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Regeneration of *Lithophyllum yessoense* Foslie in culture

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The regeneration of *Lithophyllum yessoense* (Corallinales, Rhodophyta), a dominant encrusting coralline alga in Isoyake (urchin-dominated barren) area of southwestern Hokkaido, was examined by culturing crust fragments with artificially-made longitudinal fracture. In all conditions (water temperature, 5–25°C; irradiance, 25–1100 $\mu Em^{-2} s^{-1}$), regeneration occurred from the fractures. The regenerated thallus, consisted of primigenous and postigenous filaments, grew rapidly in higher temperatures, in higher irradiances, and in earlier stages of culture. The marginal and thickness growth rates of the regenerated thalli were much larger than cultured one-year-old plants and natural perennial plants previously reported for the same species.

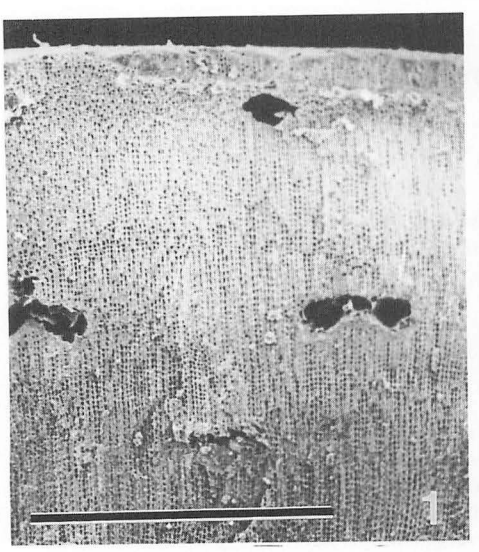
Key Index Words: Corallinaceae—Epithallial shedding—Growth rate—Isoyake—Lithophyllum yessoense—*Regeneration—Rhodophyta*

In Isoyake (urchin-dominated barren) areas along the southwestern coast of Hokkaido, subtidal substrata have been covered extensively by nongeniculate coralline algae (Corallinales, Rhodophyta), among which the perennial encrusting species *Lithophyllum yessoense* Foslie is dominant (Noro et al. 1983; Fujita 1989). This species grows slowly (Fujita 1990a, b), but its thallus surface is comparatively clean as a result of epithallial shedding (Masaki et al. 1981, 1984). It can survive grazing by sea urchins or limpets (Fujita 1992) and may suffer physical or biological damage (e.g., collision with rolling stones; strong wave action in winter; artificial removal by 'chain-swinging' method 《Nabata and Matsuda 1983》; movement of creeping animals), and consequently a large part of the plant may be lost. Artificial injury is more serious in terms of volume of lost thallus than the slight wound caused by herbivore grazing (Fujita 1992). However, there is no information on the response of this species to heavy injury. In the present paper, results of experiments on regeneration of fragmented pieces of thalli of *L. yessoense* are reported.

Materials and methods

Thalli of *L. yessoense* were removed by chiseling from subtidal rocky bottoms (1–3 m depth) at Taisei, Hiyama Province of southwestern Hokkaido on Mar. 5, 1984 and Aug. 14, 1985. Plants were immediately brought alive in sea water to the laboratory in Hakodate. Healthy thalli of several square centimeters were selected, and were broken into 4 or 5 pieces. The pieces from one thallus were placed in a waterbath (3 *l*) filled with sea water (2 *l*).

In the first experiment (Exp. I) in Mar. 1984, triplicate waterbaths were placed in three incubators set at 5, 10, and 15°C, respectively; all of the incubators were subject to light conditions of 12: 12LD with 150 $\mu Em^{-2} s^{-1}$. After 42 days, all pieces were taken out, and either fixed and decalcified with Susa's solution (Gray 1954) for measurement of length and thickness of regenerated thallus or airdried for scanning electron



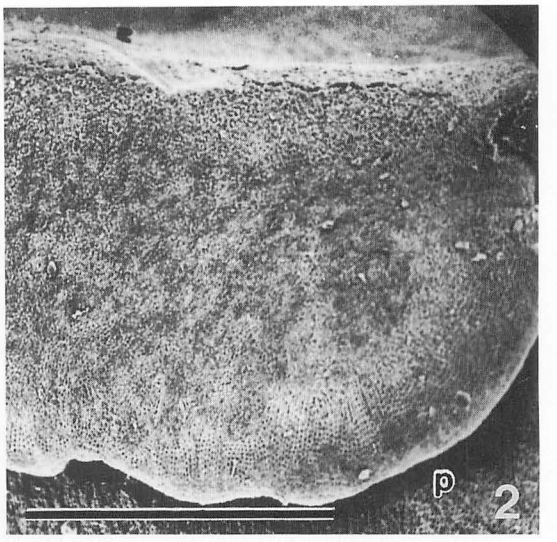


Fig. 1 Longitudinal fracture of *Lithophyllum yessoense*.

Fig. 2 The regenerated thallus extended downward the parent fragment (p) (65 days, 25°C–1100 $\mu Em^{-2} s^{-1}$). Scale bars = 100 μm

microscopy.

In the second experiment (Exp. II) in Aug. 1985, triplicate waterbaths were placed in an incubator at 25°C, and each was cultured under 3 different levels of irradiance, 25, 150 and 1100 $\mu Em^{-2} s^{-1}$, respectively. One piece of thallus from each triplicate was taken out for observation in the same way as Exp. I after 5, 15, 30 and 65 days.

Along the coast of southwestern Hokkaido, the sea water temperature is the lowest (5°C) in February and highest (25°C) in August (Fujita 1989), so that the lowest temperature in Exp. I and the highest temperature in Exp. II correspond to the winter and summer conditions, respectively.

The size (length and thickness) of regenerated thallus was measured on cross sections of paraffin-embedded decalcified pieces with a light microscopic scale. Structure of regenerated tissue was observed with a scanning electron microscope (SEM-25, Nihon Denshi K. K.), using air-dried pieces coated with gold-palladium.

Results

Morphological observation

In all conditions (water temperature: 5–25°C, irradiance: 25–1100 $\mu Em^{-2} s^{-1}$), regeneration occurred (Figs. 2–8) from fractures (Fig. 1).

At first, regeneration was initiated from one or a few distal parts where columnar cells were pigmented (Fig. 3). The regenerated thallus increased in thickness and then extended downward along the longitudinal fracture of the parent fragment (Figs. 2, 4–6). The tip of the well-developed regenerated thallus was separated from longitudinal fracture of the parent fragment.

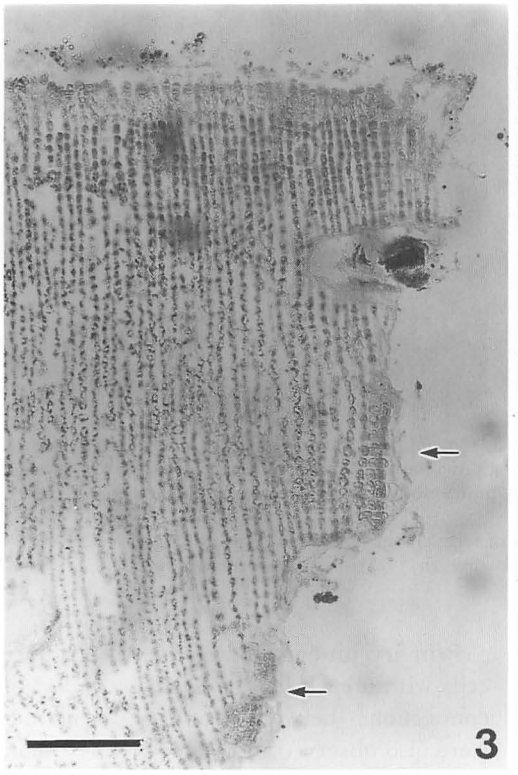
While the regenerated thallus remained at-

Fig. 3 Regenerated thallus initiated at two distal points (arrows) of a longitudinal fracture (15 days, 25°C–25 $\mu Em^{-2} s^{-1}$).

Fig. 4 Regenerated thallus (65 days, 25°C–25 $\mu Em^{-2} s^{-1}$), consisting entirely of postigenous filaments. The outermost epithallial layer is sloughing off (arrow).

Fig. 5 Regenerated thallus (65 days, 25°C–1100 $\mu Em^{-2} s^{-1}$), in which the tip is separated from parent fragment. The outermost epithallial layer was sloughing off (arrow).

Fig. 6 SEM of the free tip shown in Fig. 5, showing the primigenous cells (pr) aligned in rows from the ventral surface view. Scale bars = 100 μm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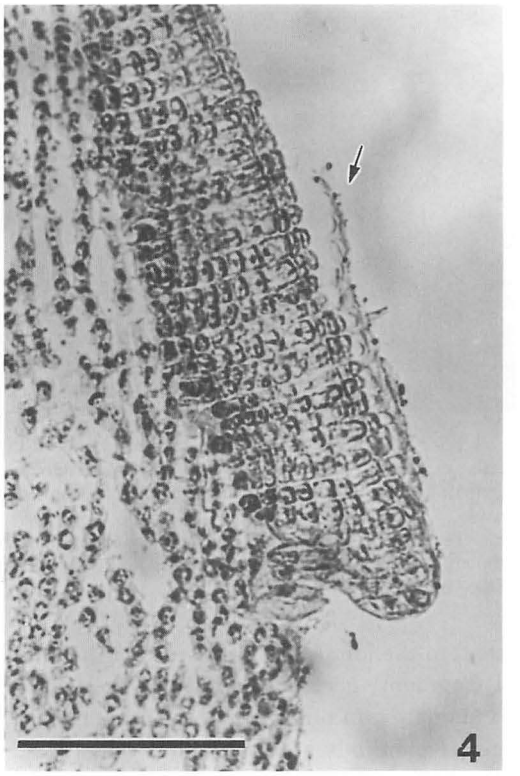
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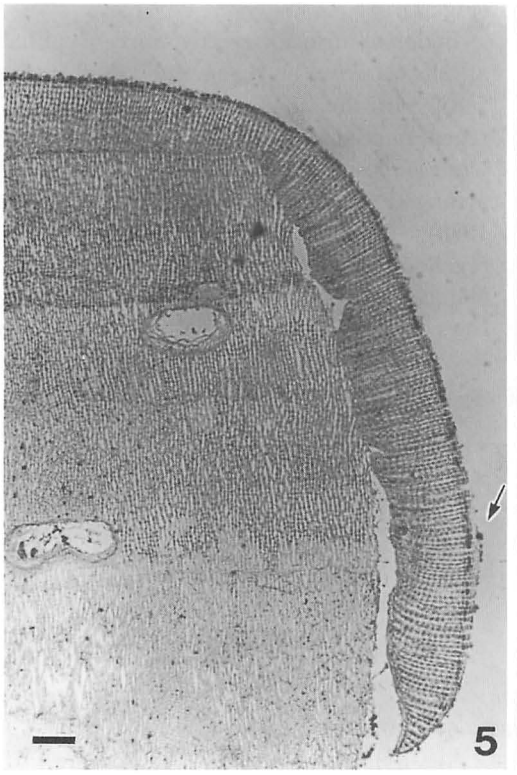
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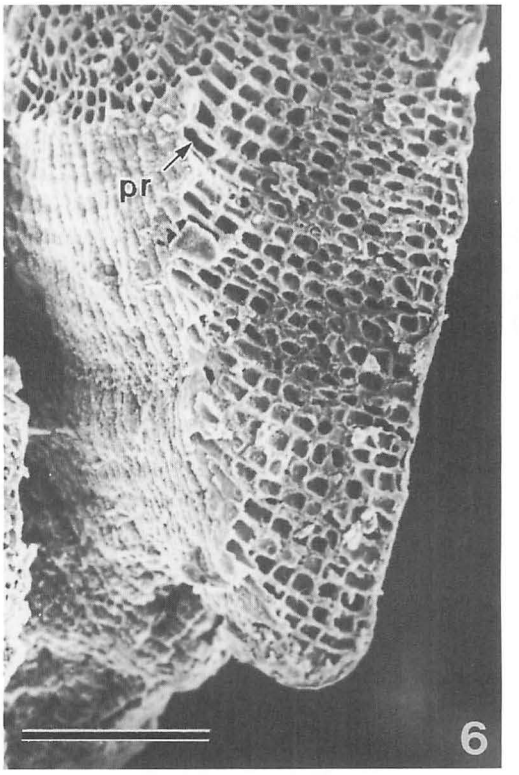
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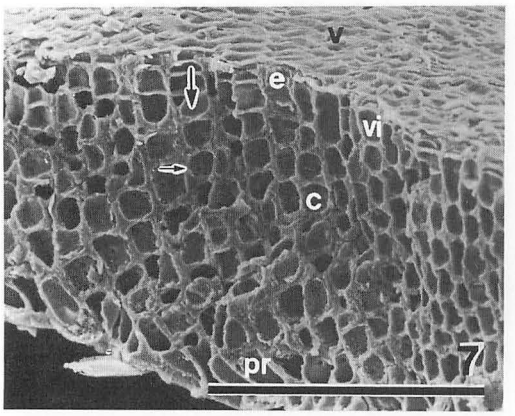

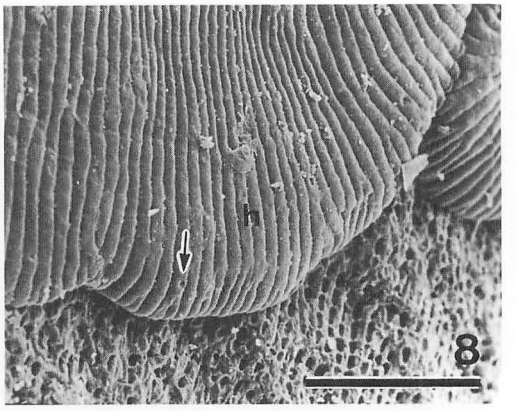

Fig. 7 Enlargement of regenerated thallus in Fig 6, showing filaments: primigenous cells (pr), columner cells (c) vegetative initials (vi) and epithallial cells (e). Epithallial cells (concavities) are arranged in vertical-growth type (v). Large and small arrows show primary pit-connections and secondary pit-connections, respectively.

Fig. 8 SEM of dorsal surface view of the margin of regenerated thallus, (65 days, 25°C–150 $\mu Em^{-2} s^{-1}$), showing epithallial cells arranged in horizontal-growth type (h). Arrows show the epithallial concavities. Scale bars = 100 μm

tached to the longitudinal fracture of the parent fragment, it consisted only of postigeneous filaments, in other words, columner cells, vegetative initials and epithallial cells (Figs. 3-4).

However, in the free tip where regenerated thallus became separated from the longitudinal fracture of the parent fragment, the ventral part was composed of newly developed primigenous filaments (Figs. 5-6) (See Woelkering 1988 for terminology of 'primigenous' and 'postigeneous'). In ventral surface view, the primigenous cells of the regenerated thallus were aligned in rows (Fig. 6).

Primary pit-connections between adjacent cells within each filament and secondary pit-connections between contiguous filaments were also observed in the regenerated thallus (Fig. 7).

In dorsal surface view, two sorts of epithallial cells occurred on regenerated thallus (Figs. 7-8). In the thickened central region of regenerated thallus, the dorsal surface (Fig. 7) was composed of honeycomb-like, randomly-arranged epithallial concavities (Garbary 1978). On the other hand, at the margin of regenerated thallus, epithallial cells were aligned in rows, and grooves between the cell rows were prominent (Fig. 8). The former

Table 1. Size (length × thickness, μm) of regenerated tissue (N = 1 in each condition) of *Lithophyllum yessoense*.

Water temperature (°C)	Irradiance ($\mu Em^{-2} s^{-1}$)	Time (days)				
		5	15	30	42	65
Exp. I						
5	150				733 × 17	
10	150				935 × 32	
15	150				1,542 × 55	
Exp. II						
25	25	341 × 33	670 × 61	—		—
25	150	414 × 29	786 × 64	1,072 × 117		1,286 × 143
25	1100	543 × 34	943 × 154	1,415 × 240		1,629 × 343

type of epithallial cell arrangement is referred to 'vertical growth-type', and the latter, 'horizontal-growth type' in the juvenile plant of the same species (Fujita 1990b). On the dorsal surface of some regenerated thallus consisting of epithallial cells of 'vertical growth-type', shedding of most distal layer of epithallial cells was observed (Figs. 4–5).

Growth of regenerated tissue

Sizes of all regenerated thalli are listed in Table 1. In Exp. I, the new thallus grew most rapidly at 15°C, and most slowly at 5°C, both in length and in thickness. And in Exp. II, regenerated thallus grew most rapidly at 1100 $\mu Em^{-2} s^{-1}$, and most slowly at 25 $\mu Em^{-2} s^{-1}$ both in length and in thickness. In the highest temperature-lowest irradiance condition examined (i.e. 25°C-25 $\mu Em^{-2} s^{-1}$), parent fragments did not survive after 15 days.

Discussion

The phenomenon of regeneration is known to occur in a wide range of marine algae (Buggeln 1981). In nongeniculate coralline algae, however, little has been known up to now, although Cabioch (1972) observed natural regeneration in many nongeniculate coralline genera. In *Lithophyllum*, she described the same pattern of regeneration in *Lithophyllum incrustans* Foslie and *Lithopyllum* sp., and called it 'reappearence of juvenile structure' (in original text of Cabioch, 'reapparition de structures juveniles'). The regeneration pattern of *L. yessoense* seems to be similar in producing primigeneous filaments which correspond to 'pseudo-hypothallium' (in original text of Cabioch, faux hypothalle').

Woelkerling (1988) noted that dimerous nongeniculate coralline algae are composed of two kinds of filaments: primigenous and postigenous filaments. The structure of regenerated thallus was dimerous like the original thallus.

The development of primary and secondary pit-connections in regenerated thallus was confirmed in the present study. These pit-connections may be important for translocation of nutrients and gas, as Steneck (1983) suggested in the case of wound-healing of grazed coralline algae, though this hypothesis requires further experimental confirmation.

Using the data of growth of regenerated thallus in Table 1, the annual marginal growth rates and thickness growth rates were calculated (Table 2). Both marginal and thickness growth rates increased at higher water temperature and at higher irradiance. These growth rates, however, decreased as the culture period became longer, probably because of reduction of exposed fracture surface.

Moreover, the annual growth rates of regenerated thallus were compared with those calculated from data of the annual plants cultured from tetraspores (Fujita 1990a) and natural perennial plants (Fijita 1990b) of the same species (Table 3). Both marginal and

Table 2. Annual growth rates (marginal × thickness, mm/year) of regenerated tissue of *Lithophyllum yessoense* calculated from data in Table 1.

Water temperature (°C)	Irradiance ($\mu Em^{-2} s^{-1}$)	Time (days)				
		5	15	30	42	65
Exp. I						
5	150				6.4×0.1	
10	150				8.1×0.3	
15	150				13.4×0.5	
Exp. II						
25	25	24.9×2.4	16.3×1.5	—		—
25	150	30.2×2.1	19.1×1.6	13.1×1.4		7.2×0.8
25	1100	39.6×2.5	22.9×3.7	17.3×2.9		9.1×1.9

Table 3. The comparison of annual growth rates (mean value and range, mm/year) of *Lithophyllum yessoense* among regenerated tissue, cultured annual plants and natural perennial plants.

Type of material	Marginal	Thickness	References
Newly generated tissue (N=13)	17.6 (6.4–39.6)	1.7 (0.1–3.7)	Present study
Cultured annual plants (N=10)	2.3 (1.6– 3.4)	0.2 (0.1–0.3)	Fujita (1990a)
Natural plants (N=100)	1.8 (0.6– 5.4)	0.5 (0.2–2.4)	Fujita (1990b)

thickness growth rates of regenerated thallus were much greater than those of cultured plants and natural plants. The comparatively high growth rates of regenerated thallus, especially in early stages of culture (in other words, just after getting damaged), must be the result of a high potential capability to recover rapidly.

The occurrence of regenerated thallus in all culture conditions suggests that this species can recover from fractures any time of year. The high growth rates of regenerated thallus in 25°C corresponds to the high photosynthetic activity at the same temperature in summer (Fujita 1988).

This study was not focused on examining the minimum thallus size for regenerating, but the recoverability of this species seems quite high, because the pieces examined in this study were much smaller than plants found naturally, which can be up to 30 cm^2 in size. In addition, the occurrence of epithallial shedding on the dorsal surface of regenerated thallus may be recognized as the recovery of the antifouling function, which was previously confirmed in the case of parent thalli (Masaki et al. 1981, 1984). Therefore, the regeneration seems to be the most important survival strategy of this nongeniculate coralline alga against various physical or biological damage as well as herbivore grazing (Fujita 1992) on the 'Isoyake' areas. The fate of regenerated thallus (e.g., reattachment to substratum of its free tip, maturity within the regenerated thallus) is unknown.

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藤田大介*・秋岡英承**・正置富太郎***：培養によるエゾイシゴロモの再生

北海道南西岸の磯焼け地帯の優占種エゾイシゴロモの細片を培養し，破砕面における再生を調べた。再生は実験範囲 (5–25°C, 25–1100 $\mu Em^{-2}s^{-1}$) 内のいずれの条件でも起こった。再生部の組織は初生的細胞糸及び後生的細胞糸で構成されており，水温及び光強度が高いほど，また，培養の初期ほど再生速度が速かった。さらに，再生した組織の縁辺成長速度及び肥厚成長速度は，以前に報告した同種の培養1年目個体及び天然産の多年個体の場合にくらべて非常に大きかった。(*936 滑川市高塚364　富山県水産試験場　**040 函館市八幡町1–2　北海道教育大学函館分校生物学教室　***041 函館市港町3–1–1　北海道大学水産学部水産植物学講座)