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Note

Effect of light intensity on the cyst germination of *Chattonella* spp. (Raphidophyceae)

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Chattonella spp. (*C. antiqua* and *C. marina*) are noxious red tide flagellates. The red tides caused by these species caused serious damage to fish culture programs in western Japan in 1970s–1980s (Okaichi 1989).

Many flagellates produce cysts, spend a dormant period, and exist in the sediments during environment conditions unsuitable for vegetative growth (e.g. Wall 1975; Anderson et al. 1983; Dale 1983; Ishikawa & Taniguchi 1996).

Chattonella cysts were found from the sediments in the Seto Inland Sea, Japan in 1986 (Imai & Itoh 1986; 1988), then many studies concerning the physiology and the ecology of *Chattonella* cysts were carried out. According to the reports, *Chattonella* cysts need low temperatures for at least four months to mature (Imai & Itoh 1987; Imai et al. 1989), the germination is most frequent at 20°C–25°C (Imai et al. 1984), and *Chattonella* cysts can germinate under low dissolved oxygen concentrations (Montani et al. 1995).

We show here the response to light intensity of *Chattonella* spp. during cyst germination. This differs from those reports of other flagellates observed previously.

Sediment samples were collected in the Harima Nada area of the Seto Inland Sea using a core sampler at Stn. NH (34°28′N, 134°24′E: about 35 m depth) in June 1991 and an Ekman grab sampler at Stn. 21 (34°15′N, 134°26′E: about 25 m depth) in May 1992. The surface sediments (upper 2 cm) were used for the experiments. Sediment samples were kept at 10°C in the dark, and germination experiments were carried out within three months of the sediment collections.

Sixty grams of sediment sample from Stn. NH was sieved through 125 μm mesh and on to 25 μm mesh. This sediment fraction (25–125 μm) was divided into six 100-ml flasks with 10 ml of filtered seawater as a medium. Three flasks were incubated under dark conditions (0 μmol photons $m^{-2} s^{-1}$), and the other three flasks were incubated in 70 μmol photons $m^{-2} s^{-1}$ (14:10 LD cycle) at 21°C for two weeks. The 10 ml of seawater in each the flask was taken out under low irradiance (<1 μmol photons $m^{-2} s^{-1}$) using a pastuer pipet and 10 ml of new filtered seawater was added to the flask every day. The number of germinated cells of *Chattonella* spp., *Scrippsiella* spp. and *Alexandrium* spp. in the seawater was monitored under the light microscope every day (Experiment 1).

Eighty grams of sediment sample from Stn. 21 was sieved through 125 μm mesh and on to 25 μm mesh. During the sieving treatment, cold filtered seawater (10°C) was used, because an increase in temperature can stimulate cysts to germinate. After the sediment fraction was divided into eight 100 ml flasks with 10 ml of filtered seawater added to each, the flasks were placed at 10°C in the dark for two weeks. Although the preparation of the experiment was carried out under normal light conditions, we considered that any effects of irradiance on cyst germination was canceled due to the subsequent incubation under dark conditions for two weeks. Two flasks each were placed under light intensities of 0, 1, 7 and 70 μmol photons $m^{-2} s^{-1}$ (14:10 LD cycle), respectively, and incubated at 21°C for two weeks. The germination of *Chattonella* spp. was monitored every day by the same procedure described above (Experiment 2).

In Experiment 1, the total number of germinated cells of *Scrippsiella* spp. and *Alexandrium* spp. at 70 μmol photons $m^{-2} s^{-1}$ were greater than those germinating under dark conditions (Fig. 1). These results agreed with previous reports for the cysts of dinoflagellates (Endo & Nagata 1984; Anderson et al. 1987), demonstrating that germination rate and frequency for both species were significantly lower under dark conditions. On the contrary, the number of germinated cells of *Chattonella* spp. was higher under dark conditions (Fig. 1). Also in Experiment 2, the number of germinated cells of *Chattonella* spp. tended to increase with a decrease in light intensity (Fig. 2).

In such germination experiments, the growth of diatoms in the medium sometimes influences the survival of germinated flagellates, especially under illuminated condition (Imai 1990). However, diatoms did not increase significantly over the

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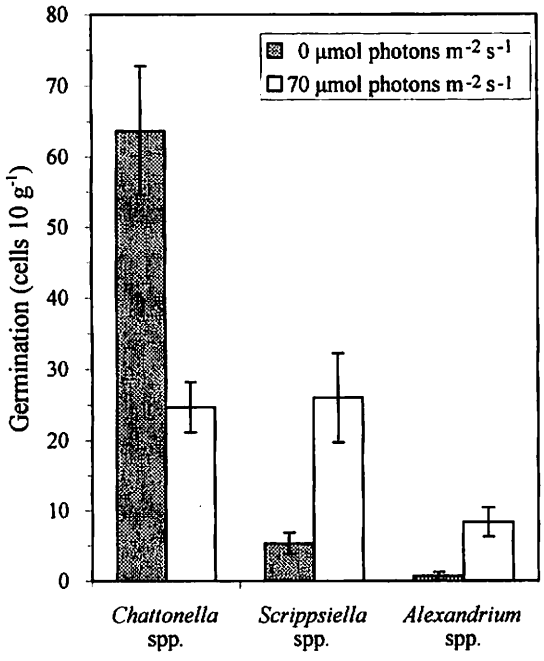

Fig. 1. Germination of natural cysts of three flagellates in sediment in the dark and under 70 μmol photons $m^{-2} s^{-1}$ illuminated conditions (solid bar = ±SD).

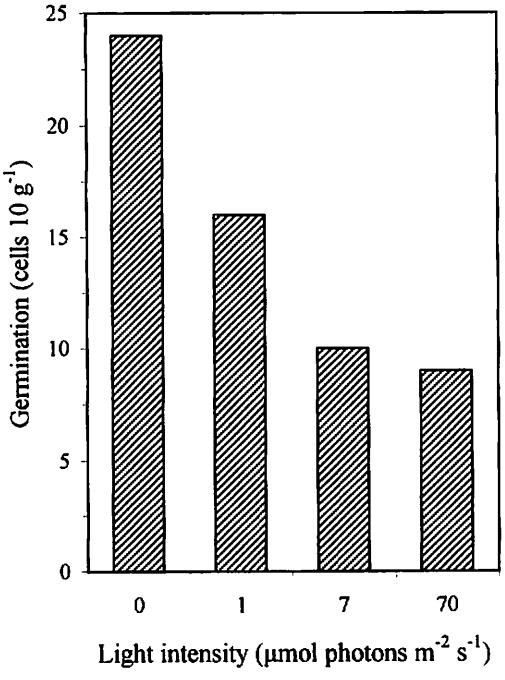

Fig. 2. Germination of natural cysts of *Chattonella* spp. in sediment at various light intensities.

experimental period. As hatched zooplankters were sometimes observed in the medium, there is a possibility that germinated cells were grazed by them to some extent. However, the existence of hatched zooplankters should not affect our conclusions as they were observed throughout the 0–70 μmol photons $m^{-2} s^{-1}$ illumination range.

Germination of *Chattonella* spp. at 70 μmol photons $m^{-2} s^{-1}$ was less frequent, however, the number of germinated cells reached close to 40% of that for the 0 μmol photons $m^{-2} s^{-1}$ experiment. Experiment 1 and Experiment 2 were carried out with the sediment still present in the incubation and, therefore, many *Chattonella* spp. cysts were probably covered with sediment. As a result, *Chattonella* spp. cysts might have been exposed to lower irradiance conditions and germinated. To clarify the actual effect of high irradiance on the cyst germination of *Chattonella* spp., isolated cysts removed from sediments should be used for the investigation. Another question arises as to whether non-germinated cysts under high irradiance conditions died or not. In this connection, the flasks that were subjected to 70 μmol photons $m^{-2} s^{-1}$ conditions were re-incubated under dark conditions after the end of Experiment 1. However, germination of *Chattonella* spp. was not observed, suggesting the loss of germination ability or death of the cysts. *Chattonella* cysts have the ability to undergo secondary dormancy if they are placed in unsuitable conditions for germination (Imai et al. 1989). One possibility for the relatively infrequent germination is that many *Chattonella* spp. cysts switched to secondary dormancy. On the other hand, the other possibility, that *Chattonella* spp. cysts died, still remains. We do not have an answer to this question. However, it is certain that germination of *Chattonella* spp. cysts is more frequent under low irradiance conditions.

Several studies concerning the effects of light intensity on cyst germination of flagellates have been carried out previously. According to these reports, three types of responses to light intensity exist concerning cyst germination. These are, (1) the cysts of *Gonyaulax polyedra* can not germinate completely in the dark (Anderson et al. 1987); (2) cysts of *Gonyaulax tamarensis* (*Alexandrium tamarense*) and *Scrippsiella* sp. can germinate in the dark, but the germination rate and frequency are lower (Endo & Nagata 1984; Anderson et al. 1987); (3) the germination of cysts of *Gonyaulax rugosum* and *Peridinium* sp. are not affected by darkness (Sako et al. 1985; Anderson et al. 1987). The germination of *Chattonella* spp. cysts was, in contrast, more frequent in the dark. Although it had already been reported that cysts of *Chattonella* spp. could germinate under dark conditions (Imai et al. 1984), our results show that cysts of *Chattonella* spp. have another physiological characteristic of light inhibition effect on the cyst germination. When the cysts of *Chattonella* spp. were produced in culture, the cyst yield was decreased with an increase of light intensity (Imai 1989; Nakamura & Umemori 1991). These findings suggest that irradiance works as a negative factor against the benthic stage of *Chattonella* spp.

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