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Effects of environmental factors and metal ions on growth of the red alga *Gracilaria chorda* Holmes (Gracilariales, Rhodophyta)

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Abstract

Gracilaria is a potentially valuable source of marine biopolymers such as proteins and polysaccharides. In order to select suitable culture conditions, growth and tolerance of *Gracilaria chorda* Holmes from Shikoku Island in southwest Japan were investigated under variations of temperature (5–30 °C), photon irradiance (20–120 μmol photons $m^{-2}\,s^{-1}$), and photoperiod (12:12 h, 14:10 h light:dark regime) in a unialgal culture. *Gracilaria chorda* showed wide tolerances for all factors investigated, which is characteristic of eurythermal species. Maximum growth was observed at 18–24 °C. The optimum photon irradiance for algal growth was 60–120 μmol photons $m^{-2}s^{-1}$. Instead of using ordinary sea salt (NaCl) to prepare artificial seawater, ultra pure salt was adopted. *Gracilaria chorda* grew faster in artificial seawater made with ultra-pure salt than that made with ordinary sea salt, probably because the former medium was clear, while the latter was milky. Effects of some metal ions on the growth were tested with artificial seawater. Iron ions affected algal growth, but cobalt ions did not. This study enables us to determine suitable culture conditions for *G. chorda*. A scaled-up 30 l culture of *G. chorda* under such conditions was successful.

Introduction

The red algal genus *Gracilaria* is harvested and cultured on a commercial scale in many countries because it has considerable economic importance as an agarophyte. The total annual *Gracilaria* production in the world increased to more than 89,000 t, including 50,000 t of cultured production, in 1995. *Gracilaria* plants are also used as sources of traditional seaweed salad in Japan and feed for shellfish (abalone) in many countries. Recently some bioactive substances from *Gracilaria* spp. have been extracted and reported (Kakita et al., 2003).

Seasonality affects agar quality in various *Gracilaria* species (Oza, 1978; Hoyle, 1978, Whyte et al., 1981; Lahaye & Yaphe, 1988; Bird & Ryther, 1990, Luhan, 1992; Yenigul, 1993). For obtaining *Gracilaria* biopolymers of constant quality and quantity, a cultured strain is likely to be more suitable than a wild one.

Environmental factors including temperature, salinity and light play an important role in the growth, reproduction and distribution of marine algae (Gessner, 1970; Gessner & Schramm, 1971; Lüning, 1981; Lobban & Harrison, 1994). Temperature requirements for survival and growth of *Gracilaria* species have been extensively studied (Bird et al., 1979; Laing et al., 1989; Yokoya & Oliveira, 1992). Some *Gracilaria* species require less than 100 μmol photons $m^{-2}s^{-1}$ for optimal growth (Bird et al., 1979; Beer & Levy, 1983), while others require higher irradiance (Lapointe, 1981; Lapointe et al., 1984). However, few data on the effects of such environmental factors on the growth of Japanese *Gracilaria* species in controlled conditions are available (Orsco & Ohno, 1992; Chirapart et al., 1994; Yokoya et al., 1999). The aim of this study is to characterize the physiological responses of *G. chorda* to temperature, irradiance and photoperiod, assessing tolerance

and optimal conditions for growth in unialgal cultures.

Several metals such as iron ions are regarded as essential components for algal growth (Matsunaga et al., 1998). Thus, effects of some metal ions on the growth were also tested in artificial seawater. We therefore tested and selected suitable culture conditions and prepared a scaled-up model of an artificial seawater system for *Gracilaria* cultivation.

Materials and methods

Stock unialgal culture strains

Stock unialgal cultures of the red alga, *G. chorda*, were started from spores released by fertile plants that were harvested in June 1998 from Seto Inland Sea off the coast of Tokushima city, Tokushima Pref., Japan. The establishment of unialgal strains followed the methods of Yamamoto and Sasaki (1987). Stock unialgal cultures were incubated with aeration at 20 °C, in a 14:10 h light:dark regime, in salinity of about 33‰, at photon irradiances of just 40 μmol photons $m^{-2}s^{-1}$ under cool-white lamps to inhibit growth in storage. Provasoli enriched seawater (PES) (Provasoli, 1968) was made using sterilized Yashima surface seawater (Yashima, Kagawa Pref., southwest Japan) without the addition of vitamins, and this medium was used for the stock unialgal culture (Yamamoto & Sasaki, 1987). Medium renewal was carried out bi-weekly.

Temperature, irradiance, and photoperiod

The growth of *G. chorda* was compared among various culture conditions. Variations of temperature (5–30 °C) and irradiance (20–120 μmol photons $m^{-2}s^{-1}$) were tested. Algal growth rates in long-day (14:10 h light:dark) regime were compared with short-day (12:12 h light:dark) regime at three different temperatures (10, 20, and 30 °C). Irradiances were measured with a LI-250 photometer equipped with a LI-193SA spherical quantum sensor (LI-COR, Inc). The controlled conditions were the same as described above for stock unialgal cultures except that irradiance was increased to 60 μmol photons $m^{-2}s^{-1}$ to promote growth.

For each experiment, five replicates of six apical segments (5 mm long and approximately 1.4 mg fresh weight) cut from the stock unialgal culture strains were inoculated into 200 mL conical flasks containing 200 mL of PES. The measurement of total fresh weight of the six apical fragments and the renewal of culture media were carried out weekly in a clean booth. The data on algal growth rate, which were measured from day 14 to day 21 of cultivation, were analyzed by one-way ANOVA (for temperature and irradiance experiments) followed by Tukey's multiple comparison test (Winer et al., 1991) or t-test (for photoperiod experiment). Relative growth rates *R* were calculated using the formula:

$$R = [\ln(W_t) - \ln(W_0)]t^{-1},$$

where W_0 is the initial fresh weight, W_t is the fresh weight after *t* days and t is the number of days (Kain, 1987). Growth rate (%) was defined as $R \times 100$.

Artificial seawater

Ordinary sea salt (sodium chloride; NaCl) contains magnesium and other ions as contaminants (Niino et al., 1993). Some adsorbents, such as chelate resins and zeolites, are known to adsorb magnesium ions. Thus, an ultra pure salt (NaCl) was purified from ordinary sea salt by passing a 5.844 % solution of ordinary sea salt through a column of $Na_6Al_6Si_{30}O_{72}{\cdot}24H_2O$-type zeolite (clinoptilolite: Sun-Zeolite Co., Ltd, Akita Pref., Japan) at 27 °C. The solution passed was re-crystallized only once and dried to obtain an ultra pure salt as a white powder. Atomic absorption spectrochemical analysis of the ultra pure salt showed that it contained only about 0.00015 % (w/w) of magnesium ions on average. On the other hand, ordinary sea salt (before adsorption treatment) contained about 0.00753 % (W/W) of magnesium ions.

Artificial seawater solids, Sample A, were made up of 548 g of ultra pure salt (NaCl), 250 g of $MgCl_2{\cdot}6H_2O$, 92.5 g of Na_2SO_4, 35.0 g of $CaCl_2{\cdot}2H_2O$, 15.8 g of KCl, 4.5 g of $NaHCO_3$, 2.25 g of KBr, 0.75 g of H_3BO_3, 0.25 g of $SrCl_2$, 0.13 mg of $FeCl_3{\cdot}6H_2O$, 8.75 mg of glycerophosphate disodium salt pentahydrate ($C_3H_7Na_2O_6P{\cdot}5H_2O$), and 4.0 mg of $NaNO_3$. Artificial seawater solids, Sample B, were a similar composition to Sample A, with the exception of the substitution of ordinary sea salt (NaCl) for ultra pure salt. After mixing of the components mentioned above, several batches of each sample of artificial seawater solids were sealed in laminated bags and stored for 30 days at 20 °C.

After storage for 30 days at 20 °C, 40 g of each sample of artificial seawater solids were dissolved in 1 L of distilled water. The transparency of each