ホソメコンブ配偶体の生長・成熟に及ぼす窒素・リンの影響

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Effects of Nitrate and Phosphate on the Growth and Maturation of Gametophytes of Laminaria religiosa Miyabe (Phaeophyceae)

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Abstract: Zoospores of Laminaria religiosa were cultured for 81-97 days in different nitrate (0-100 μM) and phosphate (0-10 μM) concentrations in order to examine the effects of nitrate and phosphate on the growth and maturation of gametophytes in batch culture. The female gametophytes matured and formed sporophytes at concentrations of more than 5.0 μM nitrate and more than 1.0 μM phosphate. These are the critical levels necessary for allowing the development from a gametophyte into a sporophyte, in cases when the maturation of the gametophyte is limited by both nitrate and phosphate. The critical nitrate concentration decreased to 1.0 μM when the phosphate concentration was sufficient, and the critical phosphate concentration similarly decreased to 0.5 μM when the nitrate level was sufficient, indicating that the critical concentration is affected by the synergy between the concentrations of two nutrients. The effects of irradiance (0.76-14.8 μE/m²/s) and water temperature (5-15 °C) on gametophyte maturation and sporophyte formation were also examined in six nutrient concentrations. Low nutrient concentration or low irradiance independently delayed maturation and formation, showing that the reproduction of L. religiosa gametophytes is controlled by the accumulation of nutrients in a plant and by quantum dose.

Key words: Laminaria religiosa; Gametophyte; Nutrient; Irradiance; Water temperature

The effects of light intensity, light quality, and water temperature on the growth and maturation of gametophytes of Laminariales have been reported by some researchers1-3. Nutrients also influence the growth and maturation of gametophytes of L. saccharina (L.) Lamour4 and Lessonia nigrescens Bory5. In Japan, Okada and Sanbonsuga6 have examined the effects of water temperature on the growth of Laminaria gametophytes. Recently, Iizumi and Sakanishi7 described the physiological characteristics of the photosynthesis of gametophytes of Laminaria religiosa Miyabe. However, few reports have described the effects of nutrients on the growth and maturation of gametophytes of Laminaria from Japan. This study examines the effects of nitrate and phosphate on the gametophyte maturation and the sporophyte formation of L. religiosa, and discusses the relationship between nutrient concentration and other environmental factors such as water temperature and irradiance.

Materials and Methods

Fertile sporophytes of L. religiosa Miyabe were collected at Kumaishi in southwestern Hokkaido, Japan, in September 1999. The sorus parts were cut off and rinsed in sterile seawater with 0.1% popiyodon solution (Yoshida Seiyaku Co.) for 30 min to remove epiphytes and mucilage. These sorus parts were wrapped in paper and stored at 4 °C overnight, and then used for making zoospores release in sterile seawater. The seawater used in the experiments was filtered through a Whatman GF/C glass fiber filter and autoclaved at 120 °C for 15 min. The zoospore solution was distributed onto microplates at a

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volume of 2 mL each. After zoospores had settled at the bottom of microplates, the medium was changed from seawater to artificial seawater based on ASP 2M \[8\]. Zoospores were cultured in 42 combinations of 7 nitrate (0-100.0 μM) and 6 phosphate (0-10.0 μM) concentrations. Water temperature and irradiance were set at 10°C and 14.8 E/m²/s (12L:12D). The media were changed every week. The germination of spores and the development of gametophytes were observed using a microscope after 26, 54, and 97 days of culture.

To examine the effects of water temperature and irradiance in the different nutrient concentrations, the zoospores were cultured at three temperatures (5, 10, and 15°C) and under three irradiances (0.76, 7.74 and 14.8 E/m²/s). The media consisted of nutrient-poor seawater, which was collected from the plant sampling site in the summer (nitrate + nitrite=0.76 μM, phosphate=0.21 μM), with added 1/100x, 1/50x, 1/10x, 1/5x, and 1x Provasoli’s enriched solution (PES). The maturation of gametophytes and the formation of sporophytes were observed after 34, 53, and 81 days of culture.

The developmental process from zoospores to sporophytes of L. religiosa could be divided into six stages, with the most advanced stage in each culture condition shown in the results: stage I showing the generation of germling tubes (Fig. 1 A) from embryospores (Fig. 1 B), stage II showing the development into male and female gametophytes consisted of 1 to 5 cells (Fig. 1 C and D), stage III growing to filamentous gametophytes, stage IV forming eggs on female gametophytes (Fig. 1 F), stage V differentiating into the sporophytes from fertilized eggs (Fig. 1 G), and stage VI growing to a visual level of more than 50 cells (Fig. 1 H).

**Results**

The zoospores formed germling tubes under all the conditions within 3 days of culture. Even in 0 μM of nitrate and phosphate, 37.6% of the zoospores germinated but did not develop into gametophytes. The growth and maturation of gametophytes under different nutrient concentrations are summarized in Tables 1 and 2. The zoospores developed into male and female gametophytes in more than 5.0 μM nitrate and 1.0 μM phosphate after 26 days of culture. In 100 μM nitrate and 10 μM phosphate, 28.8% of female gametophytes formed eggs which developed into sporophytes. After 54 days of culture, male and female gametophytes were observed in more than 1.0 μM nitrate and 0.5 μM phosphate. However, sporophytes were formed only in 100 μM nitrate and 10 μM phosphate (57.4%). After 91 days of culture, no gametophytes formed in less than 0.5 μM nitrate and 0.25 μM phosphate. Particularly, all the zoospores, even if they had germling tubes, died with the collapse of pigments in 0 and 0.1 μM phosphate.
regardless of the presence of nitrate. At the end of the culture, gametophytes were almost matured in more than 1.0 mM nitrate and 0.1 mM phosphate. Sporophytes became visual in more than 5.0 mM nitrate and 1.0 mM phosphate. In this culture, some of the small gametophytes matured without growing into filamentous large gametophytes, i.e., those with more than 10 cells (Fig. 1 E).

The effects of irradiance on gametophyte growth are summarized in Table 3. After 34 days of culture, the sporophytes occurred under high irradiances (7.74 and 14.8 μE/m²/s) and in nutrient-rich media (1/10 x, 1/5 x, and 1x PES), but the gametophytes became large under low irradiance (0.76 μE/m²/s) in spite of the nutrient-rich conditions. Small gametophytes consisting of a few cells were observed at 1/100 x and 1/50
x PES under all the irradiances, and zoospores never developed into filamentous gametophytes in nutrient-poor media. After 53 days of culture, the sporophytes became larger than 50 cells. Small gametophytes in 1/50x PES became larger under the high irradiances and in nutrient-rich media. In other nutrient conditions, particularly in nutrient-poor media, no development was observed. After 81 days of culture, sporophytes became larger than 50 cells.

Small gametophytes in 1/50x PES became larger under the high irradiances and in nutrient-rich media. In other nutrient conditions, particularly in nutrient-poor media, no development was observed. After 81 days of culture, sporophytes became larger than 50 cells.

The effects of water temperature on the gametophytes are summarized in Table 4. After 34 days of culture, sporophytes were observed in all the water temperatures in 1 x PES. In low water temperatures (5 and 10 °C), sporophytes also appeared only in nutrient-rich media of 1/10 x and 1/5 x PES. When the nutrient was less than 1/5 x PES, gametophytes were still few-celled. After 53 days of culture, sporophytes were newly formed in 1/50 x PES at 5 °C and 1/5 x PES at 15 °C. In addition, filamentous gametophytes were observed in 1/50 x PES under 14.8 E/m²/s. Filamentous gametophytes newly appeared in 1/100 x and 1/50 x PES media under 14.1 E/m²/s. Even under low irradiance (0.76 E/m²/s), some sporophytes appeared only in 1/5 x and 1 x PES, but almost all gametophytes remained unchanged in 1/50 x and 1/10 x PES.

**Discussion**

Many zoospores of *L. religiosa* gametinated after settlement even in nitrate-free and phosphate-
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free media, indicating that they already had sufficient energy to germinate and that germination was not greatly influenced by nutrient concentrations. The growth of gametophytes was strongly limited by low nutrient concentrations, particularly regarding that of phosphate (<0.25 μM). Irradiance and water temperature had negligible effects on the development and maturation of gametophytes in nutrient-rich media.

Low irradiance often produced filamentous gametophytes without maturing in nutrient-rich conditions. The filamentous gametophytes also formed in low nutrient conditions (Table 4). In contrast, small gametophytes matured without growing into filamentous gametophytes under high irradiance (Table 3). Large filamentous gametophytes have been observed by several researchers\textsuperscript{1,4,5,9}. Hoffman and Santelices\textsuperscript{5} reported that the number of gametophytes in Lessonia nigrescens progressively decreased as the amounts of nitrate and phosphate increased. Kain\textsuperscript{1} also suggested that large gametophytes were observed if nutrients were depleted. Lüning\textsuperscript{9} reported that the large filamentous gametophytes could be adaptive to low irradiance. Accordingly, the filamentous gametophytes probably occur in nutrient-poor or low-irradiance conditions, yet a limited amount of nutrients does not always produce filamentous gametophytes because they could die in the early developmental stage in nutrient-depleted media.

Gametophytes matured optimally at 10 °C, followed by 5 °C and 15 °C. These results support earlier findings in which showed that the optimal water temperatures to be 8-13 °C in L. japonica, L. ochotensis, L. diabolica, L. religiosa and L. angustata var. longissima from Hokkaido, including L. religiosa\textsuperscript{6}. However, water temperature hardly inhibited the maturation of gametophytes or the development of sporophytes in conditions of sufficient nutrients and irradiance. On the other hand, low irradiance strongly limited the growth of gametophytes and delayed their maturation even in enriched media. From this point of view, irradiance proved to be a more important factor than water temperature. In addition, even under high irradiance, the maturation of gametophytes was limited in low nutrient concentrations. These results indicate that certain irradiance and nutrient levels are necessary for the early maturation of gametophytes. The induction of maturation depends on quantum dose (radiant exposure) and not on photon flux density (irradiance)\textsuperscript{22}. Accordingly, radiation time controls the maturing initiation of gametophytes. Even under low irradiance (0.76 E/m\textsuperscript{2}/s), the gametophytes of L. religiosa matured after 81 days of culture, showing that maturation and the formation of sporophytes depend on quantum doses. Moreover, the critical quantum dose is correlated with nutrient concentration. Low nutrient concentration delayed the maturation of gametophytes, and severe nutrient limitation prevented the gametophytes from maturing even when the quantum dose was enough, showing that nutrient concentration is an important factor. Mizuta et al.\textsuperscript{10} have reported that L. japonica sporophytes need to accumulate nitrogen for maturation. The gametophytes also probably accumulate nutrients. These results indicate that both quantum dose and nutrient accumulation trigger the maturation process of gametophytes and the resulting sporophyte formation.

The critical concentrations are useful for elucidating the nutritional conditions of seaweeds in the field. The minimum nitrate and phosphate concentrations for the maturation of gametophytes followed by the formation of sporophytes were 5.0 μM nitrate and 1.0 μM phosphate in batch culture, indicating each is a critical concentration. However, the sporophytes were also formed even in 1.0 μM nitrate and 10 μM phosphate or in 100 μM nitrate and 0.5 μM phosphate. In these cases, the limiting nutrient was either nitrate or phosphate, showing that each critical concentration could change due to the synergy between the two. Water motion is generally known to stimulate algal metabolic and nutrient uptake rates\textsuperscript{11-13}. This suggests that the critical levels of nitrate and phosphate are lower in running water.
References


ホソメコンブ配偶体の生長・成熟に及ぼす窒素・リンの影響
水田浩之・鳴海日出人・山本弘敏

ホソメコンブの遊走子を異なる硝酸態窒素（0〜100μM）およびリン酸態リン濃度（0〜100μM）の人工海水増で培養し、生成や成熟に及ぼす影響を調べた。雌性配偶体は硝酸およびリン濃度がそれぞれ5.0μM，1.0μM以上の場合に成熟し、胞子体を形成した。この濃度は配偶体世代から胞子体世代へ移行する際の閾値を示している。しかし、これは窒素とリンの制限を同時に受けている場合の場合でなく、どちらか一方が十分に供給されると、それぞれの閾値は1.0μM，0.5μMへ低下した。このことは、閾値は制限する栄養塩が複数の場合、その相乗作用によって変動することを示している。また、栄養塩海水および5段階の栄養塩添加海水で光量（0.76, 7.74 and 14.8μE/m²/s）および水温（5〜15℃）の影響を調べたところ、栄養塩あるいは低光強がそれぞれ単独に配偶体の生長・成熟を遅らせた。この結果は、栄養塩の体内への蓄積と積算光量がホソメコンブの配偶体の生長・成熟を制御していることを示してい