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Effects of temperature and irradiance on early development of *Chondrus ocellatus* Holm (Gigartinaceae, Rhodophyta)*

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Abstract *Chondrus* is a type of commercially produced red seaweed that widely used for food and carrageen extraction. Although the natural life history of the alga had been well understood, the factors influencing development of the tetraspore and carpospore remain poorly understood. In the perspective of seedling resources, the regulation of early development is crucial for the seedling nursing; therefore, it is necessary to understand the physiological influences during its early development. In this study, we studied the effects of temperature and irradiance on the early development of *Chondrus ocellatus* Holm under laboratory conditions. The released tetraspores and carpospores were cultivated at different temperatures (10–28°C) and irradiances (10, 60 μmol photons $m^{-2}s^{-1}$) with a photoperiod of 12L:12D. The results indicate that both tetraspores and carpospores are tolerant to temperatures of 10–25°C, and have the highest relative growth rate at 20°C. Irradiance variances influenced the growth of the discoid crusts, and the influence was more significant with increasing temperature; 60 μmol photons $m^{-2}s^{-1}$ was more suitable than 10 μmol photons $m^{-2}s^{-1}$. The optimum temperature and irradiance for the development of seedlings was 20°C and 60 μmol photons $m^{-2}s^{-1}$, respectively.

Keyword: *Chondrus ocellatus*; temperature; irradiance; early development

1 INTRODUCTION

Chondrus ocellatus Holm is one kind of commercially produced red seaweed, which mainly inhabits coastal areas of Korea, Japan, China and Taiwan (Taylor et al., 1994). Its niche is in substrates from high to low tide regions. Usually, the juvenile seedlings appear in autumn and mature in summer; the alternative life history includes the following stages: tetrasporophyte, gametophyte and carposporophyte (Hori, 1993).

Because of its significant yields of carrageen and other industrial materials, many studies have been conducted on this genus (Burns et al., 1972; Noda, 1990; McLachlan, 1991). However, there are very limited reports on *C. ocellatus*, especially in regard to its developmental and physiological properties. Enright et al. (1981) reported that the growth rate of *C. ocellatus* increased at temperatures of 2–14°C and growth decreased at 23°C. Ji et al. (1992) reported that 15–20°C was suitable for development of *C. ocellatus* seedlings, and the diameter of the discoid crust increased at 20°C. Kim et al. (2006) investigated the growth and reproductive phenology of *C. ocellatus* in Busan, Korea. Wang et al. (2006) observed the early development of *C. ocellatus*, and hypothesized that development could be divided into three stages: the division stage, discoid crust stage and seedling stage. However, the exact physiological parameters are not completely understood; therefore, intensive studies on the effects of light and temperature on early development of the tetraspore and carpospore are required. In addition, the physiological characteristics of the

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species from different origins may be different.

The objective of our study is to investigate the effect of temperature and irradiance on the early development of *C. ocellatus* collected from natural habitats in Qingdao, China. We aim to understand the physiological properties, and to establish the optimal culture conditions for the seedlings to provide the possibility of artificial seedlings in the future.

2 MATERIALS AND METHODS

Mature *C. ocellatus* specimens were sampled in an intertidal zone of the seashore (36°02′40″N, 120°20′08″E), Qingdao, China, from Nov. to Dec. 2008. The collected mature gametophytes and tetrasporophytes (Figs.1a, 1b) were brushed and washed with sterilized seawater for elimination of epiphytes, then immersed in 1% KI for 20 min for axenic treatment. They were then rinsed thoroughly with sterilized seawater. After drying for 1 h, the spores were released from the mature tetrasporangia and cystocarps of the matured thalli. After incubation for one night, the thalli were removed and the released tetraspores and carpospores were transferred to new petri dishes containing culture medium (PES) (Provasoli, 1968). The culture media was renewed every 3 days.

The petri dishes containing the spores were cultured in incubators (Jiangnan, China) at controlled light and temperature conditions. The tetraspores and carpospores were cultured at six different temperatures (10, 15, 20, 22, 25, 28°C) and two irradiances (10, 60 μmol photons $m^{-2}s^{-1}$), with a photoperiod of 12L:12D. Each treatment was replicated three times for parallel tests. Observations were conducted each day under a microscope (Olympus, BX51) with a digital camera (Qimaging, MicroPublisher 5.0 RTV). Based on the comparative tests, we chose spores cultured at 20°C and 60 μmol photons $m^{-2}s^{-1}$ as the main sample for morphological observation. Growth of discoid crusts

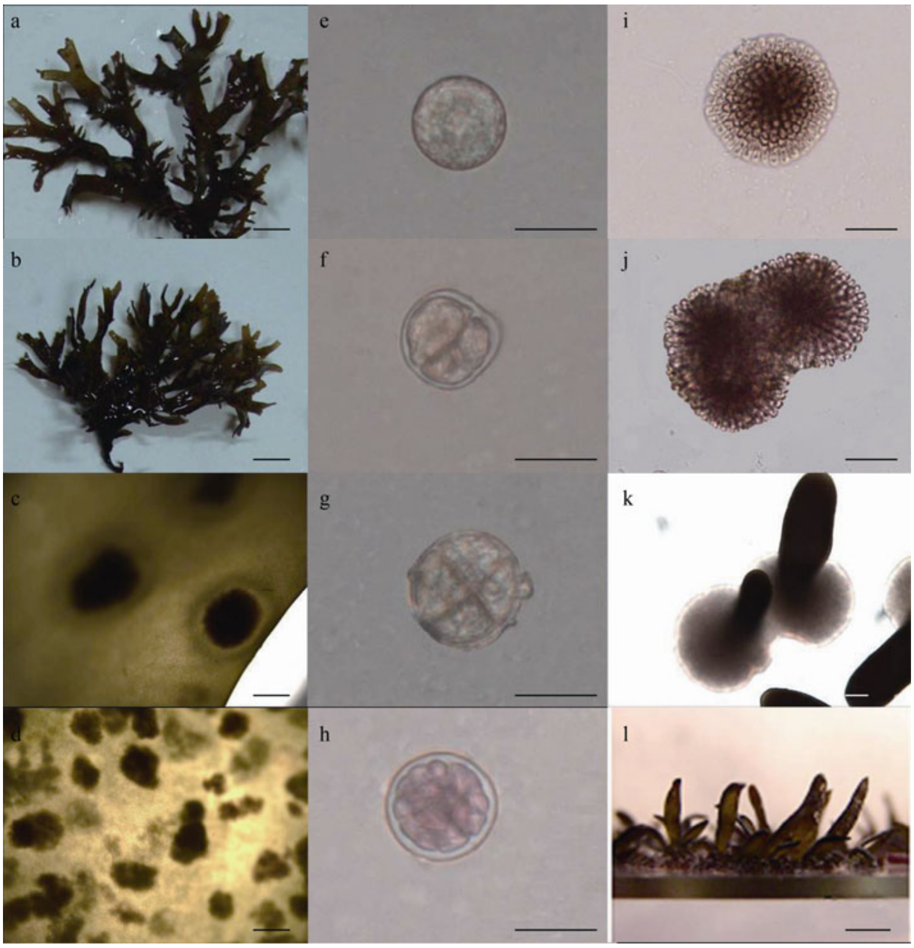

Fig.1 The morphology and early development of *Chondrus ocellatus* Holm

a, mature gametophyte, bar=1 cm; b, mature tetrasporophyte, bar=1 cm; c, cystocarp, bar=1 mm; d, tetrasporangium bar=1 mm; e, newly-released spore, bar=20 μm; f, two even cells stage (division stage), bar=20 μm; g, four cells stage (division stage), bar=20 μm; h, multicellular stage (division stage), bar=20 μm; i, discoid crust stage; bar=100 μm; j, discoid crust coalescence, bar=100 μm; k-l, juvenile seedling stage, bar=100 μm and 5 mm, respectively.

was measured each week under the microscope using a visual micro-scale. For each test, 50 samples from each petri dish were measured. The relative growth rate (RGR) was calculated using the discoid crust diameter data according to the following formula:

$$RGR= (\ln N_t - \ln N_0) / T$$

where N_0 is the initial diameter and N_t is the diameter after T days.

One-way ANOVA tests followed by Tukey's multiple comparisons were used to examine the effect of temperature on diameter variations of the discoid crust cultivated at 60 μmol photons $m^{-2}s^{-1}$. Independent-sample t tests were conducted to compare diameter variations at different irradiance treatments. All statistical analyses were performed using SPSS 13.0 with the level of significance set at $P<0.01$.

3 RESULTS

3.1 Observations on early development of *C. ocellatus*

The morphological changes that we observed during the early development of *C. Ocellatus* (Fig.1) were basically the same as those reported by Wang et al. (2006).

3.2 Effect of temperature on growth of the discoid crust of the tetraspore

The effect of different temperatures on the growth of germlings was investigated (Fig.2). Temperature significantly affected expansion of the discoid crust at an irradiance of 60 μmol photons m^{-2} s^{-1} in weeks 1, 2, 3 and 4 (ANOVA: F=26.44, 118.74, 284.78, and 292.70, $P<0.01$, respectively) (Fig.2).

Germling growth occurred between 10 and 25°C. However, at 28°C the spores divided into several cells, but did not develop any further at both 10 and 60 μmol photons $m^{-2}s^{-1}$; they turned pale or even died after cultivation for about one week. After the first week of culture, the germlings at 10–25°C only just developed or would develop into discoid crusts, and the discoid varied significantly (ANOVA: F=26.44, $P<0.01$); the difference appeared more obvious in the second week (ANOVA: F=118.74, $P<0.01$), and even more significantly after four weeks (ANOVA: F=292.70, $P<0.01$). The growth of the tetraspores increased from 10 to 20°C, and reached a maximum at 20°C, but growth decreased after 20°C (Fig.2). After four weeks culture, the largest diameter of the discoid crust was 364.3±44.1 μm, which appeared at 20°C; the next largest were those at 22°C, 15°C and then those at 25°C (Fig.2).

Although the growth rate of spores at 25°C was higher than those at 15°C at the beginning, growth at 25°C became lower than that at 15°C after three weeks cultivation, and growth and development of germlings at 10°C was the slowest (Fig.2).

3.3 Effect of temperature on growth of the discoid crust of the carpospore

Growth and development of discoid crusts from the carpospores had a broad tolerance to temperatures at 10–25°C. With increasing temperature, the growth rate increased rapidly, but declined at over 20°C (Fig.3). Temperature significantly affected growth of the discoid crust at an irradiance of 60 μmol photons $m^{-2}s^{-1}$ (ANOVA: F=12.69, 104.84, 213.69, and 217.09, $P<0.01$, respectively) (Fig.3). Generally, there was rapid growth of the discoid crust at 15–25°C, and it reached a maximum at 20°C.

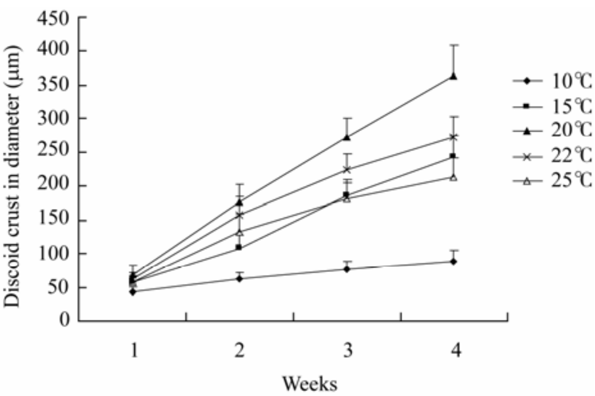

Fig.2 Growth of discoid crusts from *C. ocellatus* tetraspores cultured for 4 weeks at different temperatures and an irradiance of 60 μmol photons $m^{-2}s^{-1}$

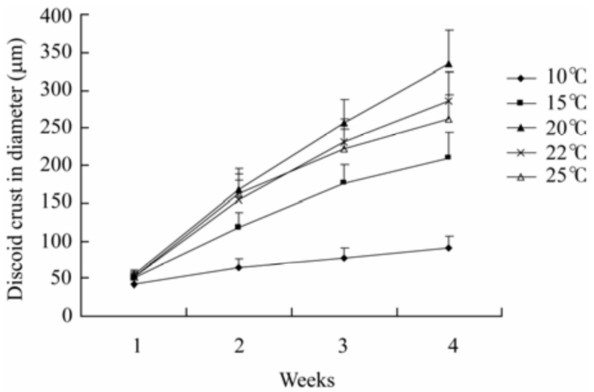

Fig.3 Growth of discoid crusts from *C. ocellatus* carpospores cultured for 4 weeks at different temperatures and an irradiance of 60 μmol photons $m^{-2}s^{-1}$

3.4 Effect of irradiance on growth of tetraspore and carpospore germlings

The effect of irradiance on the growth of the discoid crusts of the tetraspores and carpospores was also observed (Figs.5, 6). High light intensity (60 μmol photons $m^{-2}s^{-1}$) significantly accelerated the growth rate (P<0.01) compared with low light intensity (10 μmol photons $m^{-2}s^{-1}$). As indicated in Figs.4 and 5, at 15, 20 and 25°C irradiance influenced the growth of the discoid crusts, and with increasing temperature this influence was more significant. However, at 10°C irradiance had only a slight effect on the discoid crust diameter. Moreover, at over 20°C, at an irradiance of 60 μmol photons $m^{-2}s^{-1}$ the diameter was twice that at an irradiance of 10 μmol photons $m^{-2}s^{-1}$ (Figs.5, 6).

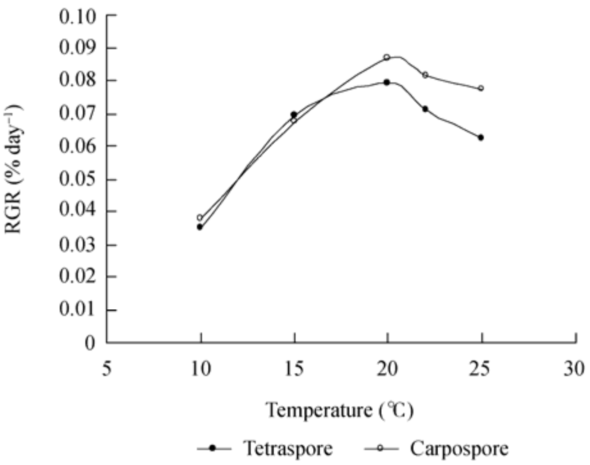

Fig.4 Relative growth rate of discoid crusts developed from tetraspores and carpospores of *C. ocellatus* at different temperatures and an irradiance of 60 μmol photons $m^{-2}s^{-1}$

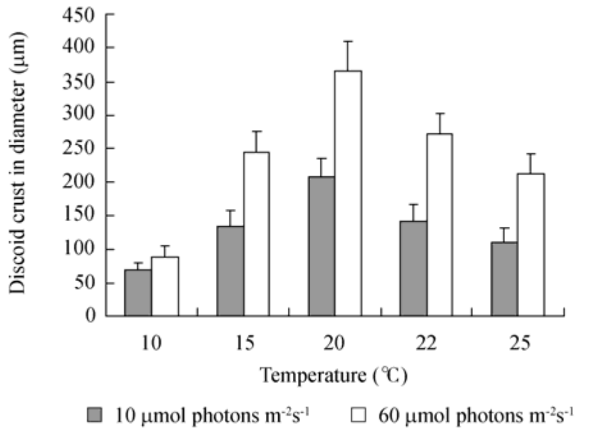

Fig.5 Growth of the discoid crust of tetraspores cultured for 4 weeks at various temperatures and an irradiance of 10 μmol photons $m^{-2}s^{-1}$ and 60 μmol photons $m^{-2}s^{-1}$

3.5 Effect of temperature and irradiance on early development of juvenile seedlings

Both tetraspores and carpospores can develop into seedlings at a wide range of temperatures (10–25°C) and irradiances (10–60 μmol photons $m^{-2}s^{-1}$) (Table 1). Generally, at the same temperature, juvenile seedlings formed earlier at a higher irradiance, and the earliest formation of seedlings occurred at 20°C and 60μmol photons $m^{-2}s^{-1}$. At 10 μmol photons $m^{-2}s^{-1}$, high temperatures enhanced discoid crust development and seedling formation (Table 1). A low temperature (10°C) and low irradiance (10 μmol photons $m^{-2}s^{-1}$) inhibited early development of the tetraspores and carpospores. At 25°C and irradiances of 10 or 60 μmol photons $m^{-2}s^{-1}$, tetraspores and carpospores developed into the seedling stage almost simultaneously.

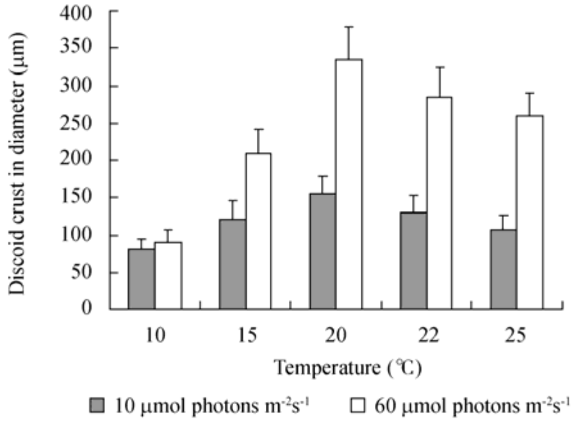

Fig.6 Growth of the discoid crust of carpospores cultured for 4 weeks at various temperatures and an irradiance of 10 μmol photons $m^{-2}s^{-1}$ and 60 μmol photons $m^{-2}s^{-1}$

4 DISCUSSIONS

In general, the tetraspores and carpospores grew and developed at 15–25°C, and the optimal growth occurred at 20°C. Our results were consistent with those reported for *C. crispus* by Burns et al. (1972); the two species have a similar tolerance to temperature. At 10 and 15°C, the relative growth rate of the tetraspores was very similar to that of the carpospores. However, within the high temperate range of 20–25°C, the growth rate of tetraspores was lower than that of carpospores (Fig.4). This is in accordance with the natural growth rhythm of *C. ocellatus*. The spore releasing peak for *C. ocellatus* appeared twice throughout the year in a natural sea area: in June-July (15–20°C) tetraspores were relatively dominant, while in July-August carpospores dominated, when seawater temperature was the highest of the whole year (Ji et al., 1992). These results suggest that carpospores are better adapted to high temperatures; ie 20–25°C.

Table 1 Formation time of juvenile seedlings cultured at various temperatures and irradiances

	Irradiance (μmol photons $m^{-2}s^{-1}$)	10°C	15°C	20°C	22°C	25°C	28°C
Carpospore	60	~50 d	~40 d	~28 d	~33 d	~35 d	--
	10	~70 d	~60 d	~40 d	~38 d	~37 d	--
Tetraspore	60	~50 d	~35 d	~28 d	~33 d	~37 d	--
	10	~70 d	~55 d	~40 d	~39 d	~38 d	--

We found that erect seedlings formed when the discoid crusts grew to a certain size; the cell mass that grows from the spores would not develop into a discoid crust until the number of cells reached a critical value. After four weeks cultivation at 20°C and an irradiance of 60 μmol photons $m^{-2}s^{-1}$, the discoid crusts reached 250 μm in diameter and formed juvenile seedlings. However, at 20°C and 10 μmol photons $m^{-2}s^{-1}$, it took 5 weeks to grow discoid crusts (250 μm in diameter) without any erect seedlings; seedlings appeared on the discoid crusts (320 nm in diameter) after 6 weeks culture. We believe that the high light intensities stimulated the rapid development of discoid crusts and the formation of seedlings, and that specimen size is a threshold for sporeling development into the next stage, possibly because of the necessary nutritional storage requirements for formation of discoid crusts and erect seedlings. The gene expression regulation mechanism during early development needs to be explored.

The attachment of spores influenced the germination and early development of germlings. In the early stage, the spores did not attach firmly. Even mild perturbations could cause them to fall off the substratum, and many unattached spores eventually failed to germinate and develop normally. Similar phenomena were reported for *Gelidium floridanum* (Bouzon et al., 2005; 2006). Fonck et al. (2008) suggested that the time of the drifting thallus, type of substratum and photon flux density affected the reattachment capacity of the thallus of *C. chamissoi*. Here, we speculate that photon flux density is a crucial factor for the attachment of spores during static culture.

In many red algae, coalescence of discoid crusts is usually observed during the early development of tetraspores and carpospores (Jones, 1956; Komiyama et al., 1957; Chen et al., 1985; Vera et al., 2008). We found that usually two or more adjacent discoid crusts merged to form a larger discoid crust and then developed into a juvenile seedling (Fig.1j). Santelices et al. (2004) reported that sporeling size determined the fate of coalescing germlings; however, we did not observe this phenomenon, possibly because of different sporeling densities, or the algal genus or species. Coalescence of the discoid crusts would be beneficial to the growth and early development of juvenile seedlings of red seaweeds.

5 CONCLUSIONS

Early development of tetraspores and carpospores of *C. ocellatus* exhibited a broad tolerance to temperatures from 10 to 25°C, and an irradiance 10 to 60 μmol photons $m^{-2}s^{-1}$. Temperature significantly influenced the growth and early development of juvenile seedlings. Optimal culture conditions were at 20°C and an irradiance of 60 μmol photons $m^{-2}s^{-1}$. A low temperature (10°C) and low light intensity (10 μmol photons $m^{-2}s^{-1}$) severely stunted growth and development.

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