# PROTECTIVE ROLE OF SODIUM CHLORIDE AND SODIUM DITHIONITE AGAINST UV-B INDUCED DAMAGE IN *CHLORELLA VULGARIS*

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## ABSTRACT

Highly useful and economic algae especially protein-rich micro- algae are highly affected, and the protein, carbohydrate and pigments are highly damaged by increased UV-B exposure in a high protein-rich (45%), green, freshwater, unicellular microalga *Chlorella vulgaris*. 1 mM sodium dithionite (SDT) partially protected survival and growth against UV-B induced damage but NaCl (0.5M) became lethal to *C. vulgaris* in supplemented condition.

**Key words**: UV-B, protection, survival, growth, *Chlorella*.

## INTRODUCTION

The appearance and increasing size of the ozone hole in the Antarctica, due to stratospheric ozone depletion and consequent enhancement in UV-B intensity reaching the earth's surface, has become a major global concern in recent years. It is not predicted to recover until 2065 (Häder *et al.* 1995, Madronich *et al.* 1998). But, the ozone hole size, 28 million square kilometer in 2000 has decreased into 18 million square kilometer over Antarctica. Now, southern Argentinean city of Ushvaia had been under very high UV levels on October 2003 (Hanson. 2003).

Because of its high energy, UV-B damages cellular protein, cytosolic and membrane bound proteins, photosynthetic pigments, genetic materials and DNA (Häder *et al.* 1998). Significant changes in solar UV on aquatic ecosystem may result in decreased biomass production, food web, species composition resulting in reduced food production for increasing global human population (Häder and Worrest 1997).

Exposure of *Chlorella vulgaris,* a protein rich microalga, to UV-B radiation inhibited the survival, growth, protein, carbohydrate, pigment contents, nitrate reductase, glutamine synthetase activity and ultimately photosynthesis (Prasad *et al.* 1998). Photoreduction of singlet oxygen in PSI of thyllakoid membrane in all oxygenic phototrophs as cyanobacterial and eukaryotic algal cells generates superoxide radicals and $H_2O_2$ and causes photoinhibition (Asada 1999).

The protein content in microalgae is higher than in any other plants, therefore in Japan *Chlorella* is commercially marketed popularly as health tablet (Venkataraman and Becker 1989).

Solar UV-B has been a significant variable in the aquatic environment throughout evolutionary

time, and photosynthetic organisms have developed a variety of defences that allow them to avoid, screen, repair, scavenge or otherwise protect their cells from long- term damage (Vincent and Roy 1993).

The damaging impacts of UV-B on *Nostoc muscorum* and *Anacystis nidulance* were also protected by amino acid, cysteine, L-tryptophan and some reducing agents such as ascorbic acid, reduced glutathions and sodium pyruvate (Tyagi 1992). Sodium dithionite prevents inactivation of succinic dehydrogenase and also the reoxidation of other compounds in the cytochrome system (Johnstone 1963).

The fluorescence inactivation of chloroplast due to ultraviolet radiation exposure might be restored by the addition of sodium dithionite (Kulandaivelu and Noorudeen 1983).Many genes in micro organisms are regulated by UV-B. It alters the gene action (Bender *et al.* 1997) and active oxygen may be one trigger for altered gene activity (Mackerness *et al.* 1998).

## MATERIALS AND METHODS

### Test Organism and Growth Conditions

*Chlorella vulgaris,* a unicellular, green microalga, member of chlorophyceae (chlorococcales) was isolated from Durga Kund pond (pH 8.0-8.5) in Varanasi. Cells were 5-16 µm, spherical, solitary, sometimes aggregated into small colonies. Each cell had a thin cell wall and a cup shaped chloroplast. Identification was made as per Philipose (1967). It was made unialgal and axenic by several transfer and grown in modified Chu-10 mediun at 25±I°C in a culture room illuminated with 14.4 $Wm^{-2}$ fluorescent light for 14 $hd^{-1}$.

### Source and Mode of UV-B Treatment

The source of UV irradiation was a UV-B lamp (Cat. No. 3-4408, Fotodyne Inc. USA) with peak emission at 312 nm. Twenty ml culture suspension was taken in 75 mm sterile petridishes (lid-removed) and irradiated under UV-B lamp. The desired intensity of UV-B (2.5 $Wm^{-2}$) was obtained by adjusting the distance between UV-B source and the sample. The intensity was measured by a Back-Ray J-221 longwave UV intensity meter (UVP-B Inc. San Gabriel, California). The suspension was stirred magnetically during UV-B exposure.

### Percent Survival

For determining the percent survival, 1 ml irradiated samples were withdrawn at known time intervals and diluted $10^4$ times. Diluted samples (0.1 ml) were plated on agar plates and after 30 h dark pre-incubation exposed to fluorescent light. Percent survival was measured by colony counts after 10 days of growth and plotted semi logarithmically.

### Protein, Carbohydrate and Pigment Estimation

Samples were withdrawn similar to survival procedure at desired intervals after UV-B exposure or after 30 h dark incubation and used for protein, carbohydrate and pigment estimation. The initial optical density of culture was kept at 0.2 (0.1 mg/ml dry wt) . Protein and carbohydrate contents were measured after Lowry *et al.* (1951) and Dubois *et al.* (1956), respectively. Chlorophylls a, b and carotenoids were extracted and measured in aqueous acetone by the method of Myers and Kratz (1955). Specific growth rate and generation time were calculated as per the method of Kratz and Myer (1955).

**Protective Assessment**

For assessing the impacts of NaCl (0.5 M) and sodium dithionite (I mM) on *C. vulgaris* two sets of experiments, percent survival and growth of *C. vulgaris* were performed. In the first set, NaCl and sodium dithionite (SDT) were directly added separately to 20 ml exponential culture suspension in petridishes and exposed to UV-B for required periods. The irradiated inocula (0.05 ml) were diluted and plated on agar plates or also transferred to the liquid medium in test tubes with or without the test chemicals. Thereafter they were incubated in light (14 $Wm^{-2}$) at 26 ± $1^0$C for 15 days after 30 h dark incubation. The test chemicals were sterilized by autoclaving.

In the second set, exponentially grown cultures were treated with the test chemicals for 48 h and exposed to UV-B for 30 and 60 minutes. The irradiated samples were washed with sterilized distilled water and plated on normal and supplemented agar plates and incubated in light.

**Growth Behavior**

Exponential grown culture suspensions of 0.2 optical density having NaCl and SDT separately were exposed to UV-B for different durations and after known interval aliquots were withdrawn and incubated in dark for 30 h and 0.2 ml was transferred into each 9.8 ml fresh sterile medium and then incubated in light for different periods. It was used for protein estimation which reveals the growth behavior.

**RESULTS**

Fig. 1 shows the survival curve of *C. vulgaris* after UV-B treatment for different time intervals. There was a linear decrease in survival with increase in duration of exposure. There was 50% survival after 10 min and complete killing occurred after 60 min UV-B exposure.

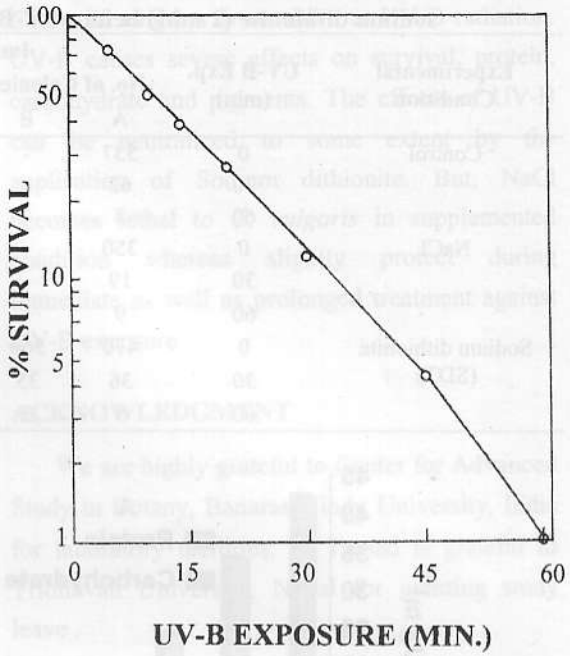


**Fig. 1. Effect of UV-B (2.5$Wm^{-2}$) on survival of *C. vulgaris*.**

Fig. 2A shows the protein and carbohydrate content decline by increasing UV-B dose but protein was more sensitive than carbohydrate. Fig 2B reveals that photosynthetic pigment contents also decline with increasing UV-B dose. Carotenoids were less affected than chl a and chl b. Chlorophyll b was found more sensitive than chlorophyll a.

Table 1 shows the protective roles of NaCl and SDT against UV-B induced damage either immediately or 48 h pretreated culture solution before UV-B exposure. Immediate supplemented SDT and NaCl did not protect against UV-B induced damage. NaCl caused death in supplemented cultures. SDT slightly protected against adverse effect of 30 and 60 min UV-B in 48 h pretreated *C. vulgaris*. NaCl slightly protected in control cultures but become lethal to *C. vulgaris* in supplemented cultures.

Table 1. Protective role of immediate treatment and 48 h pretreatment of NaCl (0.5 M) and sodium dithionite (1 mM) before UV-B exposure to *C. vulgaris*.

| Experimental Condition | UV-B Exp. (min) | Immediate | | | | After 48 h Pretreatment | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of Colonies | | % Survival | | No. of Colonies | | % Survival | |
| | | A | B | A | B | A | B | A | B |
| Control | 0 | 537 | - | 100 | - | 470 | - | 100 | - |
| | 30 | 62 | - | 11.5 | - | 53 | - | 11.3 | - |
| | 60 | 8 | - | 1.5 | - | 7 | - | 1.5 | - |
| NaCl | 0 | 350 | - | 100 | - | 172 | - | 100 | - |
| | 30 | 19 | - | 5.4 | - | 36 | - | 20.9 | - |
| | 60 | 9 | - | 2.6 | - | 8 | - | 4.7 | - |
| Sodium dithionite (SDT) | 0 | 470 | 560 | 100 | 100 | 75 | 53 | 100 | 100 |
| | 30 | 36 | 35 | 7.7 | 6.3 | 14 | 14 | 13.7 | 26.4 |
| | 60 | 5 | 3 | 1.1 | 0.5 | 4 | 3 | 5.3 | 5.7 |

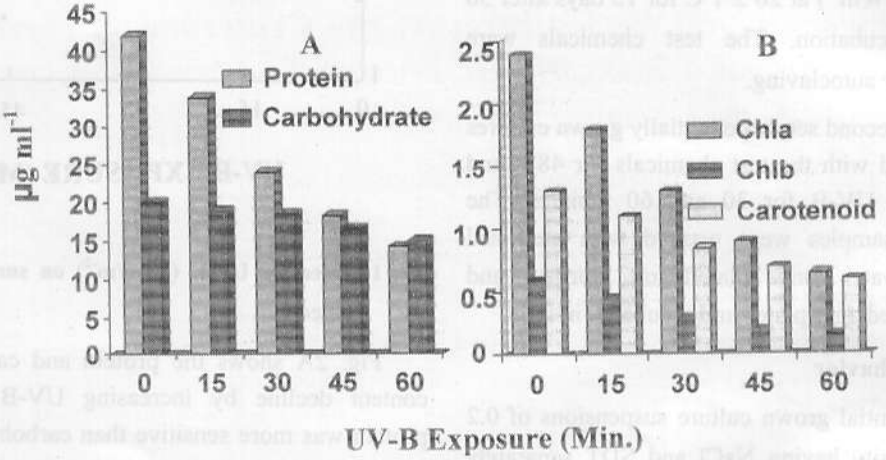


Fig. 2. Effect of immediate UV-B exposure on protein, carbohydrate, and pigment content (µg $ml^{-1}$) of *C. vulgaris*.

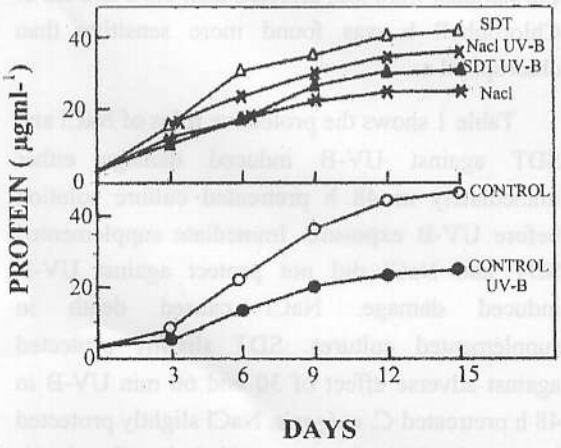


Fig. 3. Protection of growth behavior of *C. vulgaris* in supplemented NaCl and sodium dithionite in liquid medium after 30 min UV-B exposure.

Fig. 3. shows the growth pattern of *C. vulgaris* after 30 min UV-B exposure in liquid medium containing SDT or NaCl. SDT and NaCl both showed less growth of *C. vulgaris* than control but they showed significant growth against 30 min UV-B treatment. Growth pattern of *C. vulgaris* was more protected by NaCl than SDT during 30 min UV-B exposure.

## DISCUSSION

The above results suggest that *C. vulgaris* is sensitive to UV-B radiation. Killing by enhanced UV-B irradiation has been also reported in marine diatoms (DÖhler 1985), dinoflagellate (Ekelund

1991) and Antarctic phytoplankton (Smith *et al.* 1992). The possible cause of killing of cells might be due to damage of genetic materials, proteins and other biologically relevant molecules absorbing light between 280-320 nm UV band. The cells are destroyed or altered due to high oxidation by $H_2O_2$ or singlet molecular oxygen which lead to the death of organims (Asada 1999, Häder *et al.* 1998).

Protein content is more affected by UV-B than carbohydrate which might be due to utilization of reserve endogenous carbohydrate during vital metabolic processes. Protein content inhibition by UV-B is in agreement with DÖhler (1985) in marine diatoms. Pigment content inhibition by UV-B is in accord with the report of Asada and Takahashi (1987) due to photooxidation. The carotenoid is less affected due to its antioxidant property.

Very little information is so far found about the role of chemicals in protecting UV-B induced damage. The degree of protection depends on the nature of chemicals and the organisms used. SDT conferred protection in *C. vulgaris* which might be effective to neutralize the cytotoxicity resulting from interaction of singlet oxygen produced during photodynamic action of UV on biomolecules at neutral pH (Garcia 1994).

Supplementary NaCl becomes lethal to *C. vulgaris* cells. It is probably due to growth cessation and decreased efficiency of PSII or due to stimulation of fermentation related to reduced oxygen availability during increased salinity (Serraj *et al.* 1994). The growth protection by SDT and NaCl against UV-B induced damage might be due to liquid holding recovery mechanism (Harm 1980).

Our findings clearly demonstrate that *Chlorella vulgaris* is sensitive to UV-B radiation. UV-B causes severe effects on survival, protein, carbohydrate and pigments. The effects of UV-B can be neutralized to some extent by the application of Sodium dithionite. But, NaCl becomes lethal to *C. vulgaris* in supplemented condition whereas slightly protect during immediate as well as prolonged treatment against UV-B exposure.

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