8]. However, general concentrations have been reported at 1% for many applications [9]. In this study, we found that at concentrations between 1% and 2%, the local agar exhibited the capability to run and migrate the DNA fragments with 100 bp and ØX 174-Hae III Digest markers.

The cause of differences in the electrophoresis results may have been the presence of undesirable components in the local agarose that affected the separation of fragments, such as preservatives and dyes. In addition, the sulfur content and pH of each local agarose were not controlled in this study, which may have significantly affected the results. It should be noted that the electrophoresis results were affected by pH and denaturants that were present in the medium [10]. The proximate analysis showed that the selected agar contained crude proteins ranging from 1.3% to 2.1% (Table 2).

This study describes the quality of DNA fragment separation by using a local agar and shows the potential of some local agars to substitute for imported agarose. These local agars offer substantial advantages, especially in terms of low cost and easy accessibility. The quality of separation and the separation resolution from these agars can still be improved by controlling sulfur content, pH and of course the presence of certain undesirable materials. These factors will be explored further in the future. The local agar of genus *G. gigas* as well as *Gelidium*, brand "B" and brand "S" could separate DNA fragments and migrate well by using 100 bp and ØX 174-Hae III Digest markers. Better visible resolution was observed in the aforementioned agars at a concentration between 1% and 2% and at an optimum concentration of 2% w/v. Further research on local agar characterization, standard purification techniques and other factors affecting agarose resolution is still necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

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