

Developmental studies in *Porphyra vietnamensis*: A high-temperature resistant species from the Indian Coast

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Abstract

Porphyra vietnamensis Tanaka & Pham-Hoang Ho (Bangiales, Rhodophyta) is a tropical seaweed collected from the west coast of India. Thalli of the blade phase are found growing only during the rainy season between July and September. They grow on rocky intertidal or subtidal substrata or as epiphytes on other seaweeds such as *Enteromorpha flexuosa* and *Chaetomorpha media*. The gametophytic thallus is monostromatic and covered with spines at the base. The species is monoecious. Male gametangia are found in patches that are distributed in the upper part of the thallus. Archeospores are found at the thallus margins and give rise to the blade phase after one week of germination even at 30 °C. Zygotospores germinated at 25 °C into conchocelis within three days from the date of their inoculation. Conchospores were released at 30 °C. The young blades grew at 32 °C in the laboratory.

Introduction

Porphyra (Bangiales, Rhodophyta) is one of the world's most valued maricultured seaweeds, and is primarily used as food in many oriental countries. It is highly prized for its flavour and as a health food as it is rich in proteins and vitamins. Nearly 17 types of free amino acids, including taurine, which controls blood cholesterol levels (Tsuji et al., 1983) can be found within the genus, which has an annual value of over US\$ 1.8 billion (Yarish et al., 1999). The biology and ecology of *Porphyra* has been studied more thoroughly than that of any other red algal genus (Tseng & Sun, 1989; Cole, 1990; Hawkes, 1990). Recently, it has been reported that *Porphyra* has much more potential and can be used as an experimental system for modern biological research, like *Arabidopsis thaliana* (Sahoo et al., 2002). *Porphyra* is represented by more than 133 species, which are particularly abundant in cold-temperate and boreal shores of the Northern and Southern Hemispheres (Yoshida et al., 1997). In some areas, individual species grow throughout the year whereas other species are very seasonal, with big crops present

on the rocks for only one to two months of the year. So far seven species of *Porphyra*, namely *P. chauhanii* Anil Kumar & Panikkar, *P. indica* V. Krishnamurthy & Baluswami, *P. kanyakumariensis* V. Krishnamurthy & Baluswami, *P. crispata* Kjellman, *P. okhaensis* H. Joshi, Oza & Tewari, *P. suborbiculata* Kjellman, and *P. vietnamensis* T. Tanaka & Pham-Hoang Ho have been reported from different parts of the Indian coast, of which *P. vietnamensis* is the most abundant (Sahoo et al., 2001). Børgesen (1937) reported *P. vietnamensis* as *P. tenera* Kjellman from Madras Harbour. Later, Sreeramulu (1952) originally described the plants as *P. naidum* Anderson. Subsequently, Umamaheswara Rao and Sreeramulu (1963) confirmed the species as *P. vietnamensis*. Lewmanomont and Ogawa (1978) studied the life cycle of *P. vietnamensis* from Songkhla, Thailand but were not successful in obtaining the release of conchospores from the conchocelis phase. Imada and Abe (1980) were able to get conchospores from *P. vietnamensis* by using phytohormones. Lewmanomont and Chittpoolkusol (1993) studied the life history of *P. vietnamensis* from Thailand and were able to complete the life history in the

laboratory at 25 °C. Although *P. vietnamensis* has been reported from different parts of the Indian coast, no detailed studies have been undertaken on its development in culture. In the present study, it has been found that a particular strain of *P. vietnamensis* can complete its life history at 32 °C in the laboratory. Since most of the *Porphyra* species grow at lower temperatures, this Indian strain of *P. vietnamensis* could be a potential crop for mass cultivation in tropical seas.

Materials and methods

Porphyra vietnamensis was collected during July 2003 from the rocky coast of Goa in western India. Fertile blades were selected and cleaned with seawater in the field to remove visible epiphytes and other contaminants. The thalli were wrapped in absorbent cotton moistened with seawater, packed in plastic bags and transported to the laboratory in an air-conditioned railway compartment. In the laboratory, the thalli were washed thoroughly in autoclaved seawater and each thallus was observed under a stereo binocular microscope. Thallus surfaces were cleaned with the help of sterilized cheese cloth and epiphytes were removed. Subsequently, the thalli were washed 3–4 times in autoclaved seawater. Then each thallus was blotted with tissue paper. Individual thalli were then wrapped in cheese cloth, put in a polythene bag and kept in a refrigerator. After two hours thalli were taken out and individual thalli were put into petri-dishes (size 90 mm, Polylab India) separately in three different culture media: f/2 (Guillard & Rhyther, 1962), PES medium (Provasoli, 1968) and autoclaved seawater. The petri-dishes were kept at 20 °C, 25 °C, 30 °C and room temperature (32 °C) under cool white 40 W fluorescent lights at an approximate photon fluence rate of 40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The thalli in petri-dishes were observed after every two hours for the release of spores which were collected by means of Pasteur pipette and transferred to new petri-dishes separately in the above mentioned media. A drop of GeO_2 was added to each petri-dish to prevent the growth of diatoms (1 g GeO_2 dissolved in 100 mL of distilled water). All petri-dishes were kept in the above mentioned temperature and light conditions with a photoperiod of 12:12 h light: dark cycle. The salinity of media was maintained at 25 ppt. Culture media were changed after every seven days and observations on spore development were recorded. Photographs were taken through a Nikon E600 photomicroscope (Nikon, Tokoyo, Japan) using

ILFORD Black and White film. Spore terminology follows Nelson et al., 1999.

Observations

Porphyra vietnamensis was found growing predominantly on the west coast of India especially in the Provinces of Maharashtra, Goa and Karnataka. The species was strictly seasonal. The leafy thalli appeared on the rocky substratum, oyster shells (*Crassostrea gryphoides*) or as an epiphyte on seaweeds such as *Enteromorpha flexuosa* (Wulfen) J. Agardh and *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützting in the mid-tidal to spray zone from the beginning of July. The blades started degenerating from the end of August and completely disappeared by the end of September. Over the last several years the senior author has observed that the growth of *Porphyra* species in India is associated with the onset of the monsoon season. During this season the seawater is turbid and rich in nutrients due to river and land-surface discharge. The seawater temperature decreases substantially from 30–32 °C to 19–23 °C and salinity from 31–33 ppt to 19–22 ppt during these months. The sky is mostly cloudy during this season, which appears to favour the growth of the species.

Thallus morphology

Considerable variation was observed in the gross morphology of *P. vietnamensis* but in general the blades were monostromatic, membranous, lanceolate to linear-lanceolate and sometimes ribbon-like, purple to pink-purple in colour. The thalli were attached to the substratum with discoid holdfasts. Sometimes thalli were branched from a common base having several bladelets (Figure 1). Usually the thalli were 3–15 cm in height but sometimes grew up to 40 cm. They were 1.5–3 cm broad and 25–32 μm thick. The cells of the basal regions were large, with pigmented pear-shaped heads, and elongated (Figure 2). Margins were undulate with 2–3 celled spines, which were found towards the basal region (Figure 3). The vegetative cells in the central region were vacuolated with a single stellate plastid (Figure 4). The thalli were monoecious with distinct male gametangial and zygotosporangial patches found towards the margin and these were present on the same thallus. The male gametangia could be distinguished as pale-yellow patches at the outer margins. There were 64 spermatia per male gametangium arranged in 4 tiers