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Spore germination on *Polysiphonia subtilissima* Montagne from Setse and Kyaikkhami coastal areas, Thanlwin river mouth, Myanmar

Abstract

Polysiphonia subtilissima Montagne collected from Setse and Kyaikkhami coastal areas was carried out. The carpospores of *P. subtilissima* germinated at 25˚C under the photoperiod of 8L:16D and 30˚C under the photoperiod of 16L:8D, showing the early stages of cell divisions and development of thalli in culture. In the carpospores germination of *P. subtilissima*, primary rhizoid developed from lower cell after second cell division during 2days in culture. After 15days, the pericentral cells cut off and formed as polysiphonous structure in the young filamentous sprout with a total length of up to 1200µm. The carpospore germination pattern of *P. subtilissima* Montage of laboratory experiment was briefly discussed.

Keywords: carpospores, kyaikkhami, laboratory culture, *polysiphonia subtilissima*, Setse, germination

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Introduction

The genus *Polysiphonia* is widely distributed in most temperate and tropical waters of the world.[1] Recently, Guiry and Guiry[2] recorded 119 species of *Polysiphonia* reported from various regions of world oceans.

Kyaw Soe & Kyi Win[3] had also reported 9 species of *Polysiphonia*. However, Kyaw Soe & Kyi Win[3] and Soe-Htun[4,5] gave no specific description on *Polysiphonia* spp. due to lack of specimens mentioned in the previous reports, in the Department of Marine Science. Recently, Soe-Htun et al.[6,7] listed two species of *Polysiphonia* viz., *P. subtilissima* Montagne and *P. sp*, Soe-Htun et al.,[8] recorded the only species of *P. subtilissima* Montagne along Gwa coastal areas. Sein Moh Moh Khaing[9], Jar San,[10] ZayarAung,[11] Myo Min Tun,[12] Thet Htwe Aung[13] and EiEi Hlaing[14] described the habitat of *P. subtilissima* Montagne.

The carposporangia release the carpospores to the marine currents and tides. They settle down on a surface and germinate by mitosis and grow into the diploid tetrasporophyte. The tetrasporangium has four (tetra) tetraspores packed into each tetrasporangium. These meiotic products are in a tetrahedral arrangement. Each spore has a circular contact area with the other three spores in the tetrahedron, and the spore will have a tri-radiate ridge marking in this circular contact area. The purpose of this study is to know the early developmental stages in carposporegermlings of *P. subtilissima* in laboratory cultures as well as in the field.

Materials and methods

The plants of *Polysiphonia subtilissima* Montagne were collected from the mangrove swamp and rocky platform in the intertidal zone at Setse (Lat. 15°52'N, Long. 97°35'E) and Kyaikkhami (Lat. 16°05'N, Long. 97°34' E) coastal areas, Thanbyuzayat Township, Mon State (Figure 1) during the period from June 2014 to September 2015. The materials were brought in ice box to the laboratory at Mawlamyine University for the observation.

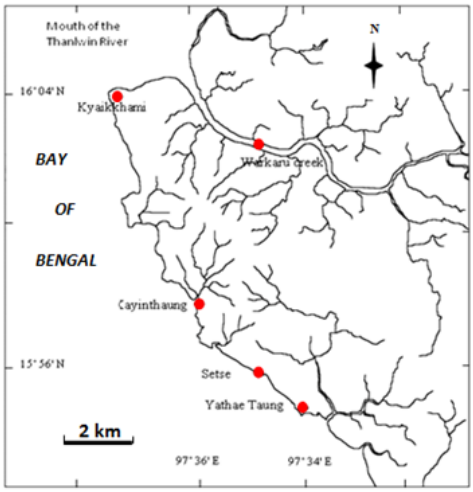


Figure 1 Map showing the collection sites of the samples.

The cultured apparatus such as Petri dishes (7.5x1.8cm) and (4x2cm), glass slides, blades, brushes, glass bowls, cover slips, pipette, pointers, cover slips (1.7x1.7cm) and forceps were washed with tap water and then sterilized again with boiling water. Sterilized seawater was adjusted to get salinity by refractometer. And then Iwasaki's SWII media was prepared for both spore liberation and germination. Germanium dioxide (GeO_2) was employed for elimination of diatoms throughout this study.

In the laboratory, the fresh and healthy carposporophyte plants were randomly selected and dried with tissue paper and were kept under the dark condition for overnight. In the next morning, both reproductive fragments were placed on cover slips in each Petri dishes (7.5x1.8cm) in 30ml and (4x2cm) in 20ml of prepared culture medium. These Petri dishes were placed under at 25˚C under the photoperiod of 8L:16D and at 30˚C under the photoperiod of 16L:8D, using cooled and ventilated incubators (Gallenkamp Cat No. IMF 781-7, volt 220/240, MHz 50). Aeration was not provided in all experiments.

Observations on liberation of spores were carried out hourly with intervals of 1 or 2hrs over a period of 24hrs to 48hrs of collection in the laboratory. Carpospores settled on the cover slips were transferred to each Petri dish containing prepared culture medium. The numbers of cell divisions were recorded and sizes of spores and germlings were measured under the compound microscope using ocular meter in 3days interval. The developmental stages of carpospores germlings were photographed by Sony DSC-W 330 digital camera, processing with Adobe Photoshop CS3. The culture medium was changed at 3days interval. Culture studies were repeated several times. This study followed the classification system of used by Guiry and Guiry.[2]

Results and discussion

A classification system of the *Polysiphonia subtilissima* Montage

Phylum: Rhodophyta

Class: Florideophyceae

Order: Ceramiales

Family: Rhodomelaceae

Genus: *Polysiphonia* Greville

Species: *Polysiphonia subtilissima* Montagne

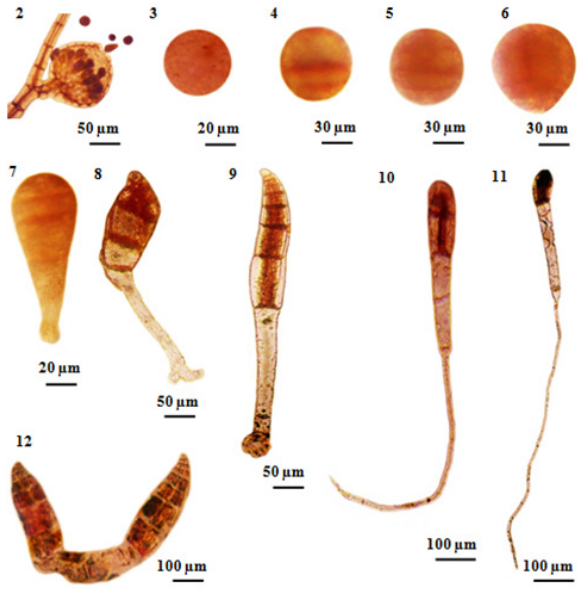
Stages in the development of carpospores germination in *Polysiphonia subtilissima* Montagne

Germination of carpospores and further development of the germlings could be studied by using a broad range of salinities 30‰ at 25°C under 8L:16D photoperiod. The carposporelings of *P. subtilissima* liberated at all salinities though the sporelings did not germinate in lower salinities 25‰ and higher salinity of 30‰. The germling of *P. subtilissima* developed into polysiphonous structures.

Liberated carpospores were globular and deep red in color; they have thin transparent walls and vary from 50µm to 70µm in diameter (Figure 2) (Figure 3) (Figure 13). The liberated carpospores were clavated and gradually transformed into globular within 5minutes. Average mean lengths of carpospores germlings of *P. subtilissima* during after fifteen days culture period were observed in (Table 1) (Figure 23).

Table 1 Average mean length of carpospores germlings of *Polysiphonia subtilissima* Montagne after 15 days culture period at temperature of 25°C under 8L: 16D photoperiod

Days	Length (µm)
1	80
2	90
3	100
5	125
6	370
7	560
10	980
12	1100
15	1200



Figures 2-12 Carpospores and their early development of *Polysiphonia subtilissima Montagne*. (2) Liberated carpospores from mature cystocarp. (3) Released carpospores. (4)Three unequal- celled stages formed within 12-24 hours. (5) The second segmentation of carpospores germling showing unequal four cells with 24-36 hours. (6) Beginning of germination three days after initiating. (7) Four-celled stage 3-5 days after initiating. (8) The rhizoidal portion of sporeling curved slightly (arrow) after 6-7 days. (9) The germling resulted in 6-8 cells stage and an expanded disc-like rhizoid; the main axis becoming slightly curved after 7 days. (10) After 7-10 days germlings, the number of 8-10 cells, lateral pericentral cells. (11) After 12 days germlings, the number of several cells and rhizoidal cells. (12) After 15 days old carposporelings.

First, isolated carpospores attached to the substratum within twelve hours and three unequal transverse walls are formed 80µm in diameter. This cell division takes place within 12 to 24hours (Figures 4) (Figure 14). After 24 to 36 hours, the carposporelings continued it division to from four-celled stage, have 100µm length and 80µm broad (Figures 5) (Figures 15).

After three days initiating, the basal portion of sporeling became the rhizoid shoot (Figure 6) (Figure 16). The rhizoidal pole was conical in shaped, continually lengthened and reached 15-30µm in length.

After 5days, germling continued four celled divisions and the rhizoid cells divided into 1-2cells reaching a length of 120-130µm in length and 50µm in width (Figure 7) (Figure 17). And then, (Figure 8) (Figure 18) shows each segment of the main axis was composed five cells and the rhizoid cell divided into 3 - 4 cells, length varied from 350-400µm in length by 6 days development.

After 7days germling, about 6-8cells in length with a longitudinal cell showing formation of the rhizoidal cells are found in five cells. The main axis was recurved and an expanded disc-like rhizoid, have 400-600µm in length 50-70µm in width (Figure 9) (Figure 19). Within 7-10days, the cells number was 8-12cells that axial cell at a position of 2-3 segments behind the apical cell were divided to form the lateral pericentral cells and the first lateral initials form the newly cutoff pericentral cell, 400-600µm in length of thallus and 400-500µm in length of rhizoid (Figure 10) (Figure 20).

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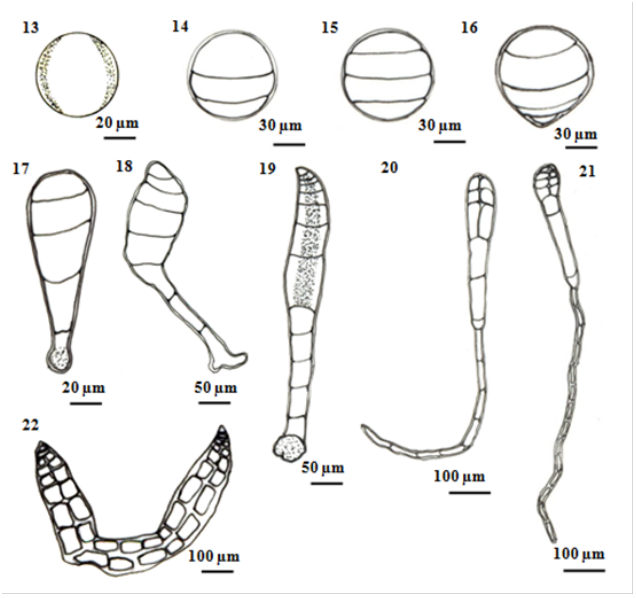
Germlings appeared the number of several cells with developed rhizoids after 12days (Figure 11) (Figure 21). After 15days inoculation, the pericentral cell cut off and formed as polysiphonous structure in the young filamentous sprout. The germling increased in length and growing, measuring a total length of up to 1200µm. The color of the germling was normal reddish brown (Figure 12) (Figure 22).



Figures 13-22 Schematic drawing showing successive stages of carpospores germination in *P. subtilissima Montagne*. (13) Released carpospores. (14) Three unequal-celled stage formed within 12 - 24 hours. (15) The second segmentation of carpospores germling showing unequal four cells with 24 - 36 hours. (16) Beginning of germination three days after initiating. (17) Four-celled stage 3-5 days after initiating. (18) The rhizoidal portion of sporeling curved slightly after 6-7 days. (19) The germling resulted in 6 - 8 cells stage and an expanded disc-like rhizoid. the main axis becoming slightly curved after 7 days. (20) After 7-10 days germlings, the number of 8-10 cells, lateral pericentral cells. (21) After 12 days germlings, the number of several cells and rhizoidal cells. (22) After 15 days old carposporelings.

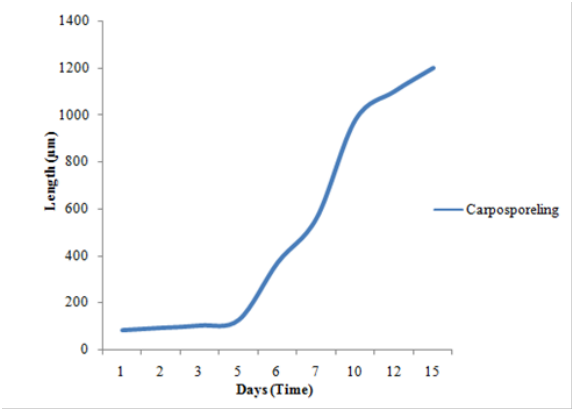


Figure 23 Average means length of carpospores germling of *P. subtilissima* Montagne during 15 days culture periods.

In the laboratory studies, the effects of temperature on spore germlings of *Polysiphonia* were investigated at 25°C and 30ºC. The growth of carposporelings was higher at 25ºC, but did not distinctly different occur at 30ºC. Kudo and Masuda[15] observed the optimum salinity for the growth of carpospores germling of *P. japonica* and *P. akkeshiensis* were found under 10 - 20°C and photoregimes (16L:8D and 8L:16D).

Carpospores discharge of *P. subtilissima* was observed within 1-8hrs whereas Kudo & Masuda[15] reported the spore discharge of *P. japonica* Harvey and *P. akkeshiensis* Segi were observed within 24hours. Kudo & Masuda[15] described, liberated carpospores were globular, deep red in color and they average 60.3µm (range 57.5-65.0µm) in diameter. In *P. akkeshiensis* liberated carpospores deep red in color and average 57.6µm (range 52.5-65.0µm) in diameter.

The species of *P. japonica* and *P. akkeshiensis* isolated carpospores soon attached to the substrate and grew into bipolar sporelings of 6-7 segments, which one day after inoculation, had differentiated into a colorless rhizoid and a pigmented main axis. These two species of germlings lateral initial were formed from each segment in a spiral line running in a counter clockwise direction toward the apex of the main axis as development of the main axis proceeded. The first lateral initial grew usually into a pseudo dischotomously divided vegetative trichoblast. However, the species of *P. japonica* and *P. akkeshiensis* formed all the ordinary branches grew indeterminately as did the main axis forming vegetative trichoblasts and ordinary branches of the second order.

According to the (Table 1), the spore germination grew quickly and the germlings gradually died after 20days. In the present study, the carpospores germination of growth rate of the *P. subtilissima* Montagne is slower than the growth of the carpospores germling of *P. japonica* and *P. akkeshiensis* Kudo & Masuda.[15] In addition, carpospores form germlings but tetraspores were not germlings in the laboratory experiments.

The local distribution ranges of *P. subtilissima* Montagne distributes along the Rakhine Coastal Region: from Sin Phyu Gyaing to Shwe Ya Gyaing; Ayeyawady Coastal Region: no data and; Tanintharyi Coastal Region from High Island to Lampi Island. The species of *P. subtilissima* Montagne is widely distributed throughout the Atlantic Ocean, the Indian Ocean and the Pacific Ocean in tropical and temperature water. Further studies are still needed to verify and revise the spore germination patterns and life cycle of the genus *Polysiphonia* Greville from Myanmar.

Conclusion

The plant of *Polysiphonia subtilissima* abundantly grew along the intertidal region of Setse and Kyaikkhami coastal areas where salinity range was 18-30‰. Field collected plants with cystocarps were selected and cultivated for the early developmental stages of spore germination of *P. subtilissima* under laboratory condition. In the carpospores germination of *P. subtilissima*, the pericentral cell cut-off and formed as Polysiphonous structure in the young filaments sprout. The germling increased in length and growing, measuring a total length of up to 1200µm after 15days. The color of the germlings was normal reddish brown in color.

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Conflict of interest

Author declares that there is no conflict of interest.

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