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

Wei-Kang Lee [a], Phaik-Eem Lim [b], Siew-Moi Phang [b], Parameswari Namasivayam [a], Chai-Ling Ho [a,*]

[a] *Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia*
[b] *Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*

## HIGHLIGHTS

- Yield and quality of agar from *Gracilaria* species collected from three natural habitats in Malaysia were evaluated.
- Species effect, habitat effect and their interactions affect the agar yield, gel strength and agar melting temperature significantly ($P < 0.01$).
- Higher agar yield and gel strength were found in *Gracilaria* species growing in the mangrove swamp compared to rocky shore and sandy mudflat.
- Mangrove swamp is a good site for seaweed farming and mariculture 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.



## 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

* Correspondence to: Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM-Serdang, Selangor, Malaysia. Fax: +60 3 89467510.
*E-mail address:* clho@upm.edu.my (C.-L. Ho).

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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 (Macler 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 and July 2013. The geographical locations of sampling sites, type of habitats, date of collection and collected samples are summarized 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 l 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 h. 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 aluminum foil and left for overnight at room temperature. The maximum weight needed to break the agar gel was determined by using a texture analyzer (TA-XT2, Stable Micro Systems Ltd., Survey, 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 $cm^{-2}$.

### 2.4. 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 aluminum 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.

### 2.5. 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.

### 2.6. 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