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Seasonal occurrences of epiphytic algae on the commercially cultivated red alga *Kappaphycus alvarezii* (Solieriaceae, Gigartinales, Rhodophyta)

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Abstract

Common problems faced in farming of the red algal genus *Kappaphycus/Eucheuma* are "ice-ice disease" and the occurrence of epiphytes. Considerable work has been documented on "ice-ice disease" and it's mode of infection but limited information is available on the emergence of epiphytes. The present study addresses the phenomenon of epiphyte infection, its prevalence in commercially cultivated red alga, *Kappaphycus alvarezii,* and their variability associated with seasonality. Cultured seaweed became susceptible to epiphytes in the dry seasons (1) between March – June and (2) September – November. Findings revealed *Neosiphonia savatieri* (Hariot) M. S. Kim *et* I. K. Lee, as the dominant infecting epiphyte, representing up to 80–85% of the epiphyte present during peak seasons. Besides *N. savatieri*, *Neosiphonia apiculata*, *Ceramium* sp., *Acanthophora* sp. and *Centroceras* sp. were observed in smaller quantities. SEM (Scanning Electron Microscope) micrographs revealed the epiphyte's attachment to the host. Further histological study showed the extent of penetration of epiphytes into the host's cortex tissues and condition of its surrounding tissues. The outbreak of epiphytic filamentous red algae also correlated with drastic changes in seawater temperature and salinity during March – June and September – November.

Introduction

In Malaysia, seaweed farming was introduced in the late 1970s and commercial cultivation started few years after, with the culture of *Gracilaria* in Peninsula Malaysia and *Eucheuma* in North Borneo. Today, after almost 35 years, the red algal genera *Kappaphycus* and *Eucheuma* are intensively cultivated in two locations; Semporna (east coast of Borneo), and Kudat (north Borneo). Concomitant with the increase in farm size and intensification of culture practices is the rise in seaweed diseases, particularly; "ice-ice disease" and invasion of epiphytes (filamentous red algae). Epiphytic invasion is not a new phenomenon and has been known to exist since the dawn of farming practices (Doty & Alvarez, 1975, 1981). However, little is known of their causative agents, seasonality, mode of action and factors causing outbreaks (Parker, 1974; Collen et al., 1995; Fletcher, 1995; Ask, 1999, Ask & Azanza, 2002; Critchley et al., 2004). Knowledge of these aspects is vital, since epiphytic algae invade regularly, at times affecting the marketability of the harvested seaweeds. Epiphyte outbreaks have also shown to weaken the seaweed, making it susceptible to bacterial attack (unpublished data).

Hence, the present study was initiated to gather substantial information and in-depth understanding of the causative agent and some indication of the factors affecting outbreaks. Dynamics of epiphyte outbreaks were monitored in relation to abiotic seawater factors such as temperature and salinity. In addition, histology and Scanning electron microscopy (SEM) investigations were carried out to determine the epiphyte's attachment and intrusion into the host.

Materials and methods

Sampling location

Infected seaweed specimens were collected from a seaweed farm in Teluk Lung, Pulau Balambangan, Kudat, Sabah (04°32′43″N, 116°58′30″E). Sampling was conducted fortnightly during the epiphyte outbreak. Specimens were transported in a cold ice-chest (4 ∼ 6 °C) and fixed in 10% formalin in seawater at the laboratory. Sections and epiphytes were removed from the host by hand under stereo microscope (Stemi-2000 CS, Carl Zeiss, Germany) using a razor blade and pith stick and stained with 0.5% (w/v) cotton blue in lactic acid/phenol/glycerol/water [1:1:1:1 (v/v)] solution and mounted in 50% glycerol/seawater microscope slides. Some of the isolated epiphytes were immediately mounted in seawater on microscope slides and observed. Epiphytes were counted randomly in an area of 1 cm × 1 cm under a stereo microscope at 2 ∼ 5 times magnification. A total of 20 individuals were observed during each observation and data presented in Figure 1 represent average numbers of epiphytes for 12 months observation.

Histological study

Sections (0.5 cm in length) of the infected algal thallus were fixed for 24 h in 4% glutaraldehyde in 0.1 M cacodylate buffer (salinity; 30 ppt). Fixed specimens were then dehydrated in the following series of ethanol; 70, 80, 90, 95 and 99%, each for 1 h. Dehydrated specimens were then cleared twice with xylene: each step lasted for 1 h. Finally, specimens were impregnated with paraffin (twice), each for 80 min, before blocking. Sections were cut at 15 μm thickness and stained in haematoxilin/eosin or pirodic acid Schiff's reagent prior to viewing.

Scanning electron microscope study

Algal thalli with epiphytes were cut ca. 1.0 cm in length and fixed for 24 h in 4% glutaraldehyde in 0.1 M cacodylate buffer before post-fixation in 1% OsO_4 at 4 °C for 2 h, followed by dehydration with a graded acetone series and finally, critical point drying. Dehydrated specimens were mounted on stubs and coated with a 10–30 nm layer of gold before observation with a Leica Cambridge S360 electron microscope.

Physical water parameters

Seawater temperature and salinity was recorded on a multi-probe water quality checker (YSI meter, Model 85–25) three times daily at 6 am, 12 pm and 6 pm. Measurements were taken at a maximum depth of 0.5 m below the seawater surface. Data are presented as averages of those measured, for each two-week interval.

Results and discussion

Seasonal variation of epiphytes

Occurrence of epiphytes was monitored from January to December 2003. The presence of epiphytes was first observed at the end of February and vegetative plants emerged 3–4 weeks later and persisted until the end of June. A second invasion was shorter: the first sign was seen in early September and the epiphyte disappeared by late November. A total of 5 epiphyte species were

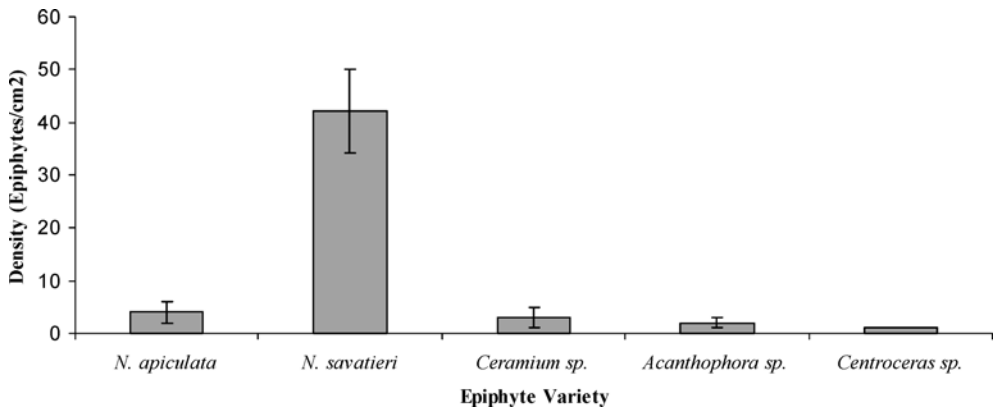

Figure 1. Average species composition of epiphytes isolated from the surface of the cultivated red alga, *Kappaphycus alvarezii*, during outbreaks.