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Full Length Research Paper

Occurrence of endosymbiont *Richelia intracellularis* (Cyanophyta) within the diatom *Rhizosolenia hebetata* in Northern Arabian Sea

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Accepted 4 January, 2010

The presence of diazotrophic cyanobacterium *Richelia intracellularis* Schmidt as an endosymbiont of *Rhizosolenia hebetata* was observed in the phytoplankton samples collected from the Northern Arabian Sea during the fog end of winter monsoon (February - March). *R. hebetata* formed 80% *Rhizosolenia* population and 60% of this species harboured endosymbiotic cyanobacterium *R. intracellularis*. This is an indication that *Richelia*-*Rhizosolenia* interaction could have a significant influence on nutrient and energy budgets of the Northern Arabian Sea.

Key words: *Rhizosolenia*, diatom, *Richelia intracellularis*, cyanobacteria, Northern Arabian Sea.

INTRODUCTION

Nitrogen is generally considered to be a major limiting nutrient for phytoplankton growth in coastal and oceanic waters. The fixation of atmospheric nitrogen in the oligotrophic ocean is an important source of new nitrogen to surface waters, stimulating phytoplankton productivity and fueling the biological pump. In tropical waters the non-heterocystous cyanobacterium *Trichodesmium* is largely responsible for nitrogen fixation (Karl et al., 2002), but the heterocystous diazotroph cyanobacterium *Richelia intracellularis* provide quantitatively substantial inputs of nitrogen on regional scales (Carpenter et al., 1999). Symbiosis between cyanobacteria and marine organisms is abundant and wide spread among marine plants and animals. *R. intracellularis* Schmidt, a heterocystous cyanophyte is common in tropical and subtropical seas. It generally appears in symbiosis with phytoplanktonic species of the genus *Rhizosolenia* and *Hemiaulus* and with benthic diatoms of the genus *Pleurosigma* (Wood, 1965, 1967) or as an epiphyte in *Chaetoceros compressus* or in *Rhizosolenia styliformis* var. *latissima* (Allen and Cupp, 1935; Sournia, 1968). The presence of

a terminal heterocyst on each *Richelia* filament suggests that these blue green algae may have the ability to fix molecular nitrogen (Fay et al., 1965; Venrick, 1974; Mague et al., 1977). Cyanobacteria contribute moderately large amount of nitrogen annually in the world ocean and are one quarter of the total input of nitrogen to the sea. The ability to utilize molecular nitrogen would seem to give a nutritional advantage in the inorganic nitrogen limited euphotic zone. Only a few workers have reported *Rhizosolenia*-*Richelia* association from Indian seas. Two records on occurrence of the species from the South East coast are found in the literature (Iyengar and Desikachary, 1944; Subrahmanyam, 1946).

The present communication reports the dominance of N-fixing (diazotroph) cyanobacterium *R. intracellularis* within the diatom *Rhizosolenia hebetata* Bailey forma *hebetata* in the phytoplankton collections from the Northern Arabian Sea.

MATERIALS AND METHODS

This observation was based on phytoplankton samples collected during the FORV Sagar Sampada cruise from Northern Arabian Sea (Lat. 20 - 22° N and Long. 64 - 68° E, Figure 1) from ten stations covering an area of 1.2 x 10⁵ sq. km at the fog end of winter monsoon (Feb - March). The phytoplankton samples collected from

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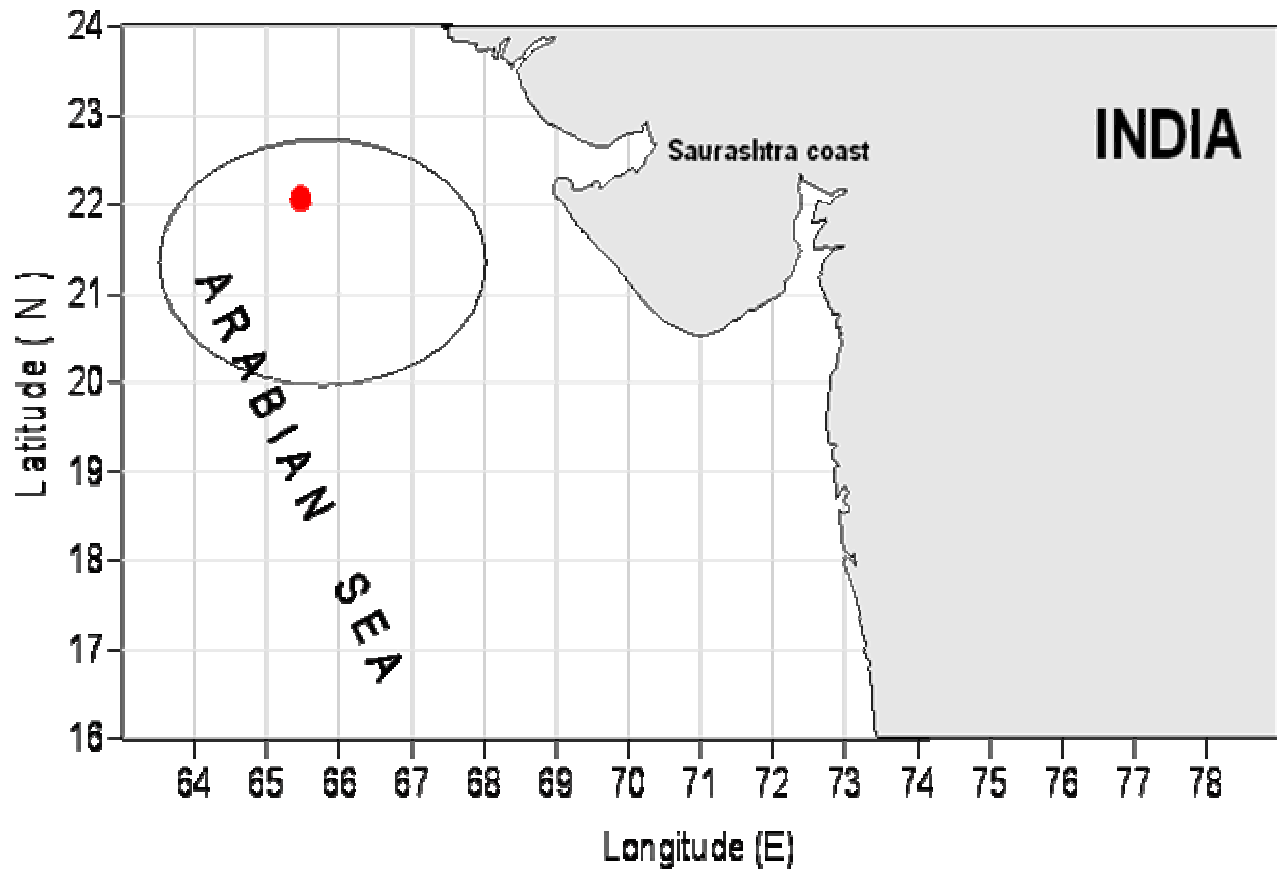


Figure 1. Map showing the location of *Richelia*-*Rhizosolenia* association observed in Northern Arabian Sea

these ten stations, one station (Lat. 22° 01.43 N, Long. 65° 20.06 E) contained *Rhizosolenia* with endosymbiont *Richelia*. The physical and chemical characteristics of the water where *Rhizosolenia*-*Richelia* association was observed were compared with another station (Lat. 22° 03.15 N, Long. 66° 45.02 E) where this species was not found. The surface samples were obtained by filtering surface sea water through a 20 μ bolting silk and the filtrates preserved in 3% buffered formalin and Lugol's iodine. Subsurface samples (up to 120 m) collected using Niskin bottles attached to CTD profiler and allowed to settle in glass sedimentation cylinders and concentrated to 50 ml after adding Formalin-Lugol's iodine solution. Physical parameters like temperature, salinity, density etc on a vertical profile were carried out employing the Seabird 911 plus CTD sensors to understand the oceanic processes. Sea surface temperature (SST) of the area was measured by ordinary thermometer. Nitrite, nitrate, phosphate and silicate were analysed using a segmented flow auto analyzer (SKALAR) onboard the vessel adopting standard procedures. Ammonium concentrations were determined by colorimetric methods. Dissolved oxygen was estimated following Winkler method. Chlorophyll *a* was measured spectrophotometrically using a double beam Perkin Elmer UV-Visible spectrophotometer following 90% acetone extraction method. The examination and photomicrographs of the samples were taken using a Nikon Eclipse microscope attached with Nikon DN 100 series digital camera. Organic matter was removed from the diatom cells using the methods of Hasle and Fryxel (1970) and the diatom species were identified with the help of the work of

Hasle and Syversten (1997).

RESULTS AND DISCUSSION

In general frustules of *Rhizosolenia* are solitary and often did not contain the endosymbiont *Richelia*. However, in the phytoplankton samples collected from the open ocean stations in Northern Arabian Sea numerous trichomes of *R. intracellularis* were observed as endosymbiont in *R. hebetata* (Figures 2 A, B and C). The presence of this endosymbiont was not total in *R. hebetata* in the collection examined. Moreover, within the *Rhizosolenia* mats individual diatoms with and without the symbiont co-occurred. The concentration of *Rhizosolenia* sp. was 91070 cells l^{-1} and the genus was represented by four species viz. *R. hebetata*, *R. styliformis*, *Rhizosolenia imbricata* and *Rhizosolenia clevis*. Notwithstanding this *R. hebetata* formed the 80% of *Rhizosolenia* population and around 60% of this species harboured endophytic cyanophyte *Richelia*. The *Richelia* occupied the periplasmic space between the plasmalemma and the cell wall of the host. This symbiotic relation is more



Figure 2(A). *Rhizosolenia hebetata* with *Richelia intracellularis* (×400).



Figure 2(B). *Rhizosolenia hebetata* without *Richelia* (×400).

facultative than obligatory for *Rhizosolenia* can produce carbohydrate when enough nitrogen is available in the environment. The frustules of *Rhizosolenia* containing *Richelia*, in general had one trichome per cell or at most two, located at the ends of the cell and with the heterocysts oriented to the valve.

The *R. hebetata* cells were varied in their size with diameter ranging from 8 - 29 µm and length 210 - 490 µm. Each trichome was composed of 8 - 13 similar cells and

one spherical heterocyst, with a well differentiated polar nodule. The diameter of the heterocyst varied between 9 - 13 µm and the total length of the trichome between 40 - 71 µm. *Richelia* apparently did not persist outside its host; it was rarely seen as a free living trichome.

Richelia does not possess gas vesicles, it probably benefits from the buoyancy provided by the host diatom. The vertical distribution of *Richelia* corresponds to that of the host diatom. Viable cells of both have been recorded



Fig. 2 (C): *Richelia intracellularis* ($\times 400$).

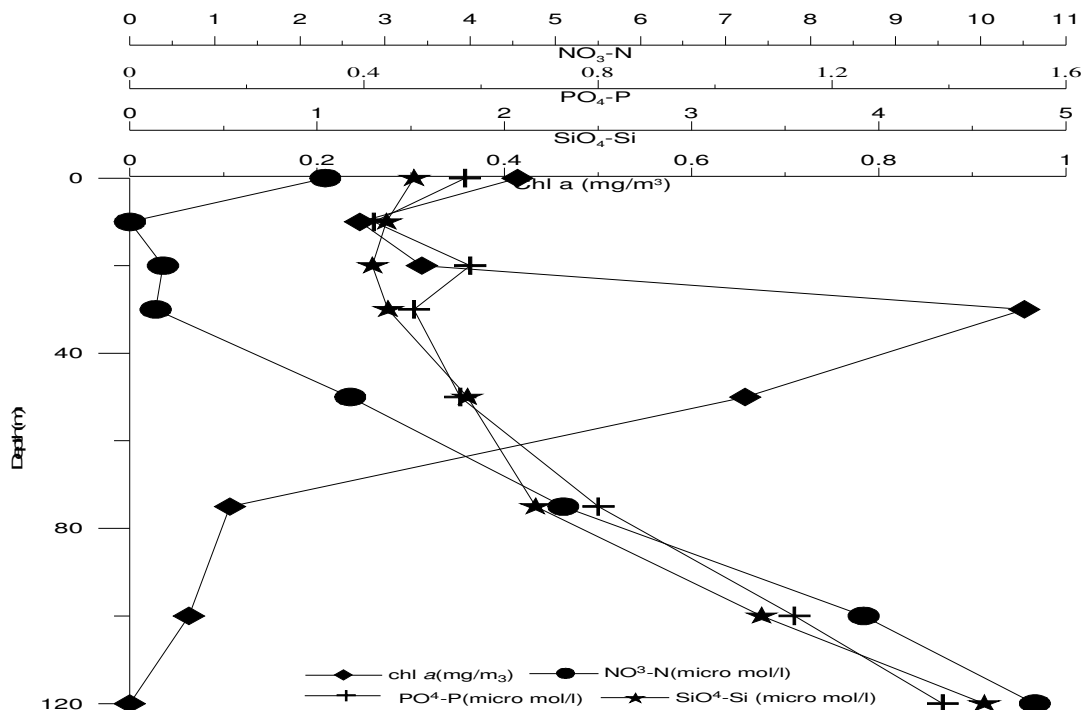


Figure 3. Physico-chemical parameters of the *Richelia-Rhizosolenia* association observed area.

recorded from the phytoplankton samples up to 100 m depth. The majority of *Richelia-Rhizosolenia* associations have been recorded from the phytoplankton samples collected from 30 m or less. This is the reason for relatively high chlorophyll *a* (0.956 mg m^{-3}) at 30 m depth. Sea surface temperature and salinity of the area was 26.9°C and 36.48 psu respectively. Nutrient concentrations recorded in this area were as follows: $2.30 \mu\text{mol l}^{-1} \text{ NO}_3\text{-N}$, $0.57 \mu\text{mol l}^{-1} \text{ PO}_4\text{-P}$ and $1.52 \mu\text{mol l}^{-1} \text{ SiO}_4$. It is evident that the nitrate concentration ($2.30 \mu\text{mol l}^{-1}$) in the area where *Rhizosolenia-Richelia* asso-

ciation was observed was higher than that of the area where such a phenomenon was not observed ($0.02 \mu\text{mol l}^{-1}$). Ammonium concentration of the area was higher ($4.11 \mu\text{mol l}^{-1}$) compared to that of other open ocean stations, which could be attributed to the diazotrophic nature of cyanobacterium, which depicts the ability to produce ammonium from dinitrogen through the process of nitrogen fixation (Chang et al., 2000). Physico-chemical parameters of the area where *Richelia-Rhizosolenia* associations observed are presented in Figure 3.

Northern Arabian Sea (NAS) is oligotrophic in nature with limited source of nitrogen during all the seasons except winter monsoon (January - March). The main nutrient source in the NAS is by convective mixing during winter monsoon and such enrichment initiates multispecies phytoplankton bloom in this area (Madhupratap et al., 1996). It is likely that besides convective mixing, inorganic nitrogen resulting from *Rhizosolenia*-*Richelia* symbiotic association supports increased primary production in the NAS.

ACKNOWLEDGEMENTS

The authors are grateful to all the participants of FORV Sagar Sampada cruise no. 264 for the help rendered in sampling. This investigation was carried out under the Marine Living Resources Programme funded by Ministry of Earth Sciences, Government of India, New Delhi.

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