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Reproduction of *Rhodochorton purpureum* from Jeju Island, Korea and San Juan Island, Washington, USA in Laboratory Culture

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Rhodochorton purpureum 4187 from Jeju Island, Korea may have a sexual life history similar to that seen by other investigators working on other strains around the world. In culture short days (8:16, 11:13, 12:12 LD) at 10-15°C induced tetrasporogenesis. Discharged spores were observed with time lapse videomicroscopy. They showed a slight amoeboid movement for 2-3 minutes before rounding up and settling. Tetrasporelings develop into male and female gametophytes. No fertilisation was observed. Tetrasporangia often were borne on carpogonial clusters of females but no discharged spores were seen. Isolate 4241 from San Juan I., Washington, USA grew well in most conditions tested but did not reproduce in short days (8:16, 11:13, 12:12 LD) at 10-15°C.

Key Words: Korea, USA, *Rhodochorton purpureum*, short-day-tetrasporogenesis, spore-motility, time lapse videomicroscopy, unisexual

INTRODUCTION

Rhodochorton purpureum (Lightfoot) Rosenvinge is a red alga (Florideophyceae, Acrochaetiales, Acrochaetiaceae) that occurs in shaded upper intertidal marine habitats of temperate to cold water regions of the north and south hemispheres. Various authors have investigated the ecology, physiology and reproductive biology of *Rhodochorton purpureum*. In the field the tetrasporophytic phase is known to produce tetrasporangia in short days of winter (Breeman *et al.* 1985) and with laboratory culture it has been well documented that tetrasporangia form in short days (8-12 hrs of light) and cool temperatures (5-15°C) (Dring and West 1983; Knaggs 1968; Lee 1985; Ohta and Kurogi 1979; Stegenga 1978; West 1969, 1970, 1972). Tetraspores give rise to gametophytes that are usually smaller than tetrasporophytes and bear well defined sexual reproductive structures. The male plants have numerous branches bearing spermatangial clusters and the females have solitary carpogonia or numerous clusters of carpogonia on older plants. The fertilised carpogonium develops into a gonimoblast from which the tetrasporophyte develops directly.

In Korea *R. purpureum* is recorded on Jeju Island (Lee 1987) but its reproduction had not been investigated. We wished to determine the optimum culture conditions for tetrasporogenesis and gametophyte development as well as to determine if discharged spores and spermatia were motile using time lapse videomicroscopy.

MATERIALS AND METHODS

The specimens of *Rhodochorton purpureum* used in this study were obtained by YPL on 28 September 2001 in the supralittoral at Seongsanpo (33°N), Jeju Island, Korea and by Michael Wynne in a very shaded supralittoral recess at Cattle Point (48°N), San Juan Island, Washington, USA, on 16 August 2002. Both samples were sent by airmail enclosed in moist tissue in small plastic bags. Techniques for isolation and culture are as described in West and Calumpong (1988) and West (2005). All cultures were maintained in Modified Provasoli's Medium (MPM) (West 2005) at 30 psu (practical salinity units). Cool white fluorescent lighting at 10-30 μmol photons m^{-2} s^{-1} was used for all cultures. For induction of tetrasporangia the plants were grown in various daylengths and temperatures. To induce spore release by mature tetrasporangia, we immersed small tetrasporangial plants (2-4 mm long) from the main culture at 30 psu into fresh medium at 14, 26 and 30 psu for up to 1 hour. These salinity differences achieved little increase in spore

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release so we also tried to simulate the effects of low tide exposure by blotting small tetrasporangial plants on Whatman No. 1 filter paper for 10 to 30 seconds and then reimmersing in fresh medium at 10, 26 or 30 psu. To induce spermatial release we either transferred male plants with mature spermatangia from culture medium at 30 psu to fresh medium at 20, 15 and 10 psu or blotted male plants dry briefly for 10-30 seconds and placed

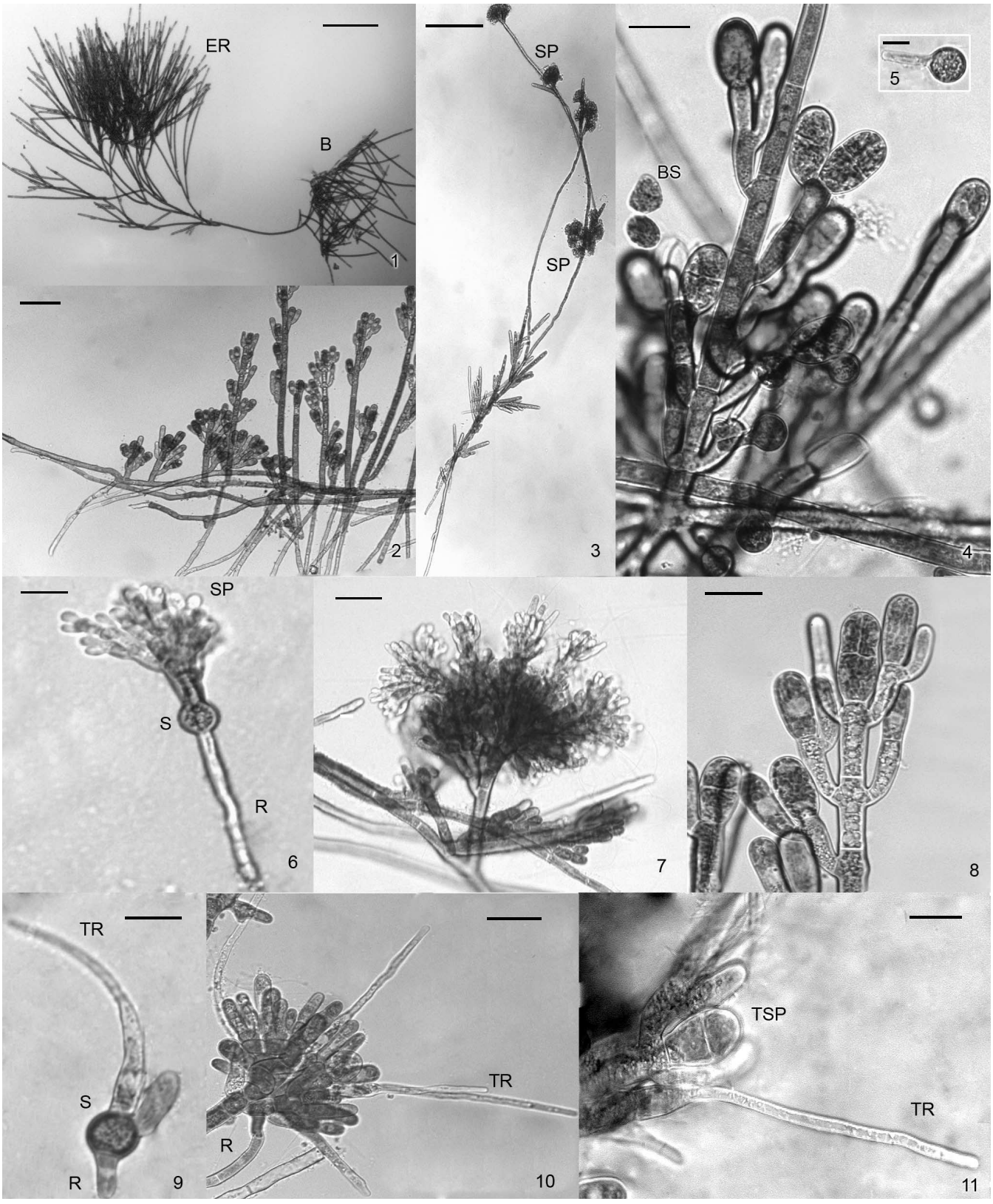

Table 1. Influence of daylength and temperature on sporulation in *Rhodochorton purpureum* 4187 in culture.

Isolate	Daylength	Temperature	Sporulation
4187			
	12:12 LD	21 ± 2°C	- (died)
	12:12 LD	18 ± 1°C	-
	11:13 LD	14 ± 1°C	+
	8:16 LD	13 ± 1°C	+
	8:16 LD	10 ± 1°C	+
4241			
	12:12 LD	21 ± 2°C	- (died)
	12:12 LD	12 ± 1°C	-
	11:13 LD	14 ± 1°C	-
	8:16 LD	21 ± 2°C	-
	8:16 LD	13 ± 1°C	-
	8:16 LD	10 ± 1°C	-

Table 2. Observations of *Rhodochorton purpureum* 4187 tetraspore release in different conditions.

Salinity Treatment	Observation
14 psu	No spore release
26 psu	Some spore release, two spores with slight amoeboid motion
30 psu	Some spore release, one with slight amoeboid motion
Blotting and salinity	
Blotted, 10 psu	No spore release
Blotted, 26 psu	Some spore release (not much), with some slight amoeboid motion
Blotted, 30 psu	Some spore release, one tetrad with one spore showing amoeboid motion

them to fresh 30 psu medium. The videomicroscopy methods were given in Pickett-Heaps et. al. (2001). The 4187 samples were sealed under a coverslip with VALAP (equal weights Vaseline, lanolin, paraffin wax melted together) and the spore release was recorded at room temperature (16-22°C) in time lapse on a videodisc recorder.

OBSERVATIONS

Table 1 indicates the results for daylength and temperature conditions in which the two isolates were cultured.

The 4241 isolate from San Juan Island, Washington USA (48°N) was tested in the complete range of conditions available but no tetrasporogenesis occurred. Growth was satisfactory in all conditions except 12:12 LD, 21 ± 2°C, 20-30 μmol photons m^{-2} s^{-1} in which the tetrasporophyte died. Normal stolons attached to the glass substrate and formed erect branched shoots at regular intervals (Fig. 1).

The 4187 plants from Jeju Island, Korea grew well in 12:12LD at 18 ± 2°C without reproduction but died 12:12LD at 21 ± 2°C (Table 1). Tetrasporogenesis was induced in two weeks by growing the plants in 11:13LD and 8:16LD at 14 ± 2°C and 8:16 at 12 ± 1°C and 10 ± 1°C (Figs 2 & 3). Tetrasporangia and bisporangia were 24-29 μm long and 17-19 μm in diameter, releasing 4 or 2 spores respectively (Figs 4 and 8). Free spores were 12-15 μm in diameter. Spores oozed out of the sporangia and once released they showed slight amoeboid deformation for 2-3 minutes, after which the spores simply rounded up and showed no further movement (such as gliding).

Table 2 shows the results for salinity and blotting treatments for spore releases of the 4187 isolate. Significant spore release occurred in 30 psu in both treatments, compared to 10, 14 and 26 psu. No spore release

Fig. 1. Habit of *R. purpureum* 4187 tetrasporophyte at 12:12 LD, 18 ± 1°C. Mature tetrasporangia developed in two weeks on the erect shoots. Scale bar is 100 μm.

Fig. 2. Habit of *R. purpureum* 4241 tetrasporophyte at 8:16 LD, 13 ± 1°C. No reproduction occurred in conditions tested. (ER, erect shoots; B, basal system). Scale bar 50 μm.

Fig. 3. Habit of *R. purpureum* 4187 male gametophyte at 8:16 LD, 13 ± 1°C. (SP, spermatangial clusters). Scale bar is 150 μm.

Fig. 4. Tetrasporangial and bisporangial formation, empty sporangia with spores and sporelings. (BS, released bispores.) Scale bar is 25 μm.

Fig. 5. Tetraspore germination two days old 4187 at 8:16 LD, 13 ± 1°C. Scale bar is 10 μm.

Fig. 6. Male gametophyte 4187 at 8:16 LD, 13 ± 1°C. (SP, spermatangia; R, rhizoid; S, spore). Scale bar is 15 μm.

Fig. 7. Mature male gametophyte with spermatangial cluster (cf Fig. 2). Scale bar is 30 μm.

Fig. 8. Tetrasporangial formation of 4187 at 8:16 LD, 13 ± 1°C. Scale bar is 25 μm.

Fig. 9. Two-week-old female gametophyte of 4187 at 8:16 LD, 13 ± 1°C. Carpogonium with trichogyne (TR), rhizoid (R) arising from a spore (S). Scale bar is 15 μm.

Fig. 10. Mature female gametophyte with a carpogonial cluster. Trichogynes (TR) and developing carpogonia. Rhizoid (R) growing from base of cluster. Scale bar is 25 μm.

Fig. 11. Female gametophyte with tetrasporangia. Scale bar is 20 μm.

was observed in 10 and 14 psu treatments. The blotting technique appeared to slightly increase the spore release in both 26 and 30 psu.

The spores typically had bipolar germination with the narrow (ca. 5 μm) rhizoid developing first (Fig. 5), followed by the erect shoot with a larger diameter (ca. 7 μm) at the opposite pole (Figs 6 and 9). The stolons and erect branches of male and female plant were slightly smaller in diameter than those of the tetrasporophyte. The sporelings became sexually mature in about 2 weeks under optimal conditions (8:16LD, 12 ± 2°C, 15-20 μmol photons m^{-2} s^{-1}). Typical young male plants were about 130 μm in length. The rhizoid was about 60 μm long, while the erect shoot and spermatangial cluster were about 50 μm in length (Fig. 6). On old longer male plants lateral branches with spermatangial clusters developed on stolons and erect shoots (Fig. 7).

We tested various ways to induce spermatial release to determine if spermatia were motile. Transferring male plants with mature spermatangia from culture medium at 30 psu (salinity) to 20, 15 and 10 psu was unsuccessful. Blotting and reimmersing male plants was also unsuccessful in stimulating release. The blotting process caused no visible damage to the plants.

Female plants often were shorter than the males when first reproductive. When the erect shoot was only one cell long a carpogonium sometimes developed (Fig. 9) and a lateral cell formed below that formed a new apical cell and further carpogonia. The carpogonial base was 8-9 μm in diameter and 17-24 μm long tapering into the trichogyne that was 3 μm in diameter and up to 200 μm long. The older carpogonia collapsed and decomposed. We observed no post-fertilisation development of carposporophytes even though free spermatia occasionally were seen attached to trichogynes. Larger older female plants had well defined stolons on which single carpogonia or short clusters bearing numerous carpogonia were seen on short erect branches (Fig. 10). Often we saw developing tetrasporangia in the carpogonial clusters (Fig. 11). These did not appear to be derived from a carposporophyte but directly from cells of the female gametophyte. No spore discharge was observed from these tetrasporangia.

DISCUSSION

Four different life history patterns are described for *Rhodochorton purpureum* from different locations.

The first and most common pattern seen is sexual- a tetrasporophyte producing tetrasporangia and the tetraspores developing into male and female gametophytes. Fertilised carpogonia form reduced gonimoblasts that give rise directly into new tetrasporophytes (Knaggs 1968; Lee 1985; Ohta and Kurogi 1979; Stegenga 1978; West 1969, 1970). One isolate from Chile produced monoecious gametophytes (West 1970) and one isolate from Muroran, Japan also had monoecious gametophytes (Ohta and Kurogi 1979). These also resulted in direct development of the tetrasporophyte from the gonimoblast.

The second pattern is asexual- the tetrasporophyte producing tetrasporangia and tetraspores form new tetrasporophytes. This occurred in some isolates from Muroran and Oshoro, Japan (Lee 1985).

The third pattern is mixed-phase-gametophytes bearing carpogonia and tetrasporangia or spermatangia and tetrasporangia occurred in isolates from Muroran, Japan (Lee 1985). Mixed-phase reproduction was also seen in our isolate from Jeju I. Korea but it was not possible to determine if the spores from mixed-phase plants were viable.

The fourth pattern is no reproduction. Only vegetative growth occurred in isolates from Nemuro, Japan but this may have been because the temperatures and photoperiods were not suitable to induce tetrasporogenesis (Lee 1985). That may be the problem with our isolate 4241 that did not reproduce in any conditions tested. However, another isolate from San Juan Island formed tetrasporangia at 8:16, 10:14 and 12:12 LD in 10 ± 1°C but no tetrasporangia were seen at 8:16, 10:14 and 12:12 LD in 15 ± 1°C (West 1972). Isolates from Amchitka, Alaska (52°N) and Concepcion, Chile (37°S) sporulated well at 10°C. An isolate from Bodega, California (38°N) sporulated well at 8:16, 10:14 and 12:12 LD in 15 ± 1°C.

Although we didn't see significant spore release or any spermatium release in treatments used here we can report *R. purpureum* from Jeju Island, Korea has bispores and tetraspores with slight amoeboid motility. By contrast, monospores of *Colaconema caespitosum* (J. Agardh) Jackelman, Stegenga & J.J. Bolton (as *Audouinella botryocarpa* (Harvey) Woelkerling) exhibited very active amoeboid or rotating movement (Guiry *et al.* 1987). Amoeboid motility of monospores was also observed in *Acrochaetium pacificum* Kylin [as *Audouinella pacifica* (Kylin) Garbary] and *Colaconema proskaueri* (J.A. West) P.W. Gabrielson [as *Audouinella proskaueri* (West) Garbary] by Pickett-Heaps *et al.* (2001).

Bisporangia frequently occur in various red algae

(Guiry 1990) and West *et al.* (2001) but their formation and functions seem to vary greatly in different taxa. The bispores in *R. purpureum* from Washington USA (West 1969) evidently germinated and developed into gametophytes but we have not substantiated that in the Korean isolate.

It has not been possible to induce spermatium release in 4187. Consequently no observations were made on spermatium motility and fertilisation. However, in *Phyllopora membranifolia* (Goodenough et Woodward) J. Agardh, spermatium motility was reported by Rosenvinge (1927) but no spermatium motility was observed in videomicroscopy investigations on fertilisation in *Murrayella periclados* (C. Agardh) Schmitz (Wilson *et al*. 2003) and *Bostrychia moritziana* (Sonder ex Kuetzing) J. Agardh (Wilson *et al.* 2002).

To continue our investigation it will be necessary to test other techniques and conditions to enhance spore and spermatium release and movement.

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