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[Note]

Laboratory, Field and Deep Seawater Culture of *Eucheuma serra* - a High Lectin Yielding Red Alga

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The red seaweed *Eucheuma serra* is a high yielding source of lectins. The plants were collected from a depth of 5-6 meters and cultured in the laboratory, field and deep seawater. A Daily Growth Rate (DGR) of 3.5% was observed at 18°C with a low light of 30 μmol photon $m^{-2} \cdot s^{-1}$ in the laboratory. When the plants were cultured in the field at different depths during winter months of December and January, best growth was observed at 1 m depth and a DGR of 2.14±0.04% was recorded. The plants grown in the tank with a continuous supply of deep seawater showed a DGR of 8.2%. The results indicate that *E. serra* can be cultivated in large scale both in deep seawater in the tank and in the field for the extraction of lectins at a commercial scale.

Key Words: *Eucheuma serra,* Deep seawater culture

INTRODUCTION

Different species of *Eucheuma and Kappaphycus* are commercially cultivated as a source of raw materials for carrageenan (Sahoo 2000). These carrageenanophytes usually require a high temperature and high light intensity for their growth. So the plants are mainly cultivated in tropical and sub tropical waters (Parker 1974; Adnan and Porse 1987; Mollion and Braud 1993; Lirasan and Twide 1993; Ohno *et al.* 1994; Gerung and Ohno 1997). Due to the industrial importance of *kappa* and *iota* carrageenan a large number of laboratory and field studies have been conducted in *K. alvarezii*, *K. striatum* and *E. denticulatum* (Dawes and Koch 1991; Dawes *et al.* 1993 and 1994). The presence of high amount of lectins have been reported in the tissue of *E. serra* (Kawakubo *et al.* 1997). More recently lectins in substantial quantity have been extracted from a few other species of *Eucheuma* (Kawakubo *et al.* 1999). Lectins are carbohydrate-binding proteins of non-immune origin and have several important uses (Sharon and Lis 1990). The tissue of *E. serra* contains high amount of lectins and the yield is higher compared to other macroalgae and marine organisms (Kawakubo *et al.* 1999).

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Since the plants of *E. serra* are not available in harvestable quantity in nature and they grow at a depth of 5-10 meters, it is difficult to commercially exploit them. So in the present investigation we tried to grow *E. serra* at different light and temperature conditions in the laboratory to find out the optimum conditions for its growth. We also cultured the plants in the field at different depths to find out suitable conditions for the mass cultivation. A significant purpose of the experiment was also to find out whether deep seawater can be used to culture algae to achieve high growth rate.

MATERIAL AND METHODS

Eucheuma serra was collected by SCUBA diving from the Pacific Ocean, Mugi Bay, Tokushima Prefacture, Shikoku, Southern Japan. The plants were brought to the laboratory and cleaned up of all visible epiphytes. 10 gms of thalli were then transferred to a tank containing deep seawater and cultured in the running deep seawater under natural day:night condition at Kochi Prefactural Deep seawater Research Laboratory, Muroto. The alga was allowed to multiply for several months to produce enough biomass for further experiments.

Laboratory culture

200 gms of fresh material were brought to the labora-

tory at Usa Marine Biological Institute located at Tosa Bay for the laboratory and field experiments. All visible epiphytes were removed by hand and then with a brush under a Nikon stereomicroscope. The plants were kept in a 2 liter culture jar under running seawater in a low light condition for a week for acclimation. Young and healthy thalli were cut into small pieces weighing 5 gms each. Five replicates were selected for each set of experiments. The thalli were placed in 2 L of autoclaved seawater at pH 8.4 and salinity 34 ppt. The plants were incubated at 15, 20, 25, 30°C at a light intensity of 80 μmol photon $m^{-2} \cdot s^{-1}$ with 12:12 light: dark cycle. One set of culture was also kept under the shade in natural day: night condition during the month of November. Since most of the plants in the 2 liter jars bleached, degenerated and subsequently died within a week except the experimental set up kept under the shade, so two more experiments were conducted. In the next attempt, a second set of plants were incubated at 12, 15, 18, 20, 25°C with a light intensity of 30 μmol photon $m^{-2} \cdot s^{-1}$ and a third set of plants were incubated at the same temperature with a light intensity of 40 μmol photon $m^{-2} \cdot s^{-1}$. Since the plants grew well at a combination of 18°C and 30 μmol photon $m^{-2} \cdot s^{-1}$ light, we set up a final experiment at 18°C with a light intensity of 7, 15, 20, 30, 40 and 50 μmol photon $m^{-2} \cdot s^{-1}$.

The increase and decrease in weight of plants were measured after every week and the media were changed. Daily Growth Rate (DGR = percent growth d^{-1}) were calculated using a linear growth rate formula because growth rate was observed to be linear in the laboratory cultures:

$$DGR = W_f - W_i / W_i \, 100 / \#d$$

Where W_f = final weight, W_i = initial weight and d = number of days between W_f and W_i.

Field culture

During the month of December, five replicates weighing 5gm each were taken in small plastic net bags and were hung at 1, 2 and 3 meter depth from the floating experimental station in the Uranouchi inlet of Tosa bay. The pH and salinity of seawater were measured in the laboratory. The temperature and light intensity of seawater at different depths were measured by a thermometer and photometer respectively. The plants were brought to the laboratory at weekly intervals, cleaned up of epiphytes and after taking the fresh weight again put back in the field. The field experiment was conducted for a period of 8 weeks.

Table 1. Water analysis data of Deep seawater and Surface seawater

	Surface seawater	Deep seawater
Temp (°C)	18-31	13-15
PH	8.3	8.3
Salinity (ppt)	34	34
DO ($mg \cdot L^{-1}$)	8.33	7.28
Na (%)	0.97	1.00
Mg (%)	0.13	0.133
Ca ($mg \cdot L^{-1}$)	42.1	42.6
K ($mg \cdot L^{-1}$)	40.6	41.9
Br ($mg \cdot L^{-1}$)	79.1	80.8
Sr ($mg \cdot L^{-1}$)	7.91	8.03
B ($mg \cdot L^{-1}$)	4.75	4.69
Ba ($mg \cdot L^{-1}$)	0.025	0.045
F ($mg \cdot L^{-1}$)	0.53	0.5
SO_4 ($mg \cdot L^{-1}$)	268	2770
NO_3-N ($\mu g\text{-at} \cdot L^{-1}$)	**1.49**	**25.9**
PO_4-P ($\mu g\text{-at} \cdot L^{-1}$)	**0.34**	**1.65**
SiO_2-Si ($\mu g\text{-at} \cdot L^{-1}$)	**13.6**	**64.2**
Pb ($\mu g\text{-at} \cdot L^{-1}$)	0.099	0.111
Cb	0.009	0.029
Cu	0.320	0.173
Fe	0.371	0.281
Mn	1.214	0.153
Ni	0.33	0.375
Zn	0.66	0.71
As	0.33	0.41
Mo	7.81	7.73

Culture in Deep seawater

Five gms of plants were transferred to transparent culture tanks with the continuous supply of deep seawater and bubbled with an aerator. The tanks were kept in the shade under the natural day: night condition at 18°C. (The deep seawater was pumped from a depth of 320 meter at Kochi prefactural deep seawater laboratory at Muroto). The thalli were cleaned at weekly intervals and weight was recorded.

Daily Growth Rate for both the field and deep seawater cultured materials were calculated using a formula that takes into account exponential growth:

$$DGR = \ln (W_f - W_i) \, 100 / \#d$$

Where In = normal log of the qutient of final over initial weight.

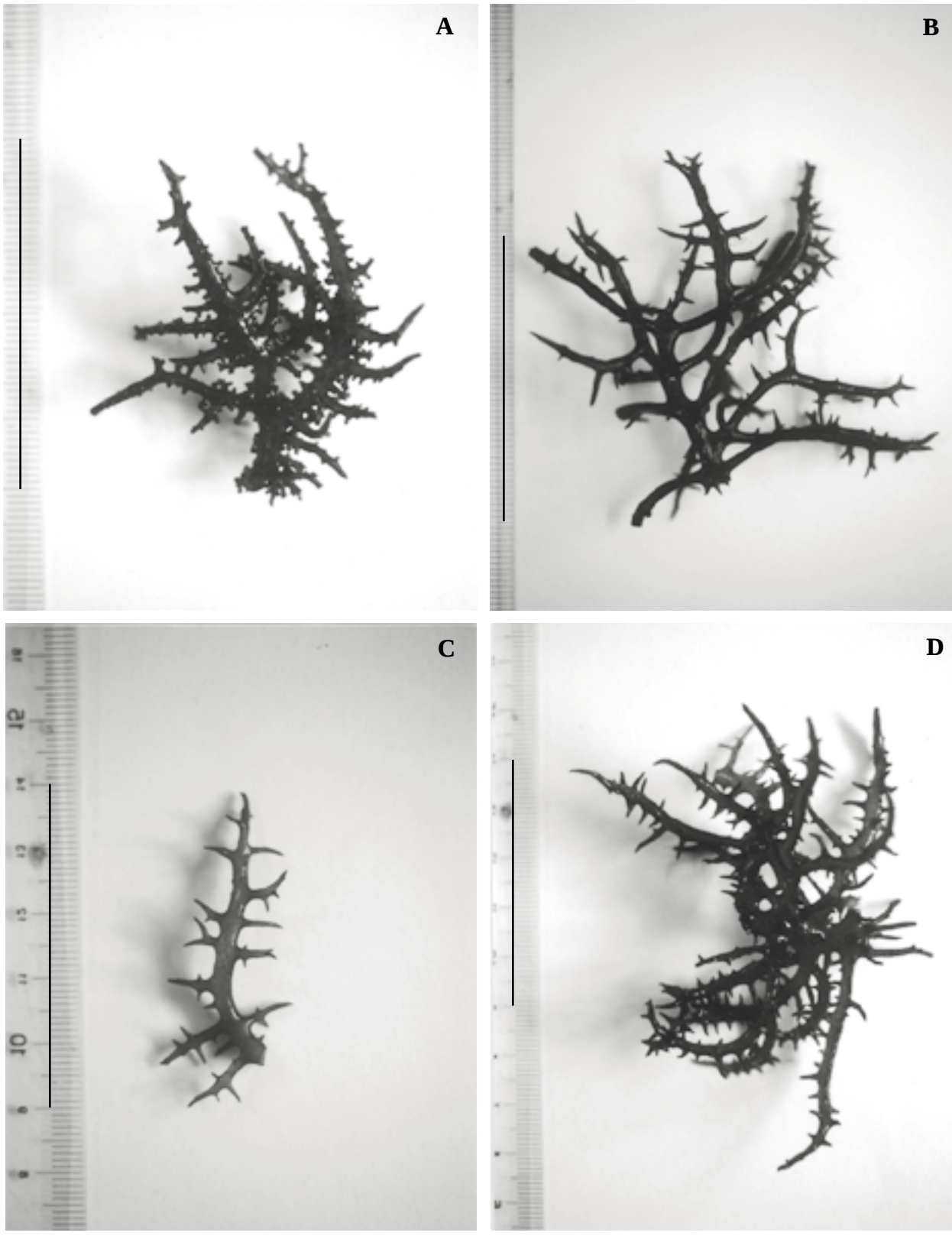

Fig. 1. Thallus of *Eucheuma serra* J. Agardh. (scale bar = 5 cm)
A. Morphotype I showing wort like protrubances on thaullus. B. Morphotype II which is compressed, showing distinct spines. C. A small piece of thallus before culture. D. Thallus showing extensive growth after cultured in deep seawater.

Table 2. Daily Growth Rate (DGR) of *E. serra* in the laboratory at different light and temperature conditions

Light intensity (μmol photon $m^{-2} \cdot s^{-1}$)	Temperature (°C)	DGR (%)	Colour of the thallus
30	12	0	Normal colour
30	15	1.87 ± 0.69	Normal colour
30	18	3.5 ± 1.4	Normal colour
30	20	1.62 ± 0.56	Normal colour
30	25	Negative growth	Discoloured
40	12	0	Slightly light colour
40	15	1.62 ± 0.56	Slightly light colour
40	18	1.87 ± 0.69	Slightly light colour
40	20	0.82 ± 0.75	More light colour
40	25	Negative, Bleached	Complete bleaching
7	18	0	Normal colour
15	18	0	Normal colour
20	18	0	Normal colour
50	18	Bleached	Discoloured

RESULTS

The water analysis data of normal seawater and deep seawater showed a significant difference in the nitrate, phosphate and silicate concentration. The Nitrate concentration is nearly 20 times higher in deep seawater as compared to normal seawater where as the phosphate and silicate concentrations is almost 5 times higher (Table 1).

In the field, two morphotypes of *E. serra* was observed. In Type 1, the thallus is dark red in colour, and branches are compressed with small wort like protrubances distributed on the surface (Fig. 1A). On the other hand in Type 2, the thallus is light red in colour, slightly compressed to cylindrical and distinct spines are present on the margin of the thallus (Fig. 1B). No reproductive structures were observed in both the specimens.

In the first set of experiment when the thalli were cultured at 15, 20, 25, and 30°C at a light intensity of 80 μmol photon $m^{-2} \cdot s^{-1}$, the degeneration of the thalli were observed at the cut ends of the thallus on the second day while the tips remained healthy. Subsequently, bleaching of tissue all over the thallus caused heavy damage and ultimate death of plants. The thalli cultured in the shade under natural day:night conditions remained very healthy after a week. A temperature range of 12-18°C and a light intensity of 20-40 μmol photon $m^{-2} \cdot s^{-1}$ were measured during the month of November. The low light intensity and low temperature were due to the cloudy conditions in the atmosphere during the month. This result prompted us to predict the temperature and light range for the further experiments. When the plants were cultured at 12, 15, 18, 20 and 25°C under a light intensity of 30 μmol photon $m^{-2} \cdot s^{-1}$, almost no growth was observed at 12°C, whereas, a DGR of 3.5 ± 1.4% was observed at 18°C (Table 2). But at 25°C the plants showed a negative growth. But in none of the cases thallus discolouration was observed except at 25°C. The thalli cultured at the above temperature but at 40 μmol photon $m^{-2} \cdot s^{-1}$ light intensity showed a decreased growth rate at 15°C and 18°C and the bleaching and negative growth rate at 20 and 25°C. No growth was observed at 12°C (Table 2). The plants cultured at 18°C with low light of 7,15 μmol photon $m^{-2} \cdot s^{-1}$ produced zero growth. Whereas at 18°C and 20 μmol photon $m^{-2} \cdot s^{-1}$ the growth was very slow. But at 50 μmol photon $m^{-2} \cdot s^{-1}$ and 18°C the thallus started bleaching. During all these experiments in the laboratory the best growth was observed at 18·C and 30 μmol photon $m^{-2} \cdot s^{-1}$.

In the field, a temperature of 18°C was recorded during the experiment period at all the three depth 1 m, 2 m, 3 m. The salinity and PH of the seawater at these depths were also nearly same at 34 ppt and 8.4 respectively. However light intensity varied at different depth having 30-35 μmol photon $m^{-2} \cdot s^{-1}$ at 1 m, 24-30 μmol photon $m^{-2} \cdot s^{-1}$ at 2 m and 25-29 μmol photon $m^{-2} \cdot s^{-1}$ at 3 m depth. At one-meter depth the plants showed a Daily Growth Rate of 2.14%, where as at 2 meter and 3 meter depth it showed a DGR of 1.14% and 0.87% respectively (Table 3).

Usually when the deep seawater is being pumped, the water temperature is between 13-15°C. For the seaweed culture, the water temperature is brought to 18°C. In the

Table 3. Daily Growth rage (DGR) of *E. serra* in the sea at different depth

Depth (m)	Light intensity (μmol photon $m^{-2} \cdot s^{-1}$)	Water temperature (°C)	DGR (%)	Colour of thallus
1	30-35	18	2.14 ± 0.04	Normal colour
2	24-33	18	1.14 ± 0.02	Normal colour
3	24-28	18	0.87 ± 0.06	Normal colour

tank when the plants were cultured in the deep seawater (Fig. 1C) surprisingly the thalli showed unusual high growth (Fig. 1D). A DGR of 8.2% was observed for the month of November and December. The thallus of both the morphotypes maintained deep purple red colour.

DISCUSSION

In the present study, two morphotypes of *Eucheuma serra* were reported, which show distinct morphological and colour variations. However, no difference in the growth rate was observed in response to light, temperature and depth. Both the morphotypes showed high rate of growth in "deep seawater". Similar morphotypes of various *Eucheuma and Kappaphycus* species were earlier reported by various workers, but differences in one growth of morphotypes were obserwed (Hurtado-Ponce 1995; Dawes 1995).

Light, temperature and nutrients are three important factors which play an important role for the growth and development of seaweeds. Besides, these factors, salinity, season, depth of water and water motion also play an important role in seaweeds growth (Harrison and Hurd 2001).

Results of our study indicated that *E. serra* prefers low light and low temperature for its growth in contrast to other species such as *E. denticulatum* and *Kappaphycus alvarezii.* The latter needs temperature of at least 25°C and above and a light intensity of 125 μmol photon $m^{-2} \cdot s^{-1}$ (Ohno 1977; Dawes *et al.* 1994; Gerung and Ohno 1997). *E. serra* seems to be more sensitive to the light in comparison to temperature as a higher photon fluxes beyond 40 μmol photon $m^{-2} \cdot s^{-1}$ bleached the thallus beyond repair. This may be due to breakdown of photolabile pigments such as phycoenythrin. Jones (1959) found bleaching and death of *Gracilaria verrucosa* thallus when exposed to high light and temperature. Dawes (1995) found that the red form of *Eucheuma denticulatu*m showed a positive Daily Growth Rate (DGR) compared to green form when exposed to higher photon fluence level. But in the present study no such difference of growth in two morphotypes could be noticed. A low light intensity below 30 μmol photon $m^{-2} \cdot s^{-1}$ prevented the growth of the thallus but the pigments were ratained. The present study concludes that a temperature of 18°C with 30 μmol photon $m^{-2} \cdot s^{-1}$ of light intensity is the most suitable condition for growth of *Eucheuma serra.*

In the field grown material, best growth was observed at a 1 meter depth. Although the water temperature remained at 18°C at all 3 different depths, the light intensity varied substantially. The availability of light at 1 meter depth was between 30-35 μmol photon $m^{-2} \cdot s^{-1}$. The field result was substantiated by the laboratory data, which reported the best growth at 18°C and 30 μmol photon $m^{-2} \cdot s^{-1}$. Molloy and Bolton (1996) studied the effect of season and depth on growth of *Gracilaria gracilis* wherein they found the optimal growth of the thallus between 0.5-2.5 meter. The most obvious explanation for the pattern of growth with depth is light availability. Temperature did not play a major role. Similar observations were also made in different species of *Gracilaria* where light density played a major role rather than the temperature at different depths (Friedlarder *et al.* 1987; Engledow and Bolton 1992). Hurtado-Ponce (1995) cultured *Kappaphycus alvarezii* at two different depths of 0.5 and 1 meter depth and found significant difference in the growth rate. Result of the present study supports observations by other workers that depth plays a significant role for seaweed growth.

In the present study a high growth rate of nearly 8.2% was found in *E. serra* when cultured in deep seawater. Such a high growth rate can be attributed to the availability of high amount of nutrients such as nitrate and phosphate in deep seawater. It has been found that *Kappaphycus alvarezii* also grow much faster when cultured in deep seawater (Sahoo and Ohno unpublished). Species of *Laminaria*, *Ecklonia* and *Undaria* have been cultured in deep seawater at the deep seawater research institute, Muroto, Japan. Although they grow healthy but did not report a very high growth rate (Yamaguchi *et al.* 1994). In general, nutrient requirements of seaweeds

are divided into three categories, macronutrients (e.g. N, P, C, etc.), micronutrients or trace elements (e.g. Fe, Zn, Co, Mn, Mo, etc.) and (Vitamins B_{12}, Thiamin, and Biotin). (Harrison and Hurd 2001). Interestingly all these nutrients are found in deep seaweter. Nitrogen and phosphorus are the two nutrients that limit most of the seaweeds growth. It has been found that seaweeds may grow faster if NO_3^- and PO_4^{3-} are added to the seawater. The effect of NO_3^- and PO_4^{3-} has been well studied in several species of seaweeds. Previous studies demonstrated that nitrogen supply has significant effect on the growth rate of various agarophytes and carrageenophytes (Rui *et al*. 1990; Chopin and Wagey 1999; Ryder *et al*. 1999). In recent years it has been found that deep seawater which contains a high concentration of NO_3^- and PO_4^{3-} has wider applications including aquaculture (Sahoo and Ohno 2001). Our study suggests that *E. serra* can be commercially cultivated at a depth of 1 meter during winter and can be cultured in mass scale in tanks in deep seawater during summer season and other parts of the year when the water temperature is higher than 18°C. Since deep seawater is much colder (13-15°C) compared to surface seawater (18-31°C), it can be mixed in different proportions to get a desired temperature to culture *Euchuma*. Similarly, nutrient concentrations can be manipulated by the mixing of different proportions of both surface seawater and deep seawater. Such manipulation of water temperature and nutrient conditions can be created to manipulate algal growth and reproduction at any time of the year. Since, deep seawater is clean, non-contaminated and rich in nutrients, it can be used as a cheap source of culture medium.

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