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Artificial UV-B induced changes in pigmentation of marine diatom *Coscinodiscus gigas*

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Abstract: *In vitro studies in marine diatom Coscinodiscus gigas revealed that artificial UV-B radiation (313 nm) at a dose level of 0.4W m^{-2} for a continuous period of 3 hours in a UV treatment chamber caused disbursement of chromatophores from their normal loci and resulted in clumping / aggregation of chromatophores exhibiting a phenomenon called UV-B induced syntrophism . It is also understood that such clumping could cause only insignificant reduction in photosynthetic oxygen release.*

Key words: *UV-B radiation, Marine diatom, Chromatophore distribution and changes*

Introduction

Due to widening of ozone hole in the stratosphere over Antarctica, the influx of UV-B radiation (280-320 nm) to the surface of the earth, is likely to rise further. The effect would more pronounced in macro algae and unicellular organisms. Among unicellular organisms phytoplankton of the marine environment which occupy the upper layer of the photic zone, is much exposed to UV radiation. Several studies have been undertaken both *in vitro* and experimental model studies to assess the possible impact on the photosynthetic production rate in marine plants. It is understood from the reports that UV-B rays are capable of affecting cell division, photosynthetic activity, altering photosynthetic pigmentation, impairing photosynthetic process particularly at PS II level, leaching or depletion of photosynthetic pigments and disrupting membrane structure in the photosynthetic apparatus (Wood, 1987; Smith *et al.*, 1992; Strid and Porre,1992; Strid, 1993; Haeder and Schafer, 1994; Neilsen *et al.*,1995; Sinha *et al.,* 1996 and Haeder *et al.*, 1997). Most of these studies are done in terrestrial as well as aquatic plants and macro algae. Studies in marine phytoplankton are very scanty in the line of UV induced structural and physiological changes in the photosynthetic pigmentation (Cullen and Lesser, 1991; Dohler *et al.,* 1991; Larkman and Wood, 1993; Herrmann, 1996; Yogamoorthi, 1996, 2002 and Zudaine, 2001). Moreover, studies pertaining to the impact of enhanced UV-B radiation on marine diatom *Coscinodiscus gigas*, one of the common resident of upper photic zone of marine environment is not available except the work of Yogamoorthi (2002). Therefore, an *in vitro* study was undertaken to find out the impact of artificial UV-B radiation on the chromatophores of cultured diatom, *Coscinodiscus gigas*.

Materials and Methods

Marine diatom *Coscinodiscus gigas* required for the study, was collected from inshore waters of Pondicherry region using plankton net. To avoid silt content in the net and to ease the segregation of required diatom species, sampling were done at a distance of 500m away from the shore line. Phytoplankton were separated and transfered to conical flasks containing filtered habitat water. In the laboratory, cells of *Coscinodiscus gigas* were isolated under binocular microscope and transfered to 500 ml culture flask containing TMRL medium (a suitable culture medium for the healthy growth of Coscinodiscus gigas). These culture flasks inoculated with diatoms were incubated by providing optimum temperature (22.0$\pm$0.5°C) and ambient light source. The visible light source was provided by 2 numbers of 4 feet fluorescent tubes and the light source was tuned in such a way that the culture cells get 12 hr of light period and 12 hr of dark period. The culture was continued for three days. At the end of 3rd day culture flasks were taken out. Known number of diatoms using Sedgwick Raft Cell, were taken out and transferred to sterilized petri dishes containing TMRL medium. Totally 4 sets of petri dishs were prepared; one set was kept as control and others as experimental samples required for 3 different irradiation period. Out of 4 sets of petri dishes 3 sets were kept inside the UV-B treatment chamber and one kept as control. The UV chamber was fitted with 2 nos.40W 4 feet fluorescent tubes for visible light source and one Phillips 40 W UV tube - 313 nm (Patel Co., USA) for artificial UV radiation source. Using LSE Radiometer, the quantum of visible light and UV radiation available to the diatoms kept in the Petri discs, were measured as 5.0 and 0.4W m^{-2} respectively. The culture dishes were irradiated continuously for a period of 12 hr. One set of control was also maintained separately. After 1 hr irradiation, one set of Petri dishes were taken out. The irradiated diatoms were observed under trinocular microscope fitted with SLR camera fitted under magnification. Cells showing striking changes, were photographed. Another set of culture discs were taken out at the end of 2 hr irradiation and the last set after 3 hr of irradiation. These two sets of samples as well as controls were also examined as it done for the first set of sample (diatoms).

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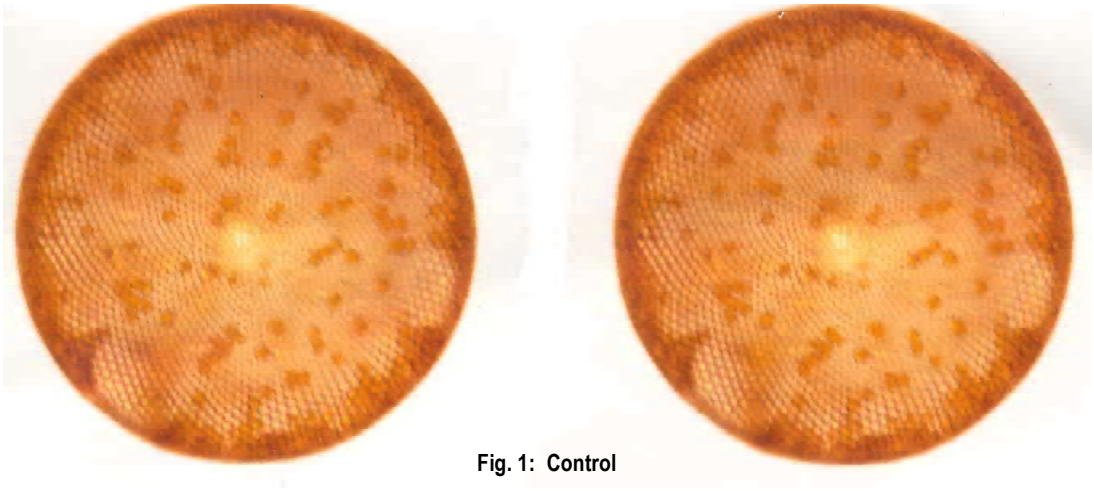

Fig. 1: Control

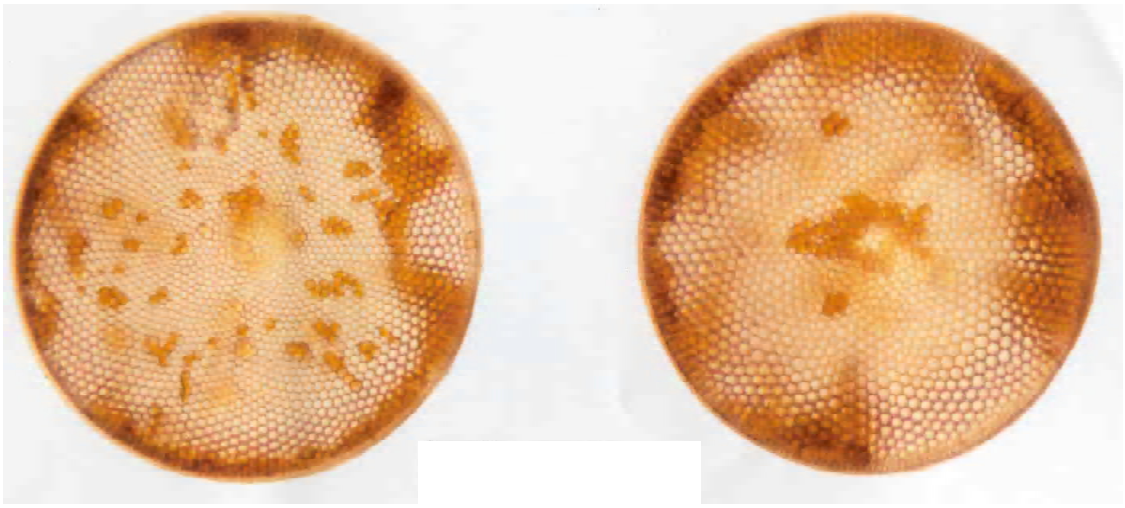

Fig. 2: Diatoms irradiated for 2 hr

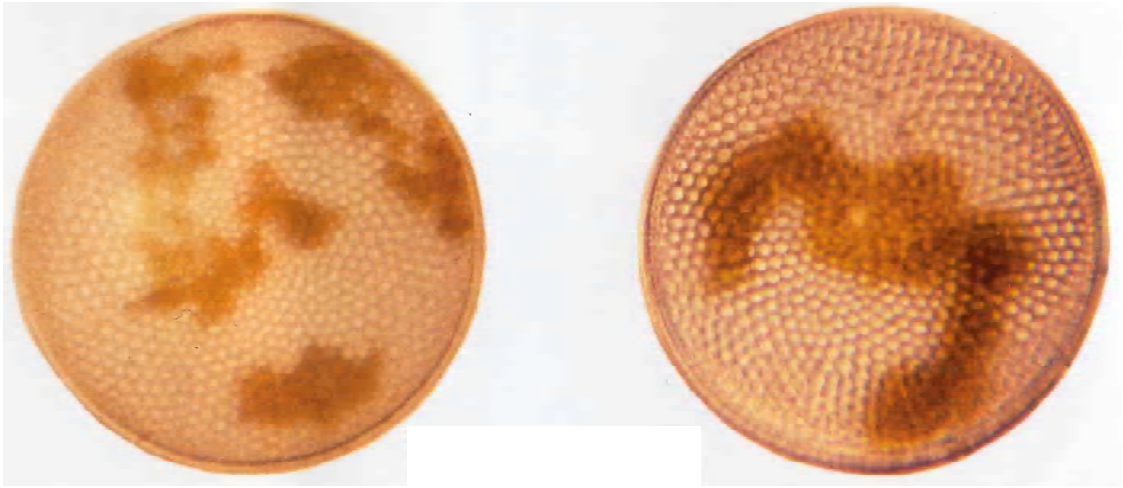

Fig. 3: Diatoms irradiated for 3 hr

Results and Discussion

In the control (normal cells) the chromatophores are distinctively arranged on the inner side of the epitheca and hypotheca exhibiting more or less uniform spacing in the inner surface (Fig. 1). The diatom cells that are irradiated for 1 hr showed no significant change in distribution pattern of the chromatophores. The cells that are irradiated for 2 hr showed significant changes/alteration in the distribution pattern of chromatophores (Fig. 2). In the 3 hr irradiated cells, the changes were quite obvious where the cells exhibited irregular clumping / aggregation of chromatophores (Fig. 3). The disbursement of chromatophores from their usual loci started from the 2 hr of irradiation and leads to clumping / aggregation at the end of 3 hr of irradiation.

Marine phytoplankton populates the upper layer of the water column and thus may be affected by the increased solar UV radiation (Santas *et al.,* 1996). Due to the great ecological importance of phytoplankton in the marine environment, UV effects on the phytoplankton productivity have been the subject of concern in view of the expanding ozone hole in the stratosphere. Even though reports are available on the influence of UV radiation on marine macro algae, studies pertaining to influence of enhanced UV-B radiation on the photosynthetic capsule / chromatophores in marine diatoms particularly *Coscinodiscus gigas* are not undertaken so far. To start with, an attempt was made to understand the influence of artificial UV radiation on the pigmanetation pattern / chromatophore distribution in the diatom. Normally the chromatophores are distinctively arranged on the inner sides of epitheca and hypotheca (diatom valves) exhibiting more or less a uniform spacing in their distribution (Fig. 1). But in the diatoms that are irradiated for a continuous period of 2 hr, exhibited very significant changes in chromatorphore distribution or position. It is quite obvious from Fig. 2 that chromatophores distributed along the periphery of the theca started moving off from their normal loci. Such alternation in the chromatophore distribution is quite obvious in the cells that are irradiated for 3 hr where chromatophores exhibited striking changes / alterations by showing very conspicuous clumping / aggregation (Fig. 3). Such phenomenon could also be called as syntroph. Previous reports on these lines also indicated that any external stress would result alteration/ changes in the photosynthetic apparatus particularly in phytoplankton and microorganisms as they lack epidermal layers, a naturally occurring biological screen in higher plants (Strid, 1993; Donkor and Haeder, 1997). As far as diatoms are concerned, their cell walls are made up of silica and pectin hence UV rays directly incident on the theca exerting a stress on the organism. Further, it is also understood from the reports of Yogamoorthi (2002) that such change / alteration in the chromatophore distribution (clumping) has no significant effect on the photosynthetic efficiency of the diatom. It is reported that the diatom showed only 8-10% reduction in its photosynthetic production during 3 hr of irradiation. These observations are quite contrast to the reports of Bidigare (1989); Gerber and Haeder (1995); Haeder *et al.* (1997) and Wiegman *et al.* (2002) where loss of photosynthetic pigments and chlorophylls have been observed. Therefore, it is presumed from the present experimental studies that as a function of UV stress in diatom *Coscinodiscus gigas*, the chromatophore responded exhibiting clumping or aggregation without causing any significant reduction in their photosynthetic production in terms of oxygen release. Though diatoms are unicellular and their cell wall is made up of silica and pectin unlike macro algae and higher plants, the enhanced UV-B could exert only stress rather than destructing photosynthetic pigments in diatom *Coscinodiscus gigas.* Further, phytoplankton communities occurrying at the upper layers of the photic zone may be capable of adjusting to changes in environmental stress such as increased solar UV-B radiation (Santas, 1996). Reports of Zudaine (2001) on the photoprotection and long term acclimation to UV radiation are also in close conformity with present findings. These findings indicate that the increasing UV radiation is bound to augment the stress factor of the radiation on phytoplankton. However, further attempts to examine the impact of long term irradiation on diatom as well as photosynthetic pigments is very much required to understand the photoresponse of these unicellular organisms and the consequential implications on the primary producers of the marine environment.

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