Agar properties of *Gracilaria* species (Gracilariaceae, Rhodophyta) collected from different natural habitats in Malaysia

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Abstract

The yield and quality of agar from *Gracilaria* species collected from distinct natural habitats (mangrove swamp, rocky shore, sandy mudflat) along the west coast of Peninsular Malaysia were evaluated in this study. The agar content was found to be significantly higher in *G. changii* and *G. edulis* growing in the mangrove swamp, while the lowest agar content was recorded for *G. changii* and *G. edulis* collected from the sandy mudflat. Higher agar gel strength was obtained from the three *Gracilaria* species collected from the mangrove swamp compared to those that live in the sandy mudflat and rocky shore. The intraspecific variations found in gelling temperature were well correlated with the trend of changes in agar gel strength, except for *G. changii* collected from the sandy mudflat and rocky shore. The intraspecific and interspecific variations of agar melting temperature did not show a consistent trend for all *Gracilaria* species tested. The agars of *Gracilaria* spp. collected from the rocky shore showed a significantly higher gel syneresis while the lowest gel syneresis was recorded for the agars of samples collected from the mangrove swamp, except for the agars of

G. salicornia from different habitats which showed no difference. In conclusion, the mangrove swamp is a natural habitat which produces *Gracilaria* with good agar properties, in terms of agar yield, gel strength and gel hysteresis, thus it can be considered as a potential site for seaweed farming and mariculture for the agar industry in Malaysia.

Keywords: agar, agar yield, gel strength, *Gracilaria*, habita

1. Introduction

Gracilaria spp. are red seaweeds (Rhodophyta) which produce phycocolloid agar, a gelatinous polysaccharide complex with high commercial importance in biotechnology, and the cosmetics, medical, pharmaceutical and food industries (Marinho-Soriano, 2001). They account for more than 50 % of global agar production, replacing *Gelidium* agar which has been gradually depleted due to over-exploitation (McHugh, 2003). *Gracilaria* spp. are cultivated worldwide on a commercial scale, with Chile and Indonesia contributing to about 38 % of agar in the current market (Bixler and Porse, 2011).

Agar is composed of repeating agarobiose units of alternating 1, 3-linked-D-galactose and 1, 4-linked-3, 6-anhydro-L-galactose residues (Rebello *et al.*, 1997). Methoxyl, sulfate esters and pyruvate ketal groups usually occur in various combinations or positions on the disaccharide chains (Rees, 1969; Duckworth and Yaphe, 1971). *Gracilaria* agars are known to have a higher level of sulfate substitution, which results in weak gels with lower gel strengths (Murano, 1995). However, this can be overcome by desulfation of agar with sodium hydroxide (Freile-Pelegrin and Murano, 2005) or enzymatic treatment with sulfohydrolase (Shukla *et al.*, 2011).

In Malaysia, 20 agarophytic *Gracilaria* species have been recorded, with many of them growing at mangrove, mud, rock, sand, coral and as driftweed (Lim and Phang, 2004; Phang, 2006; Yow *et al.*, 2013). The types of habitats and environmental conditions may affect the populations of these seaweeds and their agar production. Biomass, agar content and quality of seaweeds can be affected by environmental parameters such as nutrient supply, water temperature, water salinity, light condition, photoperiod, and motion of water in the natural habitats (MacIer and Zupan, 1991). However, there is no published record for the agar properties from *Gracilaria* species grown under different habitat backgrounds.

In this study, three common agarophytic species, *Gracilaria changii* (Xia et Abbott) Abbott, Zhang et Xia, *Gracilaria salicornia* (C. Agardh) Dawson and *Gracilaria edulis* (S.G. Gmelin) P.C. Silva were collected from three locations in Peninsular Malaysia with different habitats and their agar properties were evaluated. The information of the agar characteristics of *Gracilaria* species from different habitats could be useful for large scale industrial exploitation and cultivation of algal material for agar industry.

2. Materials and Methods

2.1. Collection of algal samples

Fresh and healthy *Gracilaria* samples were collected from natural populations at three locations along the west coast of Peninsular Malaysia between June-July 2013. The geographical locations of sampling sites, type of habitats, date of collection and collected samples are summarised in Table 1. All seaweed materials were collected during low tides. They were found at the water-logged area and attached to the hard substrata in the natural habitats. Salinity, pH and temperature of the seawater at the collection site were measured with a salinity refractometer (S/Mill-E, Atago, Japan), pH meter (Crison Instruments, SA, Spain) and temperature meter (HI 8424, Hanna Instruments Inc., Romania), respectively. Dissolved oxygen (DO) and nutrient levels of seawater were provided by the Department of Environment (DOE) Malaysia. The data were measured at their monitoring stations located within 700 m away from the sampling sites.

The macroalgae with cystocarpic structures were found in low abundance or absent in some habitats at the time of collection, thus they were excluded in this study. The collected seaweeds -were placed into a plastic bag containing seawater, covered with towel, stored in a polystyrene box filled with ice, and transported back to laboratory. These algal samples were manually cleaned to remove rocks, sand, mud and epiphytes before they were rinsed with distilled water. The seaweeds were dried under the sun to remove excessive water followed by oven drying at 60 °C until the weight of the seaweeds was constant. The dried *Gracilaria* samples collected from sandy mudflat and mangrove swamp were divided into triplicates, while *Gracilaria* samples from the rocky shore were sufficient for only one replicate.

2.2. Agar extraction

Extraction of native agar was done by modifying the extraction method described by Phang *et al.* (1996). Firstly, dried *Gracilaria* thalli were hydrated with distilled water (in a ratio of 1:50 w/v) at pH 7 in 1 L conical flasks and autoclaved at 103 kPa for 15 min. The hot algal extracts was pre-filtered through gauze before filtered with a vacuum pump and polysulfone filter set (Nalgene, Rochester, NY) equipped with a glass microfiber filter discs (Sactorius Stedim Biotech, Grade MGC, Goettingen, Germany). The filtrate was cooled, allowed to solidify in trays at room temperature and frozen at -20 °C for 16 hours. The frozen agar was thawed and impurities were discarded. The agar was washed with 85 % (v/v) and 99 % (v/v) isopropanol, respectively, before it was oven-dried at 50 °C to a constant weight. The agar yield was expressed as the average percentage of agar dry weight from replicates of *Gracilaria* samples.

2.3. Measurement of agar gel strength

A 1.5 % (w/v) agar gel with a gel depth of approximately 15-20 mm was made in a beaker, covered with aluminium foil and left for overnight at room temperature. The maximum weight needed to break the agar gel was determined by using a texture analyser (TA-XT2, Stable Micro Systems Ltd., Surrey, UK) fitted with a 10 mm diameter cylindrical plunger (P/10 CYL Delrin probe) and a 5000 g load cell, operated with a penetration speed of 1 mm s⁻¹ for a distance of 5 mm. Gel strength was calculated and expressed in g cm⁻².

Measurement of gelling and melting temperatures

Gelling temperature was measured by preparing 10 ml of hot 1.5 % (w/v) agar solution in a 50 ml test tube which was allowed to cool at a rate of 0.5-1 °C min⁻¹. A thermometer was placed into the hot solution and the test tube was slanted by 45° before it was returned to a vertical position for every 0.5 °C drop in temperature. The gelling temperature was recorded when the meniscus of the agar solution failed to return to its initial position. To determine the melting temperature, 10 ml of 1.5 % (w/v) agar gel was prepared in a 50 ml test tube, covered with aluminium foil and kept for overnight at room temperature. A 5 mm glass bead was placed on the gel surface. The test tube was placed in a bath with its temperature increased gradually from 60 °C to 100 °C (with a rate of 1 °C min⁻¹). The melting temperature was recorded when the glass bead dropped to the bottom of the test tube.

2.4. Determination of gel syneresis

Gel syneresis index (%) was measured with a modified method from Villanueva *et al.* (2010). Twenty milliliter of 1.5 % (w/v) gel was prepared in a 100 ml beaker and kept for overnight at room temperature. The initial weight of the agar gel was recorded before it was placed on a Whatman (No. 1) filter paper (Whatman International Ltd, Maidstone, UK) for two hours at room temperature. The final weight of the gels was measured to determine the amount of exudates lost from the agar hydrogel. The syneresis index of the agar gel samples was calculated as the percentage of loss in gel weight.

2.5. Statistical analysis

Statistical analysis was performed using SAS statistical programme Version 9.3 (SAS Institute Inc., Cary, North Carolina, USA). A two-way analysis of variance (ANOVA) was performed to determine the species effects and habitat effects on agar properties. Duncan's Multiple Range Test (DMRT) was carried out at $P < 0.05$ as a post hoc analysis to determine statistical significant interspecies variation per habitat and intraspecies variation between habitats, separately.

3. Results

The three locations along the west coast of Peninsular Malaysia were visited within a period of less than 30 days (between June and July 2013) for seaweed collection, to minimize the seasonal variations such as amount of rainfall, day length and effect of monsoon winds.

Salinity, pH, temperature and nutrient levels of seawater from all collection sites were shown in Table 1. The DO levels were the highest and the lowest at rocky shore (7.45 mg/L) and mangrove swamp (5.89 mg/L), respectively, while the nutrient levels were comparable for all habitats except for rocky shore with a higher nitrite level (1.9 mg/L). Between June-July, a high abundance of seaweed was observed for the three *Gracilaria* species from the mangrove swamp but a low and limited algal population was found at the rocky shore. Hence, the samples from the rocky shore were only sufficient for one biological replicate in this study.

Species and habitat effects, and their interactions on agar yield were statistically significant ($P < 0.01$) in this study (Table 2). The agar yields of the *Gracilaria* species differ interspecifically at the rocky shore and mangrove swamp (with significant difference ($P < 0.05$))

between species from the mangrove swamp) but no significant difference ($P < 0.05$) was found between the agar yields of *G. changii* and *G. edulis* from the sandy mudflat (Fig. 1A). *G. edulis* collected from the rocky shore and mangrove swamp had the highest agar yield (23.11-28.37 %), followed by *G. changii* (18.77-22.56 %) and the lowest agar yield was recorded for *G. salicornia* (13.63-13.94 %). The agar yields of the *Gracilaria* species were found to vary intraspecifically among different types of natural habitats, except for *G. salicornia* samples from both mangrove swamp (13.63 ± 0.86 %) and rocky shore (13.94 %) which did not show any notable difference in their agar contents (Fig. 1A). *G. edulis* and *G. changii* from the mangrove swamp have a significantly higher ($P < 0.05$) agar content (28.37 ± 0.12 and 22.56 ± 0.59 %, respectively) compared to the sandy mudflat (18.77 ± 0.93 and 19.88 ± 0.65 %, respectively) (Table 3). The *G. edulis* and *G. changii* samples collected from the rocky shore have a slightly higher agar yields (23.11 and 20.50 %, respectively) than those of samples collected from the sandy mudflat (19.88 ± 0.65 and 18.77 ± 0.93 %, respectively).

At the same habitats, consistent species effects on agar gel strength were observed and were statistically significant ($P < 0.01$), with *G. changii* and *G. edulis* having the highest (205.85-360.33 g/cm²) and lowest agar gel strength (78.05-166.66 g/cm²), respectively (Fig. 1B, Table 2). All *Gracilaria* species collected from the mangrove swamp showed a significantly higher ($P < 0.05$) agar gel strength compared to those of samples collected from other habitats (Fig. 1B, Table 3). *G. edulis* collected from the mangrove swamp had the highest agar gel strength (166.66 ± 8.62 g/cm²), followed by the sandy mudflat (93.15 ± 11.31 g/cm²) and rocky shore samples (78.05 g/cm²), respectively. *G. changii* from the mangrove swamp has the highest gel strength (360.33 ± 22.46 g/cm²), followed by samples collected from the rocky shore (283.51 g/cm²) while the lowest gel strength was from sample growing on sandy mudflats (205.85 g/cm²). *G. salicornia* collected from the mangrove swamp had a lower agar gel strength (270.50 ± 23.66 g/cm²) than those of *G. salicornia* collected from the rocky shore (215.98 g/cm²).

The types of habitat and species were found to cause intraspecific variations in gelling and melting temperatures of *Gracilaria* agars but the interaction between these two factors on agar gelling temperature was insignificant (Fig. 2, Table 2). Consistently, all three *Gracilaria* species collected from the mangrove swamp have a higher gelling temperature while the lowest gelling temperature was recorded for samples collected from the rocky shore. The pattern of variation in gelling temperature for *Gracilaria* species was similar with that in agar

gel strength, except for *G. changii* collected from sandy mudflat which has a lower gel strength, but had a higher gelling temperature than those of *G. changii* collected from the rocky shore. Among the species, the agars of *G. changii* collected from all habitats had a lower gelling temperature (27-32 °C), compared to that of *G. edulis* agar samples which had an intermediate gelling temperature (36-40 °C) and *G. salicornia* agar samples which are viscous, sticky, set at a higher temperature (39-43 °C) to form solid gel.

Intraspecific variation of agar melting temperature was not consistent for all three *Gracilaria* species in this study (Fig. 2B). *G. edulis* grown in the mangrove swamp showed a significantly higher ($P<0.05$) melting temperature (92.67 ± 0.29 °C) compared to that of *G. edulis* from sandy mudflat (81.50 ± 0.50 °C) (Table 3), while samples collected from the rocky shore had a slightly higher melting temperature (83.00 °C) than those of samples collected from the sandy mudflat. However, *G. changii* recorded the highest melting temperature for samples grown on sandy mudflat (91.33 ± 0.76 °C) and the lowest melting temperature for the sample collected from the mangrove swamp (88.17 ± 0.58 °C). No remarkable difference can be found for agar melting temperatures between the *G. salicornia* samples harvested from both rocky shore (93.50 °C) and mangrove swamp (94.05 ± 0.50 °C). Among these agarophytes, *G. edulis* has the lowest agar melting temperature which was below 85 °C except for *G. edulis* collected from the mangrove swamp, whereas *G. changii* and *G. salicornia* have a higher agar melting temperature, in the range of 88-91 °C and 93-94 °C, respectively.

The agar hydrogel of *G. edulis* and *G. changii* samples collected from the rocky shore demonstrated the highest gel syneresis (13.64 and 13.09 %, respectively) while the lowest gel syneresis was from the respective species grown at mangrove swamp (9.55 ± 0.85 and 10.69 ± 0.25 %, respectively) (Fig. 3). The agar of *G. salicornia* grown in the mangrove swamp had a slightly higher syneresis index (14.30 ± 0.71 %) than that of samples collected from the rocky shore (13.55 %). No clear interspecific variation for agar gel syneresis was observed for *Gracilaria* samples from both rocky shore and sandy mudflat. However, *G. salicornia* at the mangrove swamp had a significantly higher ($P<0.05$) agar gel syneresis than *G. changii* and *G. edulis*.

4. Discussion

Our study revealed that the agar content of the *Gracilaria* samples was dependent on the type of habitats along the west coast of Peninsular Malaysia. The highest agar contents in this study were recorded for *G. changii* and *G. edulis* grown in the mangrove swamp. Israel *et al.* (1999) showed that *G. tenuistipitata* var. *liui* cultured at pH 7.0 had a higher carbon dioxide concentration (222.4 μM) compared to those cultured at pH 8.0 (14.3 μM), with water salinity remained constant at 30 ppt. Mangroves have a slightly lower pH (pH 7.64) compared to other habitats (pH 8.02-8.04), which might correspond to a higher carbon dioxide concentration. The growth rates of seaweeds could be higher in mangroves which are a sink of sediments and nutrients, with a lower pH and DO, and result in better agar production than those that inhabit rocky or sandy areas which are dependent on nutrients and minerals from the seawater (Krishna Prasad and Ramanathan, 2008).

Based on our field observations, *G. changii* and *G. edulis* which are attached to the roots of *Avicennia* spp. were sheltered from strong light and had thalli with a smaller diameter, which was approximately 15-30 % thinner than that of samples collected from other habitats. Seaweeds are well-known for their high morphological plasticity, with the same species having different morphological features when grown under different environmental conditions. Boardman (1977) reported that seaweeds may have thinner thalli as a light-shade adaptation strategy to reduce the non-photosynthesizing tissue and to capture maximum sunlight radiation. It is not known whether thinner thallus will increase the photosynthetic efficiency and channel more sugar precursor into agar biosynthesis pathway. However, *G. salicornia* collected from different habitats do not have significant variation in their thallus size.

A higher agar gel strength was recorded for *Gracilaria* spp. growing in the mangrove swamp compared to other habitats. Chandramohan *et al.* (1974) showed that the arylsulfatase activity originating from arylsulfatase producing bacteria was significantly higher in mangroves (24.30-32.61 μg phenolphthalein/g) compared to that in the sea (1.10-15.51 μg phenolphthalein/g) and estuarine (2.00-5.30 μg phenolphthalein/g) habitat. Dhevendaran *et al.* (1985) also found that the arylsulfatase activity in mangrove sediment samples was 2-fold higher than that of samples from backish water and estuarine areas. It is unknown whether the exogenous arylsulfatase released in the soil, or symbiotically attached to the seaweeds, or endogenously produced seaweed sulfohydrolase could act on the sulfated polysaccharides in

Gracilaria spp. by hydrolyzing the sulfate esters and subsequently improving the agar gel strength.

A higher gel strength in *Gracilaria* samples was associated with a higher gelling temperature, except for *G. changii* collected from the sandy mudflat and rocky shore. Both gelling temperature and gel strength are indicative of agar quality, and they are mainly dependent on the chemical structure of the agar polysaccharides. The major factor which affects the gelling temperature is the degree of substitution with methoxyl groups on agar polysaccharide chain (Guiseley, 1970) while the amount of sulfate substitution and its position were also suggested to inhibit or delay the gelling mechanism (Lahaye and Rochas, 1991). Although the sulfate and methoxyl contents are not measured in this study, the increase in gelling temperature and gel strength in *Gracilaria* samples collected at mangrove swamp could be due to a higher amount of methoxyl group and lower sulfate substitution in the agar, respectively (Praiboon *et al.*, 2006).

The melting temperature of agar was known to be positively correlated with its molecular weight, with higher agar molecular weight increases the stable interactions between agar polymer structures, thus a higher temperature is required to melt the agar gel (Rodriguez *et al.*, 2009). Although Freile-Pelegrin and Robledo (1997) proposed that there is a positive correlation between melting temperature and gel strength, our results did not support their findings. However, seaweed source, extraction method and agar purity may affect the melting properties of agar gel (Gertrudes, 1990). All *G. changii* and *G. salicornia* samples had a melting temperature higher than 85 °C, which fulfill the requirement of U.S. Pharmacopeia (USP) standards for agar.

Gel syneresis index measures the water holding capacity of agar hydrogel upon aging and reflects the overall gel matrix stability. In this study, *G. changii* and *G. edulis* collected from the rocky shore showed the lowest gel stability with the highest syneresis index while those collected from the mangrove swamp have the lowest syneresis index and a stable agar hydrogel. There is limited knowledge on which and how the chemical substituents of agar affect the gel syneresis. Hence, a sensitive chemical and structural analysis using Nuclear Magnetic Resonance (NMR) spectroscopy can be carried out in the future to study the relationship between gel syneresis and chemical composition.

5. Conclusions

In conclusion, intraspecific variation in agar properties of *Gracilaria* species collected from different natural habitats, reflect the significant effects of different geological and environmental factors on agar polysaccharides. Our results also confirmed previous findings (Hoyle, 1978; Marinho-Soriano, 2001) that the agar gel strength was species dependent, whereby different *Gracilaria* species collected from the same natural site have different agar gel strength. *G. changii* had a significantly higher agar yield and gel strength compared to that of *G. salicornia*, which is consistent with previous reports (Siow *et al.*, 2012; Lee *et al.*, 2014). All in all, our finding suggested that native agar from *G. changii* collected from the mangrove swamp had a good gel strength which is equivalent to the first grade food agar set by Japanese Specifications for Processed Agar (JSPA). Hence, the mangrove swamp may have great potential to be an aquaculture cultivation site of for *Gracilaria* spp., for higher yield, gel strength and gel hysteresis of agars.

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References

- Bixler, H.J., Porse, H., 2011. A decade of change in the seaweed hydrocolloids industry. J. Appl. Phycol. 23, 321-335.
- Boardman, N.K., 1977. Comparative photosynthesis of sun and shade plants. Annu. Rev. Plant Physiol. 28, 355-377.
- Chandramohan, D., Devendran, K., Natarajan, R., 1974. Arylsulfatase activity in marine sediments. Mar. Biol. 27, 89-92.
- Dhevendaran, K., Chandramohan, D., Natarajan, R., 1985. Seasonal distribution of arylsulfatase in the marine-environment at Porto-Novo, east coast of India. Indian J. Mar. Sci. 14, 192-195.

- 1 Duckworth, M., Yaphe, W., 1971. The agar characteristics of *Gracilaria* species. Carbohydr.
2 Res. 18, 1-9.
- 3 Freile-Pelegrin, Y., Murano, E., 2005. Agars from three species of *Gracilaria* (Rhodophyta)
4 from Yucatan Peninsula. Bioresour. Technol. 96, 295-302.
- 5 Freile-Pelegrin, Y., Robledo, D., 1997. Influence of alkali treatment on agar from *Gracilaria*
6 *cornea* from Yucatan, Mexico. J. Appl. Phycol. 9, 533-539.
- 7 Gertrudes, A.S., 1990. A Manual for the processing of agar from *Gracilaria*.
8 ASEAN/UNDP/FAO Regional Small-Scale Coastal Fisheries Development Project,
9 Manila, Philippines.
- 10 Guiseley, K.B., 1970. The relationship between methoxyl content and gelling temperature of
11 agarose. Carbohydr. Resour. 13, 247-256.
- 12 Hoyle, M.D., 1978. Agar studies in two *Gracilaria* species (*G. bursapastoris* (Gmelin) Silva
13 and *G. coronopifolia* J. Ag.) from Hawaii. II. Seasonal aspects. Bot. Mar. 21, 347-352.
- 14 Israel, A., Martinez-Goss, M., Friedlander, M., 1999. Effect of salinity and pH on growth and
15 agar yield of *Gracilaria tenuistipitata* var. *liui* in laboratory and outdoor cultivation. J.
16 Appl. Phycol. 11, 543-549.
- 17 Krishna Prasad, M.B., Ramanathan, A.L., 2008. Sedimentary nutrient dynamics in a tropical
18 estuarine mangrove ecosystem. Est. Coast. Shelf Sci. 80, 60-66.
- 19 Lahaye, M., Rochas, C., 1991. Chemical structure and physico-chemical properties of agar.
20 Hydrobiologia 221, 137-148.
- 21 Lee, W.K., Namasivayam, P., Ho, C.L., 2014. Effects of sulfate starvation on agar
22 polysaccharides of *Gracilaria* species (Gracilariaceae, Rhodophyta) from Morib,
23 Malaysia. J. Appl. Phycol. 26, 1791-1799.
- 24 Lim, P.E., Phang, S.M., 2004. *Gracilaria* species (Gracilariales, Rhodophyta) of Malaysia
25 including two new records. Malay. J. Sci. 23, 71-80.
- 26 Macler, B.A., Zupan, J.R., 1991. Physiological basis for the cultivation of the Gelidiaceae.
27 Hydrobiologia 221, 83-90.
- 28 Marinho-Soriano, E., 2001. Agar polysaccharides from *Gracilaria* species (Rhodophyta,
29 Gracilariaceae). J. Biotechnol. 89, 81-84.
- 30 McHugh, D.J., 2003. A guide to seaweed industry. FAO Fisheries Technical Paper. No. 441.
31 FAO, Rome pp105.
- 32 Murano, E., 1995. Chemical structure and quality of agars from *Gracilaria*. J. Appl. Phycol.
33 7, 245-254.

- 1 Phang, S.M., Shaharuddin, S., Noraishah, H., Sasekumar, A., 1996. Studies on *Gracilaria*
2 *changii* (Gracilariales, Rhodophyta) from Malaysian mangroves. *Hydrobiologia* 326-
3 327, 347-352.
- 4 Phang, S.M., 2006. Seaweed resources in Malaysia: current status and future
5 prospects. *Aquat. Ecosyst. Health Manag.* 9, 185-202.
- 6 Praiboon, J., Chirapart, A., Akakabe, Y., Bhumibhamond, O., Kajiware, T. 2006. Physical
7 and chemical characterization of agar polysaccharides extracted from the Thai and
8 Japanese species of *Gracilaria*. *Sci. Asia* 32, 11-17.
- 9 Rebello, J., Ohno, M., Ukeda, H., Kusunose, H., Sawamura, M., 1997. 3, 6 anhydrogalactose,
10 sulfate and methoxyl contents of commercial agarophytes from different geographical
11 origins. *J. Appl. Phycol.* 9, 367-370.
- 12 Rees, D.A., 1969. Structure, conformation, mechanism in the formation of polysaccharide
13 gels and networks. *Adv. Carbohydr. Chem. Biochem.* 24, 267-332.
- 14 Rodriguez, M.C., Matulewicz, M.C., Nosedá, M.D., Ducatti, D.R.B., Leonardi, P.I., 2009.
15 Agar from *Gracilaria gracilis* (Gracilariales, Rhodophyta) of the Patagonic coast of
16 Argentina-Content, structure and physical properties. *Bioresour. Technol.* 100, 1435-
17 1441.
- 18 Shukla, M.K., Kumar, M., Prasad, K., Reddy, C.R.K., Jha, B., 2011. Partial characterization
19 of sulfhydrolase from *Gracilaria dura* and evaluation of its potential application in
20 improvement of the agar quality. *Carbohydr. Polym.* 85, 157-163.
- 21 Siow, R.S., Teo, S.S., Ho, W.Y., Mohd. Yunus, A.S., Phang, S.M., Ho, C.L., 2012.
22 Molecular cloning and biochemical characterization of Galactose-1-phosphate
23 uridylyltransferase from *Gracilaria changii* (Rhodophyta). *J. Phycol.* 48, 155-162.
- 24 Villanueva, R.D., Romero, J.B., Ragasa, A.L.R., Montano, M.N.E., 2010. Agar from the red
25 seaweed, *Laurencia flexilis* (Ceramiales, Rhodophyta) from northern Philippines.
26 *Phycol. Res.* 58, 151-156.
- 27 Yow, Y.Y., Lim, P.E., Phang, S.M., 2013. Assessing the use of mitochondrial *cox1* gene and
28 *cox2-3* spacer for genetic diversity study of Malaysian *Gracilaria changii*
29 (Gracilariaceae, Rhodophyta) from Peninsular Malaysia. *J. Appl. Phycol.* 25, 831-838.

Legends of tables

Table 1

Sampling locations with date of collection, type of habitats, collected samples and environmental parameters. DO, dissolved oxygen; NH₃-N, ammonia-nitrogen; NO₃-N, nitrate-nitrogen; NO₂, nitrite; PO₄³⁻, phosphate-phosphorus.

Table 2

Two-way ANOVA analysis on the species effects, habitat effects and their interactions on the properties of *Gracilaria* agar. Rocky shore was excluded from statistical test due to no replication for all *Gracilaria* species and *G. salicornia* (with only rocky shore and mangrove swamp samples) was excluded in statistical test. A=species effects, B=habitat effects and A x B=interaction between species and habitat effects. df, degrees of freedom; SS, sum of squares; MS, mean square; F, ratio of variance. *=significant (P<0.05), **=highly significant (P<0.01), ns= not significant.

Table 3

Duncan's multiple range test (DMRT) groupings to test for effects of habitats on agar properties of *Gracilaria* species. Habitats with different letters (case specific, depending on each *Gracilaria* species) are significantly different (p<0.05). Refer to Fig. 1-3 for the data. Rocky shore was excluded from statistical test due to no replication for all *Gracilaria* species and *G. salicornia* (with only rocky shore and mangrove swamp samples) was excluded in statistical test.

1 Table 1

2

Date of collection	Locations	GPS Coordinate	Type of habitats	<i>Gracilaria</i> samples	Environmental parameters							
					Salinity, ppt	pH	Temp., °C	DO, mg/L	NH ₃ -N, mg/L	NO ₃ -N, mg/L	NO ₂ , mg/L	PO ³⁻ , mg/L
26 Jun 2013	Batu Besar (BB), Malacca	02° 20.758' N 102° 02.682' E	Rocky shore	<i>G. changii</i> <i>G. salicornia</i> <i>G. edulis</i>	35.0	8.04	30.27	7.45	<5	-	1.90	0.03
	Teluk Pelandok (TP), Negeri Sembilan	02° 25.808' N 101° 53.720' E	Sandy mudflat	<i>G. changii</i> <i>G. edulis</i>	35.0	8.02	31.26	6.40	0.01	0.07	0.01	<0.01
24 Jul 2013	Morib (M), Selangor	02°45.878' N 101°25.976' E	Mangrove swamp	<i>G. changii</i> <i>G. salicornia</i> <i>G. edulis</i>	32.9	7.64	31.08	5.89	0.06	<0.01	-	<0.01

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Table 2

Sources of variation	df	SS	MS	F value	P value ³
Agar yield					
A	1	35.9148	35.9148	87.39	<0.0001**
B	1	113.2216	113.2216	275.48	<0.0001**
A x B	1	16.5675	16.5675	40.31	0.0002**
Gel strength					
A	1	70398.4645	70398.4645	322.74	<0.0001**
B	1	38985.7200	38985.7200	178.73	0.0008**
A x B	1	4917.5105	4917.5105	22.54	0.0014**
Gelling temperature					
A	1	165.0208	165.0208	344.39	<0.0001**
B	1	15.1875	15.1875	31.70	0.0005**
A x B	1	0.0208	0.0208	0.04	0.8400 ^{ns}
Melting temperature					
A	1	21.3333	21.3333	68.27	<0.0001**
B	1	48.0000	48.0000	153.60	<0.0001**
A x B	1	154.0833	154.0833	493.07	<0.0001**
Gel syneresis					
A	1	2.3941	2.3941	8.98	0.0172*
B	1	1.2288	1.2288	4.61	0.0641 ^{ns}
A x B	1	0.1728	0.1728	0.65	0.4441 ^{ns}

Table 3

Agar Properties	<i>G. changii</i>		<i>G. edulis</i>	
	Sandy mudflat	Mangrove swamp	Sandy mudflat	Mangrove swamp
Agar yield	A	B	a	b
Agar gel strength	A	B	a	b
Gelling temperature	A	B	a	b
Melting temperature	A	B	a	b
Gel syneresis	A	A	a	a

Legends of figures

Fig. 1. Yield (A) and gel strength (B) of agar obtained from three *Gracilaria* species collected from different habitats. Error bars denote $\pm$ SD, $n=3$ for all except for *Gracilaria* species collected from rocky shore ($n=1$). *Gracilaria* species with different letters (case specific, depending on each habitats) are significantly different ($p<0.05$). Rocky shore was excluded from statistical test due to no replication for all *Gracilaria* species. TP, Teluk Pelanduk; M, Morib; BB, Batu Besar.

Fig. 2. Gelling (A) and melting (B) temperatures of agar obtained from three *Gracilaria* species collected from different habitats. Error bars denote $\pm$ SD, $n=3$ for all except for *Gracilaria* species collected from rocky shore ($n=1$). *Gracilaria* species with different letters (case specific, depending on each habitats) are significantly different ($p<0.05$). Rocky shore was excluded from statistical test due to no replication for all *Gracilaria* species. TP, Teluk Pelanduk; M, Morib; BB, Batu Besar.

Fig. 3. Gel syneresis of agar obtained from three *Gracilaria* species collected from different habitats. Error bars denote $\pm$ SD, $n=3$ for all except for *Gracilaria* species collected from rocky shore ($n=1$). *Gracilaria* species with different letters (case specific, depending on each habitats) are significantly different ($p<0.05$). Rocky shore was excluded from statistical test due to no replication for all *Gracilaria* species. TP, Teluk Pelanduk; M, Morib; BB, Batu Besar.

Figure 1

Figure 2

Figure 3