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Growth rates and agar properties on some species of *Gracilaria* Grev. (Rhodophyta, Gigartinales) from Manado, Indonesia

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Abstract: Specimens of *Gracilaria edulis, G. salicornia, Gracilaria Sp.* A and *Gracilaria Sp.* B were collected from Manado, Indonesia, transported and cultured in Usa Marine Biological Institute, Kochi University, Japan. Daily growth rates (DGR) of these materials were measured under different levels of temperature, salinity and irradiance in laboratory controlled conditions using a temperature-controlled closed circulating system ("aquatron") and outdoor tank cultivation system. The maximum DGR were found on *Gracilaria edulis, G. salicornia* 5.1%±0.9, 4.9±0.8 *Gracilaria Sp.* A. 3.25±0.9 and *Gracilaria Sp.* B. 4.9%±1.1 under optimum condition. All species grew well within a temperature range of 25-30°C, salinity range of 25-35‰ and irradiance from 100-200 μ mol photon m^{-2} s^{-1}. In outdoor tank cultivation. The highest DGR of each species was found at a temperature range of 25-30°C, salinity of 30-35‰ and an irradiance of 150-300 μmol photon m^{-2} s^{-1}. The gel strength of each species was *G. edulis* 580 g cm^{-2} and *Gracilaria Sp.* B, 540 g cm^{-2} was higher than both *Gracilaria Sp* A, 520 g cm^{-2} and *G. salicornia,* 80 g cm^{-2}. The agar yield obtained were *Gracilaria Sp.* A, 26.2%, *G. edulis,* 28.3 %, *Gracilaria Sp.* B, 27% and *G. salicornia,* 8.6%.

Key words: Growth rate, agar properties, *Gracilaria,* Indonesia

INTRODUCTION

Members of the agarophyte genus *Gracilaria* are widely distributed geographically with the majority of species being reported from warm-water and tropical regions. This genus comprises more than 100 described species throughout the world (McLachlan & Bird, 1986 ; Bird, 1995). *Gracilaria* has been harvested from naturally occurring stocks in a number of countries in the developing world to become the agarophyte of choice because those under culture conditions show favourable growth rates, good quality of colloid and fairly good yields. Due to overharvesting and declining populations, cultivation is an increasingly important source of raw material. More sophisticated and expensive culture methods have been tried using a variety of land-based techniques such as tank and pond systems (Critchley, 1993; Abbott, 1994).

Studies on the outdoor tank and aquatron culture of *Gracilaria* of different geographical origin have been undertaken by many researchers (Orosco & Ohno, 1992; Chirapart *et al.*, 1994) with the main focus being agar production and quality (Rebello *et al.*, 1996; Orosco *et al.*, 1992; Chirapart & Ohno, 1993).

Indonesia has extensive marine resources including seaweeds which grow along the 180 000 km coastline. The materials used in the present study were collected from the Gulf of Manado, Indonesia. They were transported to and cultured in a outdoor tank at Usa Marine Biological Institute, Kochi University, Japan. Controlled culture experiments were also conducted in order to determine the effects of temperature, salinity and photon fluence on growth rate and

agar properties of each species.

MATERIALS AND METHODS

Aquatron and outdoor tank culture

The materials used in the present investigation were taken from a natural population in the Gulf of Manado, Indonesia. Five replicates comprising thallus segments of 5 to 10 g of each species: *G. edulis, G. salicornia, Gracilaria Sp.* A and *Gracilaria Sp.* B were cultured in a temperature-controlled closed recirculating system-"aquatron" (Ohno, 1977). Light was provided using white fluorescent tubes at 12:12h light/dark cycle at irradiances of 50, 100, 150 and 200 μmol photon m^{-2} s^{-1}. Temperatures of 20, 25, 30 and 35°C and salinities of 20, 25, 30 and 35‰ were tested as culture variables. Experiments were carried out for 60 days, at each condition. In outdoor tank culture, the culture medium supplied was unfiltered seawater from the Uranochi Inlet, Tosa Bay, southern Japan. DGR, temperature, salinity and irradiance were measured within 6 months.

Growth rate

The % DGR were measured every 7 to 10 days during the period of observation. Irradiance measurement were made using a digital photometer LI-189, Li-Cor Co. USA. Seawater temperatures were measured with a mercury thermometer and salinity using a Digi-Auto. T.S-Digital Lab. Salinometer, Model 3-G (Tsurumi Seiki, Tokyo). % daily growth rates were measured from increasing weight and presented as percentage growth per day using the formula of Penniman *et al.* (1986) : $G = [(W_t/W_0)^{1/t} - 1] \times 100$. Where : G = % increase in fresh weight per day ; W_0 = initial weight ; W_t = weight after t days determined.

Agar extraction

The agar extraction was performed according to Rebello *et al.* (1996) with some modifications. 30 g dried material was boiled for 2 h in 1.2 L of 5% NaOH solution at 80°C for 2 h and washed in tap water for 1 h to remove the exess NaOH. The alkali-treated samples were neutralized in 1 L of 1.5% H_2SO_4 solution for 1.5 h and washed in tap water for a further 12 h. The agar was extracted by boiling the sample in 1.2 L of distilled water for 2 h. The extracted agar was filtered through a vacuum pump equipped with a Buchner Funnel No 6 with 3-μm-pore-size industrial filter paper (Advantec, Toyo Roshi, Co., Japan) and kept at room temperature until gel formation. The agar gel was sliced, frozen at -35°C for 24 h, thawed in tap water, air-dried and then oven-dried at 70°C for the determination of agar yield. Viscosities of the solution at 70°C were determined using a Brookfield Viscometer (BL-No.2 spindle at 60 rpm, Tokyo Keiki). Gelling temperature was determined according to Kim (1970) and melting temperature was measured as described by Hurtado-Ponce & Umezaki (1988). Extractions were made in triplicate. Gel texture was measured using a Sun-Rheometer CR-200D equipped with cylindrical plunger of 1 cm^2 diameter operating at maximum force of 2 kg and table speed of 20 mm min^{-1}. The load deformation curve provided rheological parameters which were defined as gel strength, hardness and flexibility. All measurements of the physical gel properties were performed on a 1.5% solution after being stabilized for 24 h at 20°C. Three replicates were used for each determination.

RESULT AND DISCUSSION

Aquatron and outdoor tank culture

Observations on the effect of temperature, salinity and irradiance on each species of Indonesian *Gracilaria* under the aquatron culture system showed fluctuations of growth rates (Table 1) The maximum DGR of *G. edulis* (4.9% ± 1.10) was obtained at 30°C, *G. salicornia* at 30°C (4.9% ± 0.9) *Gracilaria Sp.* A, at 25°C (2.5% ± 0.8) and *Gracilaria Sp.* B, at 30°C (4.8% ± 1.0). All species showed poor growth when the temperature was reduced to 20°C. Observation on the effect of salinities showed that all species grew well in the 25 to 30‰ ranges. Observations on the influence of irradiance showed that the materials grew well in the range 100 - 150 μmol photon m^{-2} s^{-1} but showed poor growth at 50 μmol photon m^{-2} s^{-1}. The results of the present study showed that *Gracilaria* species could grow well under different conditions of salinity, temperature and irradiance in an aquatron culture system.

The surface seawater temperature and salinity during the period of outdoor tank culture investigation shown in Fig. 1. Temperature ranged from 17.1°C (winter) to 29.8 (summer). Salinity fluctuated between 21.97 to 34.05‰ with lower values in July during the rainy season. Irradiance of outdoor tank culture ranged from 120 to 310 μmol photon m^{-2} s^{-1} during the period of investigation. In the outdoor tank culture system, growth is influenced by natural conditions and all species demonstrated seasonal variation (Fig. 2). % DGR was high in all species when seawater temperature reached a high of 20 to 30°C in the summer and gradually decreased in winter. During the 6 month culture period, % DGR of *G. edulis* was 4.7% ± 1.1, *G. salicornia* of 4.8% ± 0.6 *Gracilaria Sp.* A of 3.7% ± 1.0 and *Gracilaria Sp.* B, of 4.4% ± 1.1. Observations on the branches of thalli showed that many epiphytes grew especially on *Gracilaria Sp.* B, although they did not appear to effect the general health of the plants.

Table 1. Daily growth rates (% ± SD) on some species of *Gracilaria* from Indonesia at different salinity level (‰), temperature (°C), irradiance (μmol m^{-2} s^{-1}) in the aquatron and outdoor culture system.

	G. salicornia	*G. edulis*	*Gracilaria sp.* A	*Gracilaria sp.* B
Salinity (‰)				
20	4.5±0.9	4.3±0.7	2.1±1.1	3.7±1.0
25	5.1±0.3	4.6±1.1	3.2±0.8	4.6±0.5
30	4.2±0.5	4.9±0.8	3.1±0.9	4.9±0.7
35	4.7±1.0	4.0±0.9	2.7±1.0	4.2±1.0
Temperature (°C)				
20	2.4±0.4	3.6±0.6	1.7±1.0	3.7±0.9
25	4.7±1.4	4.9±1.1	2.3±1.1	3.9±0.8
30	4.9±0.9	4.8±1.3	2.5±0.8	4.8±1.0
35	3.5±1.1	4.3±1.1	2.3±0.6	4.2±0.4
Irradiance (μmol m^{-2} s^{-1})				
50	2.9±1.2	3.4±0.4	2.7±0.9	3.8±0.3
100	4.8±1.4	4.7±0.6	2.3±0.7	4.0±1.1
150	3.2±0.3	4.3±0.9	3.1±0.9	4.1±1.0
200	3.7±0.9	4.6±0.8	3.0±1.1	4.0±0.8
Outdoor tank	4.8±0.9	4.7±0.8	3.7±1.1	4.4±0.8

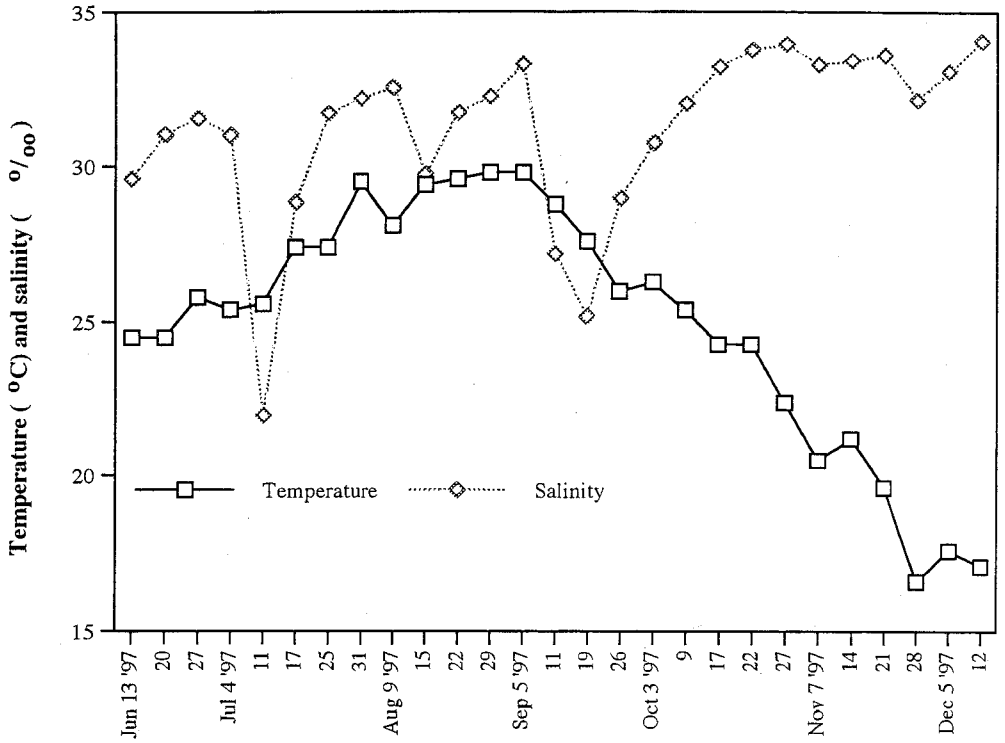

Fig. 1. Temperature and salinty during the period of outdoor tank cultivtion.

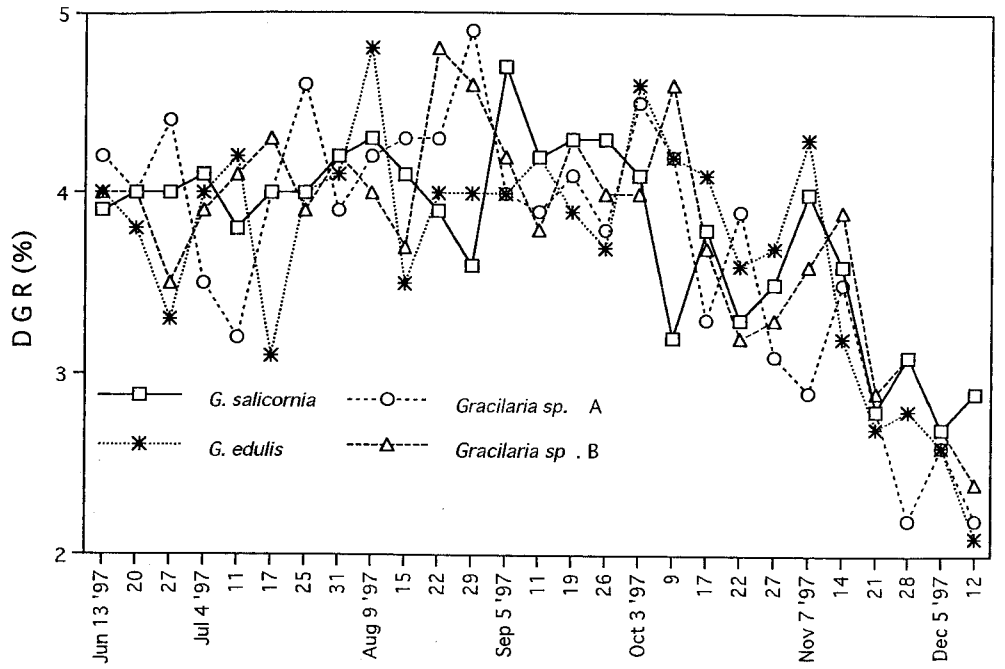

Fig. 2. Daily grouth rates on some species of *Gracilaria* in outdoor tank cultivation.

The results from the aquatron and outdoor tank cultures showed that the genus *Gracilaria* examined can grow well over wide ranges of temperature : 17.1 to 35°C, salinity 21.97 to 35‰ and irradiance of 50 to 200 μmol photon m^{-2} s^{-1}. In general, high % DGRs of all species was recorded between 4.1 to 4.9% under the different experimental conditions. This result showed that these tropical species require stable conditions of temperature and salinity approximate those of their original source.

It was interesting to note that the pattern of growth of all species were similar over the range of different experimental conditions. *Gracilaria* is a cosmopolitan genus and can grow in a wide range of salinity and temperature therefore can be found growing in many areas of the world. However, maximum growth can be find only when the seawater condition in favorable for this genus. In the tropical origin where temperature and salinity value are almost similar throughout the year, is important to consider in developing the culture technique of this genus. In southern Japan where the seawater is affected by the warm Kuroshio Current, the culture of *Gracilaria* could be improved at least during the summer and autumn seasons.

Agar quality

The material used for agar extraction was harvested from a natural population in the Gulf of Manado, Indonesia. Table 2 shows the mean yield and physical characteristics of agar from each of the species investigated. A high agar yield of 28.3% was obtained from *G. edulis*, 8.6% from *G. salicornia*, 26.2% from *Gracilaria Sp.* A, and 27% from *Gracilaria Sp.* B.

Table 2. Agar properties on some species of Gracilaria from Indonesia.

	G. edulis	*G. salicornia*	*Gracilaria sp.* A	*Gracilaria sp.* B
Yield (%)	28.3	8.6	26.2	27
Viscosity (cP)	47.5	10	22.5	37.5
Gel strength (g cm^{-2})	580	80	520	540
Melting temperature (°C)	66	27	72	69
Gelling temperature (°C)	32	21	29	28

All species seem to have similar agar properties except for *G. salicornia* which showed a much low agar yield and gel strength. Low agar yields have also been reported for *G. salicornia* from other sites : Malaysia (10% ; Phang, 1994) ; Saipan (16% ; Nelson *et al.*, 1983). Low viscosity was reported for *G.Sp.* A. which made filtration relatively easy. The agar properties of *G edulis*, from the different geographical origins were also reported by Hoyle (1978); Nelson *et al.*, (1983); Hurtado-Ponce & Umezaki (1988). In this present study, gel strength of *Gracilaria edulis, Gracilaria sp.* A and *Gracilaria sp.* B seems similar.

From the results of this study, we recognized that *Gracilaria sp.* A, *Gracilaria sp.* B, *G. edulis* except *G. salicornia* have the potential to be used as suitable material for agar extraction. To satisfy the increasing demand for agar, extraction industries should be developed in Indonesia where raw materials are available throughout the year. Further work is required on specific techniques for cultivating this genus especially in Indonesian waters.

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