CHUGOKUGAKUEN J. 2002
Vol. 1, pp. 7-12

CHUGOKUGAKUEN
Journal
http://www.cjc.ac.jp/

Original Article

Role of Symbiotic Algae on Gemmule Germination of a Freshwater Sponge, *Radiospongilla cerebellata*

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Annandale sponges (Radiospongilla cerebellata) have chlorellae as endosymbiotic algae and are distributed throughout the freshwaters of southwestern Japan. Gemmules of this sponge hatch only under an illuminated condition. The hatch is perfectly inhibited in total darkness. Although illumination is necessary for the gemmule hatching, the intensity of the light is very low and the illuminating period to induce the hatching is very short. Gemmules also fail to hatch unless the surface of incubating media is freely exposed to the atmosphere. Photosynthetic inhibitors strongly suppressed the hatching even under the optimal temperature and fully illuminated condition. To understand role of air components and photosynthesis on the gemmule hatch, we examined gaseous elements of the air and found that oxygen and carbon dioxide were responsible for the gemmule hatch. These gases are also evolved or consumed by photosynthesis. When oxygen was evacuated from the incubating media, no gemmules were hatched. On the other hand, gemmules incubated in a medium containing excess carbon dioxide did not germinate even under the optimal illuminated condition. This suggests that gemmule germination of the Annandale sponges seems to be initiated by oxygen and suppressed by carbon dioxide. This suggests that the both gasses might be evolved in or consumed from hibernating gemmule cells by photosynthesis of symbiotic algae to induce germination in these animal cells.

Key Words: Symbiosis, Sponge, Algae

Introduction

Freshwater sponges usually flourish during warm seasons and disappear in winter. They only thrive in unfavorable cold season by forming dormant gemmules that will germinate (hatch) in a warmer season the next year [2, 3]. Some of freshwater sponges are characterized by bright green coloration due to endosymbiotic chlorellae. As has been shown in other lower animal forms, such as green hydra, the symbiotic role of zoochlorellae in Polifera have also been studied mainly on account of the nutrient supply to the animal cells. However, we have previously reported that gemmules of Ananndale sponges are hatched only under illumination, but the daily dose of light to induce the gemmule hatching is very low. Daily illumination as low as 100 lx for 10 min

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will suffice to induce 100% of gemmulle hatching [1]. However, a photosynthetic inhibitor, diuron, strongly suppressed the hatch, suggesting photosynthesis of symbiotic algae is essential to the hatching of gemmules. On the other hand, electron microscopic observation of the gemmule cells (thesocytes) shows a rich source of nutrient granules in the cytoplasm. All of these observations suggest that significance of photosynthetic activity in symbiotic algae might not be relevant for the nutrient supply in the gemmule germination. Conversely, photosynthesis of symbiotic algae might evolve oxygen and deduce carbon dioxide in hibernating gemmule cells, and also might cause change in the ionic state in the cytoplasm of the thesocytes. So we designed experiments to examine the effect of oxygen or carbon dioxide concentration of incubation media on the gemmule germination.

Material and methods

Gemmules of Ananndale sponges (*Radiospongilla cerebellata*) were collected from ponds and marshes around Okayama city in August and September each year. Collected gemmules were stocked in a refrigerator in a vial containing M medium [4] or dechlorinated tap water at least for three month of dormancy. No differences were observed on the final hatching rate of the gemmules by stock media. After a certain term of dormancy, gemmules were germinated in M medium in a petri dishes at 24 °C under designated photoperiod and light intensity. All solutions or media used for storage and experiments were sterilized. One dish contained 25 gemmules and five dishes were usually used for a single experiment.

For ordinary experiments, gemmules were illuminated by light of 3,300 erg/cm^2/sec from ordinary fluorescent light at daily photoperiod of 10 h light and 14 h dark

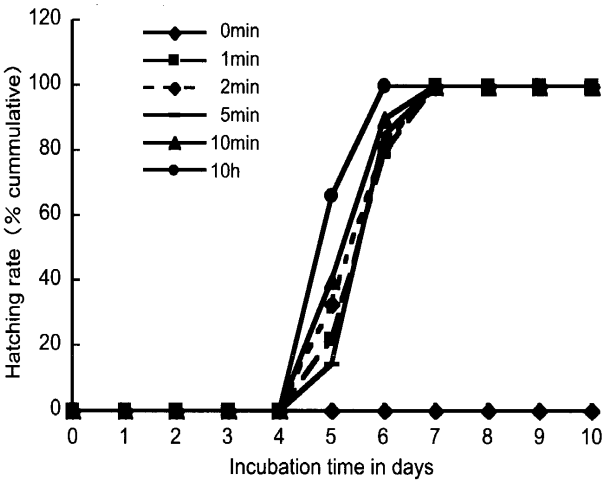

Fig. 2 Various daily photoperiods under 3300 erg/cm^2/sec. Even one minute of daily illumination brought all gemmules to hatch. No illumination caused no gemmules to hatch.

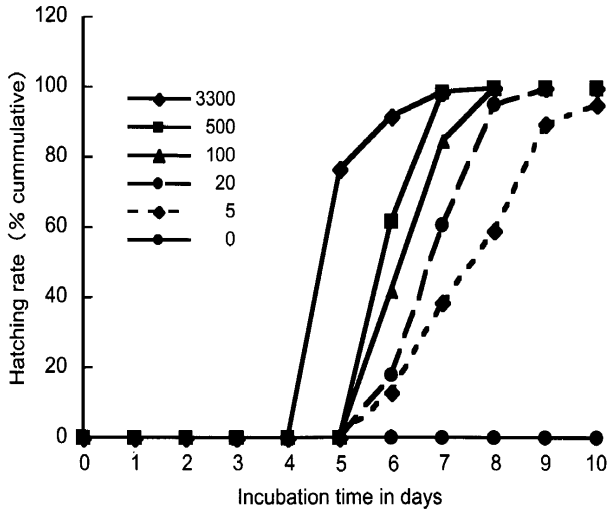

Fig. 1 Effects of illumination on gemmule hatch of Ananndale sponges. Under daily photoperiod of 10L-14D, very dim light of 5 erg/cm^2/sec was effective to induce almost perfect germination by 10 days, although under total darkness no gemmules were induced to hatch.

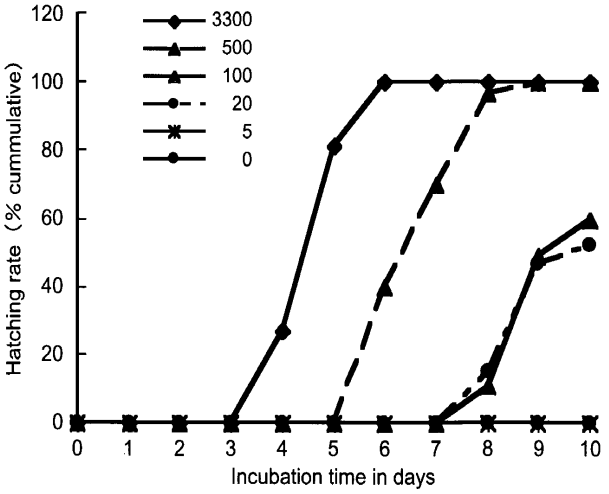

Fig. 3 Daily one minute of illumination by various light intensities. Low intensity such as 100 or 20 erg/cm^2/sec caused 50% of gemmules to hatch. Light intensity of five erg/cm failed to induce gemmule hatch.

(10L-14D). For the photoperiod experiments, various daily light exposures, such as 0 min, 1 min, 2 min, 5 min, 10 min or 10 h were adopted, and the rest of the day gemmules were kept under total darkness. For light intensity experiments, gemmules were illuminated daily for 10 h at light intensities of 5, 20, 100, 500 and 3,300 erg/cm^2/sec.

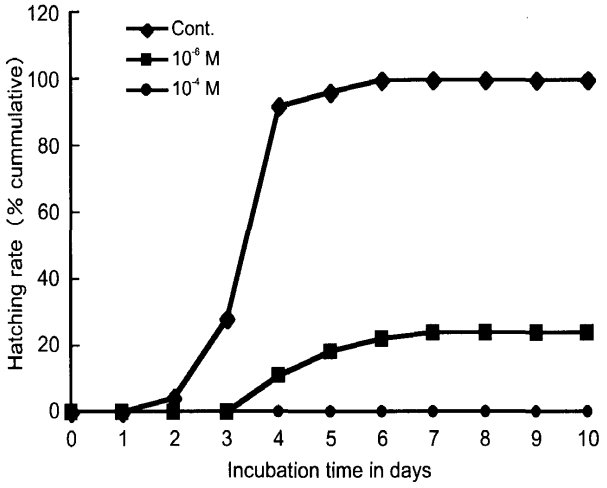

Fig. 4 Effect of atrazine, a photosynthetic inhibitor, on gemmule hatch. The gemmule hatch was strongly suppressed by administration of the agent to incubation media at concentration of 10^{-6} M or higher.

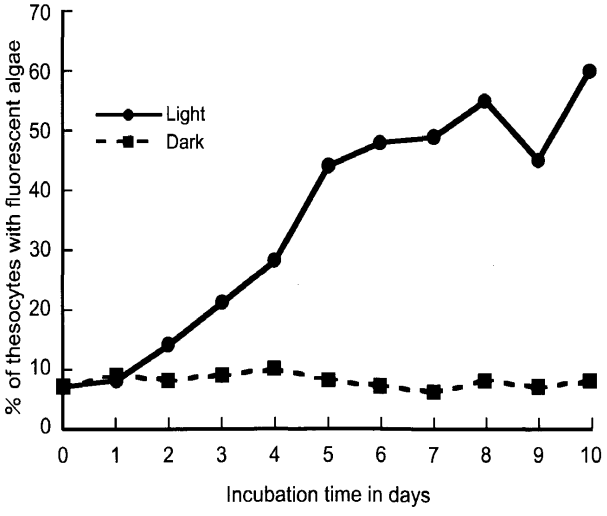

Fig. 5 Fluorescent microscopy of gemmule cells to reveal chlorophyll contents in symbiotic algae. Before incubation started no fluorescent spots was detected, while after 7 days' illumination, strong lights were observed in the cell.

Results

When gemmules were incubated in M media under the optimal condition, that is, at a temperature of 24 °C and with a daily photoperiod of 10L-14D under intensity of 3300 erg/cm^2, 100% of hatch was obtained in 7 to 8 days. However, total darkness could not induce any gemmules to hatch, even when the temperature was properly provided (Fig. 1). Even with very low light intensity, such as 5 erg/cm^2, most of the gemmules were induced to hatch under 10L-14D photoperiod. When gemmules were incubated under the ordinary light intensity of 3300 erg/cm^2, daily illumination of only one minute brought all gemmules to hatch (Fig. 2). One minute's daily illumination of very low intensity, such as 20 erg/cm^2 brought 50% of hatch in 10 days (Fig 3).

Administration of the photosynthetic inhibitor, atrazine at 10^{-6} M, in the incubating medium strongly suppressed gemmule hatching (Fig. 4). Higher concentration (10^{-4} M) of the agent perfectly inhibited the hatch. Fluorescent microscopic detection of chlorophyll showed that both of thesocytes (gemmule cells) that contained functional algae and of functional algae in a single thesocyte were increased during the incubation. In the dormant state or at the start of incubation, there were only few thesocyts that contained functional algae, that is, algae with chlorophyll fluorescence of bright red spots about one micron diameter. Most of algae in thesocytes showed yellow circumference of the cell wall due to the cellulose fluorescence. After 6 days incubation under the optimal illumination, however, most of gemmule cells contain algae with strong chlorophyll fluorescence and the number of the algae in the thesocytes was also increased (Fig. 5), that is, the red fluorescent algae in a thesocytes increased to four or five per cell, while at the beginning of the incubation there were only one or two. The rate of thesocytes that contained red fluorescent chlorellae was also increased reaching 50% of the whole gemmule cells, that is, at least half of the gemmule cells were inhabited by symbionts before they were hatched. On the other hand, in the later part of the incubation, the yellow fluorescent organelles were hardly observed. Electron microscopic observation showed two types of algae, that is, one with a rich thylacoid membranes and the other without the thylacoid membranes (Fig. 6).

When the air phase was not allowed to contact with the incubating media, that is, the surface of the media was sealed with air tight film or liquid paraffin, the

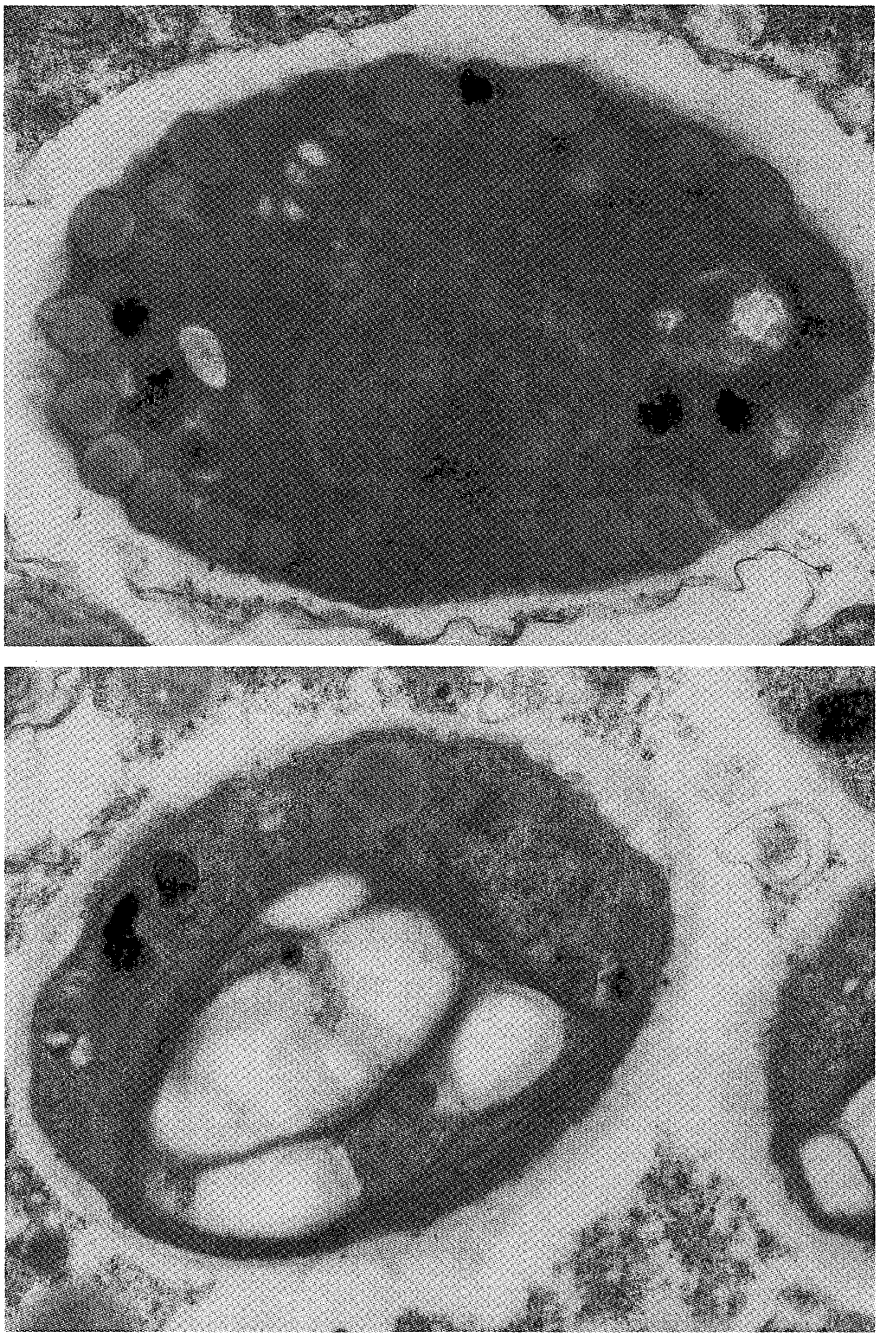

Fig. 6 Electron microscopic figures of symbiotic algae in dormant state (upper micrograph) and germinating state (lower micrograph). Thylakoid membrane was clearly visible in germinating algae, while it was obscure in dormant one.

hatching rate was significantly decreased (Fig. 7). When effect of each component of the air was tested for hatching promotion, only O_2 gas induced gemmules to hatch. On the other hand, the hatching rate was greatly decreased in media saturated with CO_2 gas (Fig. 8). A medium adjusted to pH 5.0 with a phosphate buffer, which is equivalent to the pH of CO_2 saturated medium, showed no suppressing effects on the gemmule hatching (Fig. 8). However, under the total darkness, oxygen administration could not induce the gemmule to hatch. Since there may be a problem of infiltration of the gas into the gemmule cells, the experiments were carried out again by gemmules with a small hole on the hard skin. The puncturing of the skin depressed the germinating rate of

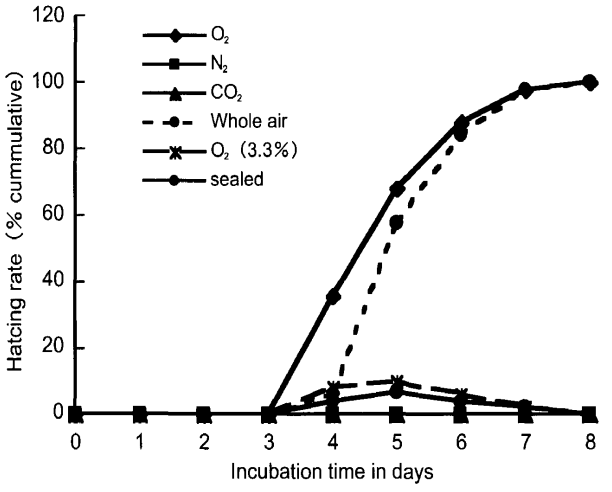

Fig. 7 Effects of air components on the gemmule hatch. Whole air or oxygen as gaseous phase of incubating vials caused gemmule to hatch, while another element, N_2 or CO_2, was failed to induce hatching. No gas phase or low concentration of oxygen in the gas phase induced very few gemmules to hatch in early part of the incubation, but these died by the eighth day.

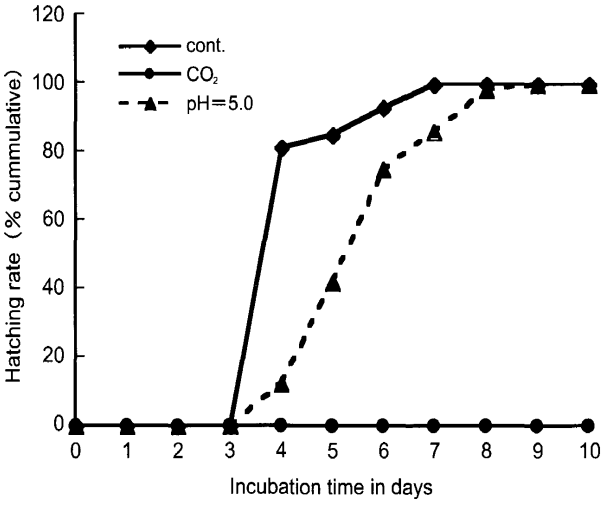

Fig. 8 Effects of carbon dioxide on gemmule hatch. Saturated CO_2 in the medium caused no gemmules hatch in 10 days. Acidity caused by saturated CO_2 (ca. pH = 5.0) had no significant effects on the gemmule hatch.

gemmules to 80%, or to 50% at most (not shown). The decrease of the maximum hatching rate by this procedure may probably be caused by the technical failure of the operation. The gas experiments on punctured gemmules also showed the same results as the intact ones, so that oxygen induced the hatch and excess carbon dioxide suppressed gemmule germination. Neither depletion of carbon dioxide nor excess oxygen caused gemmules germinate. Combined treatment of depletion of carbon dioxide and excess oxygen could not induce germination on the intact or the punctured gemmules.

Discussion

Endosymbiotic relationship between animals and unicellular algae has been intentionally studied, especially in protozoa [5] and lower invertebrates such as sponges [1], cnidarians [6-9] and molluscs [10-11]. It has been shown that algal symbionts in animal cells influence the host lives on its biochemical and physiological processes [12-14]. Symbiotic algae are contained even in eggs of an amphibia laid in poorly aerated waters to get sufficient oxygen [22]. As has been previously shown [1], gemmule germination of the Ananndale sponges was induced by illumination and proper temperature. Hatching was strongly suppressed by photosynthetic inhibitors added to incubating media (Fig. 4). The agent does not seem to damage gemmule cells, since prolonged treatment with the agent before incubation showed no negative effects on the gemmule germination. This suggests that the inhibitor may have influenced the photosynthetic processes exclusively on the symbiotic algae but causes no effects to the integrity of gemmule cells. If photosynthesis in the symbiotic algae is indispensable for the gemmule germination, the question is, what factor or factors can possibly be of inducing gemmule germination. During the photosynthesis of the algal symbionts, such factors as nutrient production, oxygen evolution and depletion of carbon dioxide will occur in the cytoplasm of germinating gemmule cells. However, nutrient supply from the algae may not be necessary, for the gemmule cells are originally provided with vitelline granules, the nutrient source of the gemmule germination. So, we examined the effect of the gas components of the photosynthetic processes on the gemmule germination. As shown in the text, excess carbon dioxide in the incubating media perfectly inhibited the germination even under the optimal illumination and temperature. Depletion of carbon dioxide from the incubating media, however, could not induce germination in darkness at all. This may show that carbon dioxide keeps the gemmules in a dormant state, that is, the depletion of carbon dioxide bring gemmules released from the dormant state, but can't induce them to germinate. We tried another gas element involved in photosynthesis, oxygen, and found that only this element could induce

gemmule germination under the light condition. However, even the oxygen could not induce the hatch in the total darkness.

It has been reported that an inhibitory substance, gemmulostasin, released from a young freshwater sponge, *Ephydatia flurivatilis*, suppresses hatching of gemmules of the same species [15, 16]. On the other hand, dormancy is shown to be maintained by the presence of cyclic AMP in Spongilla [17, 18]. In our results, carbon dioxide strongly suppressed the gemmule hatch and they maintained their dormant state (Fig. 7 and 8).

Symbiosis between algae and freshwater sponges is mainly understood as mutual benefits of photosynthesis; that is, photosynthetic products; such as oxygen and nutrients from algae to sponge, and metabolic wastes; such as, carbon dioxide and phosphate, from sponge to algae [15, 19]. Symbiotic algal contribution to the gemmule hatching was also reported in *Ephdatia* sponges [20], but no specific factor or factors from algae to induce gemmule hatch were notified. Photoperiod (the day length) is also significant in gemmule hatch of müller spongs, *Ephidatia mulleria* [21]. However, the present experiment on Ananndale sponges showed the day length is not significant to induce gemmule hatch, although illumination is essential.

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Accepted March 29, 2002.