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Effect of temperature on growth rate and agar quality of a new member of Japanese *Gracilaria* in Tosa Bay, southern Japan.

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Growth of *Gracilaria* sp. growing much in Uranouchi Inlet at Tosa Bay recently, was conducted in the aquatron culture system at various temperatures of 13, 16, 20, 23, 25 and 28°C with a photon flux density of $100\pm10\ \mu M\cdot m^{-2}\cdot s^{-1}$, in a light regime of 12L : 12D hours. Daily growth rate of thalli attained maximum and minimum values of $2.47\pm1.05\%$ at 16°C and $1.20\pm0.27\%$ at 28°C, respectively. Physical properties of agar of the cultured thalli were also determined after 2 h treatment with 5% NaOH at 80°C. Agar yields attained between 24.2 ± 0.2 and $28.4\pm0.6\%$. Measurement of the gel strength of 1.5% agar gave a highest value of $755\pm15\ g\cdot cm^{-2}$ at 16°C. The lowest gel strength recorded was $437\pm48\ g\cdot cm^{-2}$ at 25°C. Viscosities of 1.5% agar sol obtained maximum value of 73.9 ± 0.6 cP at 16°C and minimum values of 16.4 ± 0.8 cP at 25°C. Gelling temperatures ranged from 48°C to 49°C. This *Gracilaria* sp. indicated high growth rate and best agar quality at 16°C.

Key Index Words: New Japanese Gracilaria—*growth rate—agar quality—temperature.*

Gracilaria sp. occurring along the coast of Kyushu Island, south Ise Bay, and other inland seas in Japan has been reported as a new Japanese *Gracilaria* (Chirapart *et al.* 1994). In recent year, large quantities of this *Gracilaria* have been harvested (more than 2000 t dry weight per year) for the production of agar and salad vegetable. Algal biomass and physical properties of this *Gracilaria* sp. have been reported to be seasonal and high gel strength obtained from the winter collected seaweed in Tosa Bay (Chirapart and Ohno, 1993b). Property of agar commonly has been reported in relation to environmental factors of plant growth (Craigie and Wen 1984; Friedlander *et al.* 1987; Christeller and Laing 1989). Moreover, growth of *Gracilaria* spp. from Tosa Bay has been reported under different environmental factors in a close-recirculating system or aquatron (Orosco and Ohno 1992). However, there have been no report on effect of environmental factor on growth and agar quality of this new Japanese *Gracilaria* under culture conditions. This study was carried out in order to examine effect of temperature on growth and agar quality of this seaweed under controlled culture conditions.

Materials and Methods

Growth rate

Gracilaria samples were collected from Uranouchi Inlet in Tosa Bay in 1992. Growth of the algae was determined in a close-recirculating system (aquatron) at various temperatures (cf. Ohno, 1977). collected samples were allowed to adapt to each set of experimental conditions for one week. After which 0.9 to 1 g of healthy thallus (10 replicates per treatment temperature) were fixed to glass rods with fine lines and incubated at 13, 16, 20, 23, 25 and 28°C with a photon flux density of $100\pm10\ \mu M\cdot m^{-2}\cdot s^{-1}$, in a light regime of 12L:12D hours. Fresh weight was measured every 7 days and growth rates assessed. One third of the unenriched cul-

tured seawater was replaced every 2-3 days. Data represent the first week of incubation. Experiments were conducted in duplicate. Daily growth rates were calculated as the increase in fresh weight in percentage following the formula of Penniman et al. (1986)

$$G=[(W_t/W_o)^{1/t}-1]\times 100$$

where G is % increase in fresh weight per day, W_o is initial weight and W_t is weight after t days.

Agar quality

The effect of temperature on agar properties of the *Gracilaria* sp. was also determined. Healthy thalli, 50 to 60 g (10 replicates per treatment temperature), were incubated in the aquatron at the temperatures and culture conditions described above. After two weeks incubations, fresh weight was measured and the samples were sun-dried prior to extraction.

Dried samples (50 g), were incubated in 2 *l* of 5% NaOH solution in 80°C water bath for 2 hr and washed in tap water for 30 min. The samples were neutralized in 1 *l* of 1.5% H_2SO_4 solution for 1 hr, and then washed in tap water for 2 hr. The treated samples were boiled for 2 hr using a Bunsen burner with 1.5 *l* of distilled water in 2 *l* Erlenmeyer flask equipped with reflux condenser. The agar extract was filtered through muslin cloth.

A 1.5% agar solution was prepared from the extracted agar by boiling 9 g of agar powder in 600 m*l* of distilled water for 30 min. Viscosities of the solution at 80°C were determined using a Brookfield viscometer (Spindle No. 1 at 60 rpm; Tokyo keki). Gel strength (3 replicates per sample) were measured using a 1 cm^2 plunger (Nikkansui Shiki gelometer, Kiya Seisakusho, Tokyo) and gel pH was measured using an electronic pH meter. Gelling temperature was determined according to Kim (1970).

Results

The physical and chemical characteristics of seawater in the experimental chamber are shown in Table 1. Thalli were cultured in water temperatures of 13, 16, 20, 23, 25 and 28°C. Water salinity fluctuated from 33.85±0.08 to 36.40±0.04. Total dissolved inorganic nitrogen (DIN) and PO_4-P concentrations varied from 0.52±0.18 to 4.21±2.65 $\mu g\cdot at\cdot l^{-1}$ and 0.12±0.03 to 0.30±0.03 $\mu g\cdot at\cdot l^{-1}$, respectively.

Table 1. Physical and chemical characteristics of seawater in the aquatron during culture period.

Temperature treatment (°C)	Salinity	PO_4-P ($\mu g\cdot at\cdot l^{-1}$)	DIN ($\mu g\cdot at\cdot l^{-1}$)
13	34.10±0.10	0.13±0.03	0.94±0.55
16	35.53±0.04	0.30±0.03	0.58±0.22
20	33.85±0.08	0.16±0.06	1.15±0.52
23	34.32±0.06	0.18±0.06	0.52±0.18
25	33.97±0.31	0.24±0.11	1.13±0.94
28	36.40±0.04	0.12±0.03	4.21±2.65

Daily growth rates of thalli grown in the

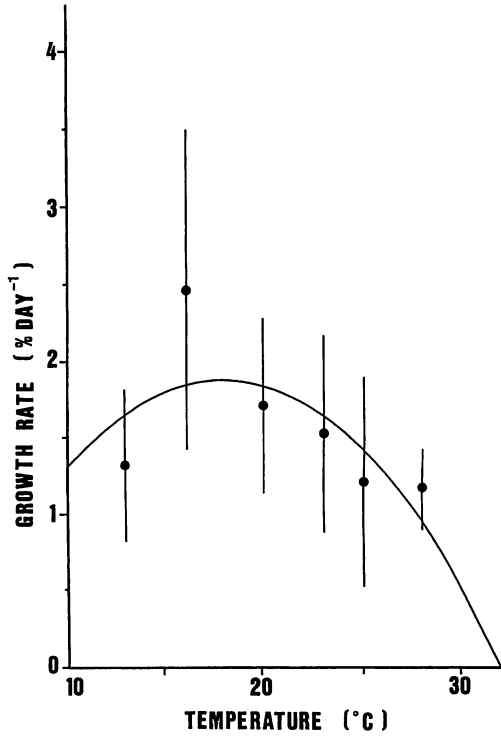

Fig. 1. Growth rates of *Gracilaria* sp. in the aquatron culture system at various temperatures. Lines represent standard deviations. Polynomial curve represent predicted growth rates.

Table 2. Agar yield, gelling temperature and gel pH of agar obtained from *Gracilaria* sp. incubated at various temperatures in the aquatron culture system, after treatment for 2 h with 5% NaOH at 80°C.

Temperature treatments (°C)	Agar yield of crude extract (%)	1.5% agar product	
		Gelling temperature (°C)	Gel pH
13	24.3±0.1	48	6.5
16	24.2±0.2	49	6.5
20	27.7±0.3	49	6.4
23	26.6±0.0	49	6.5
25	28.4±0.6	49	6.7

aquatron at various temperatures are shown in Fig. 1. The greatest growth was observed at temperature between 16°C and 20°C. The cultured plants were healthy with dark reddish color, branching, and no epiphytes. Maximum daily growth rate attained was 2.47±1.05% at 16°C. Increasing cultured temperatures over than 20°C, growth rates were gradually decreased and obtained minimum value of 1.20±0.27% at 28°C. At high temperature, the cultured thalli were not healthy, much epiphytized.

Properties of agar obtained from *Gracilaria*, after incubation in the aquatron at various temperatures, are given in Table 2. Agar yields varied between 24.2±0.2 and 28.4±0.6%. Gelling temperatures were

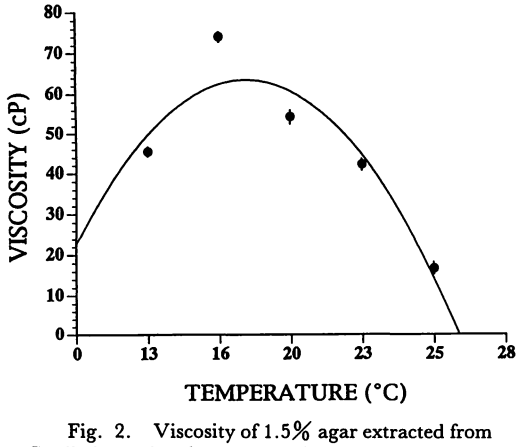

Fig. 2. Viscosity of 1.5% agar extracted from *Gracilaria* sp. incubated at various temperatures in the aquatron culture system. Lines represent standard deviations. Polynomial curve represent predicted viscosity.

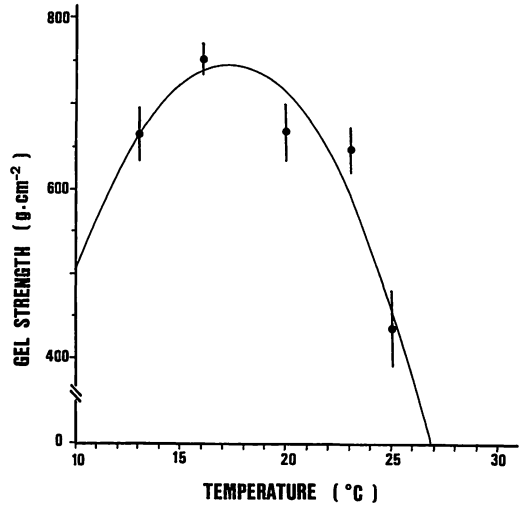

Fig. 3. Gel strength of 1.5% agar extracted from *Gracilaria* sp. incubated at various temperatures in the aquatron culture system. Lines represent standard deviations. Polynomial curve represent predicted gel strengthe.

rather stable between 48°C and 49°C and gel pH of 1.5% agar gel ranged from 6.4 to 6.7. Viscosity of 1.5% agar sol attained maximum value of 73.9±0.6 cP from sample cultured at 16°C (Fig. 2). The values were decreased after increasing water temperature and obtained minimum value of 16.4±0.8 cP in plant cultured at 25°C. The viscosity values corresponded to agar gel strength values obtained in this study. Gel strength of 1.5% agar gel (Fig. 3) obtained highest values of 755±15 $g \cdot cm^{-2}$ at a temperature of about 16-17°C while the lowest value of 437±48 $g \cdot cm^{-2}$ occurred at 25°C. Increasing water temperature over than 20°C, gel strengths were descreased.

Temperature being tested in the cultured conditions had no effect on the yield of agars and gelling temperatures whereas low temperature (16-20°C) had effect on agar gel strength and viscosity values. The higher gel strength and viscosity values also corresponded with higher growth rates of the cultured thalli.

Discussion

Growth of *Gracilaria* sp. showed positive

relationship with temperature up to 20°C, and obtained maximum growth rate at a temperature between 16°C and 17°C. This result indicated the optimum temperature similar to a previous study of *G. chorda* (15°C) in the same culture system (Orosco and Ohno 1992). However, a previous study on growth of *Gracilaria* spp. from southeast Asian water using the same aquatron system, has been reported maximum growth at 25°C for *G. "verrucosa"* and *G. fisheri* and at 27°C for *G. salicornia* and *G. firma* (Chirapart and Ohno 1993a). Present study, agar gel strength of the *Gracilaria* sp. showed inverse relationship with increasing temperature in the culture system, and positive correlation with growth rate. Similar result had been reported in long-term experiments in outdoor culture of *Gracilaria* cf. *conferta* in Israel, which showed increased gel strength under high growth rate conditions (Friedlander et al. 1987). However, the Israel strain obtained high gel strength at high temperatures in contrast to the present study wherein alkali-treated agars attained from the cultured *Gracilaria* showed the best gel at low temperatures (16–17°C). There results correspond with those of the gel of *Gracilaria* collected from natural environment, which showed a maximum gel strength value in winter, when seawater temperatures were 15–17°C (Chirapart and Ohno 1993b). This may be confirmed by the hypothesis of Craigie and Wen (1984) that growth rate may have more effect on gel strength than the particular environmental factor being tested. Craigie and Wen (1984) also suggested that chemical composition varied with the part of the thallus from which it was extracted and with the temperature at which the alga was grown. They also reported that *Gracilaria tikvahiae* showed the highest 3,6-anhydrogalactose content from alkali-modified agar of the thalli grown at 17°C, and young parts of thallus contained a higher proportion of 3,6-anhydrogalactose than mature parts (Craigie and Wen 1984). This, then, may in part explain our observed correlations between gel strength and growth rate at low temperature in the aquatron culture system. Moreover, there have been reports of gel strenghts increased with increasing temperature, and decreased with increasing nitrogen level (Christeller and Laing 1989). Conversely, lower gel strengths also have been reported during summer or in high temperatures with high level of sulfation (Cote and Hanisak 1986, Miller and Furneaux 1987).

In conclusion, optimum temperature for growth of the *Gracilaria* sp. was 16–17°C. At this low temperature condition, the algae also can produce the best gel. Both growth rates and culture temperature are considered to be effect on agar quality of this seaweed. Under certain conditions the cultured new Japanese *Gracilaria* produced comparable yields and agar properties to those of natural-grown plants and its higher agar gel strength obtained within the range (gel strength more than $500\,g \cdot cm^{-2}$) of commercial grade agars. This new Japanese *Gracilaria* species has a high potential as an essential source of agar for food industry gels.

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Anong Chirapart*・大野正夫・沢村正義*・楠瀬博三*：土佐湾の新産オゴノリ類の成長と寒天品質におよぼす温度の影響**

高知県浦の内湾に，最近大量に生育し始めたオゴノリ類の一種について，アクアトロン培養（光量は，100±10 μM m^{-2} s^{-1}，12時間照射）により，13，16，20，23，25と28°Cの温度条件で，成長速度を調べた。日間成長率は16°Cでもっとも高く，2.47±1.05%を示し，28°Cで最も低く，1.20±0.27%であった。

培養された葉体について，80°C，5%，NaOH 2時間処理により寒天の物理的特性を調べた。寒天の収率は，24.2±0.2から28.4±0.6%の範囲であった。ゲル強度は，16°Cで最も高い値，755±15 g cm^{-2}，25°Cで最も低い値，437±48 cm^{-2}をしめした。ゲル寒天の粘度は，16°Cで最も高く73.9±0.5 cP，25°Cで最も低く16.4±0.8 cP，を示した。ゲル化温度は48–49°Cであった。これらの結果から16°Cで最も成長が良く，寒天の品質ももっとも良かった。（*783 高知県南国市物部乙-200 高知大学農学部生物資源科学科，**781-11 高知県土佐市宇佐町井の尻194 高知大学海洋生物教育研究センター）

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