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DETECTION OF UV-B-INDUCED THYMINE DIMER IN A CYANOBACTERIUM, *SCYTONEMA* SP.

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ABSTRACT

DNA molecule is one of the major targets for UVR that can alter its molecular structure by forming different types of lesions leading to chronic mutagenic and even death of the cell. In comparison to UV-B, the wavelength of UV-A has poor efficiency in inducing the DNA damage; because they are not absorbed by native DNA. Before assessing the impact of UV-B radiation on DNA, we observed its effects on growth and survival of the test organism *Scytonema* sp. It was observed that growth and survival were severely affected by UV-B radiation for different durations. UV-B treatment causes loss in the cooperative binding property of DNA which is evident from the failure of complementary strands of DNA.

Keywords: DNA; UV-B; cyclobutane-pyrimidine dimmer

INTRODUCTION

INTRODUCTION

Ultraviolet is arbitrarily subdivided into three wavebands such as UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (<280 nm) that may affect the normal life processes of all organisms ranging from prokaryotes to mammals **(Friedberg *et al*., 2005)**. In all the groups of UVR, UV-C is quantitatively absorbed by oxygen and ozone in the Earth's atmosphere, hence, does not show much harmful effects on biota, but on the other hand UV-B and UV-A radiation play a significant role in producing the pronounced effects on diverse habitats, even though most of the extraterrestrial UV-B is absorbed by the stratospheric ozone **(Madronich *et al*., 1998; McKenzie *et al*., 2003)**. UVR can affect a number of physiological and biochemical processes such as growth and survival, pigmentation, heterocyst differentiation, photosynthesis, enzymes of nitrogen metabolism and total protein profiles **(Sinha *et al*., 1995; Sinha *et al*., 1998)** in diverse organisms. DNA molecule is one of the major targets for UVR that can alter its molecular structure by forming different types of lesions leading to chronic mutagenic and even death of the cell. In comparison to UV-B, the wavelength of UV-A has poor efficiency in inducing the DNA damage, because they are not absorbed by native DNA. However, UV-A is able to generate singlet oxygen ($^1O_2$) or reactive oxygen species (ROS) that can damage DNA *via* indirect photosensitization reactions **(Alscher *et al*., 1997; Mackerness, 1999)**.

Impacts of UV radiation on Cyanobacteria

Cyanobacteria are phylogenetically a group of gram-negative prokaryotes that possess higher plant type oxygenic photosynthesis. During Precambrian period (between 2.8 to 3.5 x 109 years) they were probably the main primary producers to release oxygen into the then oxygen-free atmosphere. Hence, cyanobacteria seem to be leading organisms for the development of aerobic metabolisms and rise of higher plants **(Schopf, 1993; Pace, 1997)**.
Cyanobacteria depend on solar radiation as their primary source of energy in their natural environment. The potential threat to these organisms is the continuous depletion of the stratospheric ozone layer and the consequent increase in solar UV-B radiation reaching the Earth's surface **(Blumthaler *et al*., 1990; Crutzen, 1992)**. Biologically effective doses of UV-B radiation may affect a number of physiological and biochemical processes of cyanobacteria such as growth and survival, pigmentation, total protein profiles, photosynthetic enzymes, photosynthesis, etc. UV-B mediated effects on growth and survival have been observed in several cyanobacteria **(Tyagi *et al*., 1992; Sinha *et al*., 1995; Sinha *et al*., 1996; Quesada and Vincent, 1997; Prasad and Zeeshan, 2004)**. Effects of UV-B on growth and survival depends upon the species type, however, it has been shown that exposure of UVR for 120-180 min can completely cease growth and survival of several cyanobacteria **(Tyagi *et al*., 1992; Sinha *et al*., 1995)**. Cyanobacterial strain such as *Nostoc* commune and *Scytonema* sp. whose filaments are embedded in a mucilage sheath are more tolerant to UV-B radiation than the species which do not have such coverings **(Sinha *et al*., 1996)**. Two Antarctica cyanobacteria such as *Phormidium murrayi* and *Oscillatoria priestleyi*, isolated from a common habitat show differential sensitivity to UV-B radiation.

UV-B induced DNA damage in cyanobacteria

It is apparent that DNA is the most prominent targets of solar UVR in all living organisms continuously incurs a myriad of types of damage that drastically attribute adverse effects on all living systems such as bacteria, cyanobacteria, phytoplankton, macroalgae, plant, animals and humans **(Peak and Peak, 1984; Quaite *et al*., 1992; Kripke *et al*., 1992; Pakker *et al*., 2000; Sinha *et al*., 2001; Buma *et al*., 2001; Jans *et al*., 2005)** and influence their normal life processes. DNA alteration may occur mainly by mispairing of bases during replication, hydrolytic deamination, depurination/depyrimidination, oxidative damage by ionizing radiation (IR) as well as by free radicals or reactive oxygen species (ROS) and by certain alkylating agents **(Dizdaroglu, 1992; Lindahl, 1993; Halliwell *et al*., 1999; Valko *et al*., 2006)**. The incidence of UVR, IR and certain genotoxic chemicals may result in single as well as double DNA strand breaks (DSBs). DSBs may lead to loss of genetic information. The high concentration of ROS may alter the configuration of cell structure, lipids, proteins and DNA and can cause a number of human diseases **(Valko *et al*., 2007)**. Certain antibiotics (eg. mitomycin-c, cisplatin, etc.) may cause cross-linking by the formation of covalent bonds between two bases on complementary DNA strands and interfere with DNA replication. The most potent carcinogenic forms of UV-induced DNA lesions are CPDs, 6-4PPs and their Dewar isomers **(You *et al*., 2000; Cadet *et al*., 2005)** that may impede with normal cellular capability and functional integrity, reduction of RNA synthesis, arrest of cell cycle progression, resulting in mutagenesis, tumorigenesis and apoptosis **(Van Steeg and Kraemer, 1999; Jans *et al*., 2005)**. It has been found that thymine-thymine (T-T) and thymine-cytosine (T-C) sequences are more photo-reactive than C-T and C-C sequences **(Courdavault *et al*., 2005)**. The elevated amount of CPDs are produced by cycloaddition reaction between two pyrimidine bases in single-strand DNA (ssDNA) as well as at the flexible ends of poly (dA).(dT) tracts, but not at their rigid centre **(Carell and Epple, 1998; Becker and Wang, 1989; Lyamichev, 1991)**. The yield ratio of CPDs and 6-4PPs mostly depends upon the two adjacent bases involved in the formation of dimer, though, it has been reported that the amount of CPDs and 6-4PPs are about 75 and 25 % respectively of the total UV-induced DNA damage product **(Sinha and Häder, 2002)**. It is remarkable, and

very significant in terms of UV-induced toxicity, that RNA polymerase has also been found to stall at both CPDs and 6-4PPs (**Britt, 1999**).
UV-induced formation of thymine dimers have been reported in a number of rice field cyanobacteria such as *Anabaena* sp., *Nostoc* sp. and *Scytonema* sp. (**Sinha *et al*., 2001**). UV-induced DNA degradation has been demonstrated in the unicellular cyanobacterium Synechocystis PCC 6308. Recently, UV-B-induced damage of DNA and its detection by PCR have been reported in cyanobacteria (**Kumar *et al*., 2004**). It seems probable that UV affects the DNA of cyanobacteria and the killing of these microbes might be due to the irreversible damages caused to DNA by the high energy UVR (**Kumar *et al*., 2004**). UV-B-induced formation of thymine dimer has also been reported in cyanobacteria, such as *Croococcus* sp. and *Anabaenopsis* sp.

Detection of DNA damage

An immuno-dot-blot assay was used to detect CPDs, 6-4PPs and their Dewar valence isomers in UV-irradiated mammalian cells **(Perdiz *et al*., 2000)**. To determine the frequency of thymine dimers in a variety of aquatic organisms such as cyanobacteria, phytoplankton and macroalgae, a simple and efficient quantitative method was developed by **Sinha *et al*., (2001)** which is based on use of thymine dimer-specific antibodies followed by blotting and chemiluminescence methods. In this assay a plasmid pBSK with known DNA sequence, length and number of adjacent thymine pairs was used for calibration. By using the same method it was found that there was an increase from 3.2 to 50.9 thymine dimers per mega base pair during the light period in phytoplankton assemblage exposed at the surface under natural solar radiation from a freshwater lake in Trelew, Argentina **(Klisch *et al*., 2005)**.

DNA repair mechanism

Several specific repair enzymes and proteins are present in the organisms that continuously scan the genome for the occurrence of DNA lesions. Once a mismatched base, an apurinic or apyrimidinic site or structurally altered base is encountered, an efficient DNA repair is initiated, which in most cases leads to the restoration of the genetic information **(Carell and Epple, 1998; Sinha and Häder, 2002)**.

Photo-reactivation

Photo-reactivation or photo-enzymatic repair (PER) is one of the simplest and most important DNA repair system that involves the enzyme photolyase. The enzyme binds to CPD (CPD photolyase) or 6-4PPs (6-4 Photolyase) and reverses the damage using the energy of light. Long wavelength UV-A or blue light energy is absorbed by either 5,10-methenyltetrahydrofolate (MTHF) or 8-hydroxy-5-deazariboflavin ($\lambda max \approx 380$ and 450 nm) and transferred to the catalytic cofactor, which is invariably a two-electron-reduced flavin adenine dinucleotide (FADH-). The whole process is known as photoreactivation (Fig. 1). The flavin in the excited state transfers an electron to the CPD, splitting the cyclobutane ring and the electron is transferred back to the flavin resulting in the generation of the two canonical bases (**Sancar, 1994; Kim *et al*., 1994; Sancar, 1996; Thoma, 1999**). Once the photolyase has been bound to a CPD, the efficiency of photoreactivation is extremely high with one dimer split for almost every blue light photon absorbed. The major photoreactivating factor, phrA, in the cyanobacterium Synechocystis sp. strain PCC 6803 codes for a CPD-specific DNA photolyase.

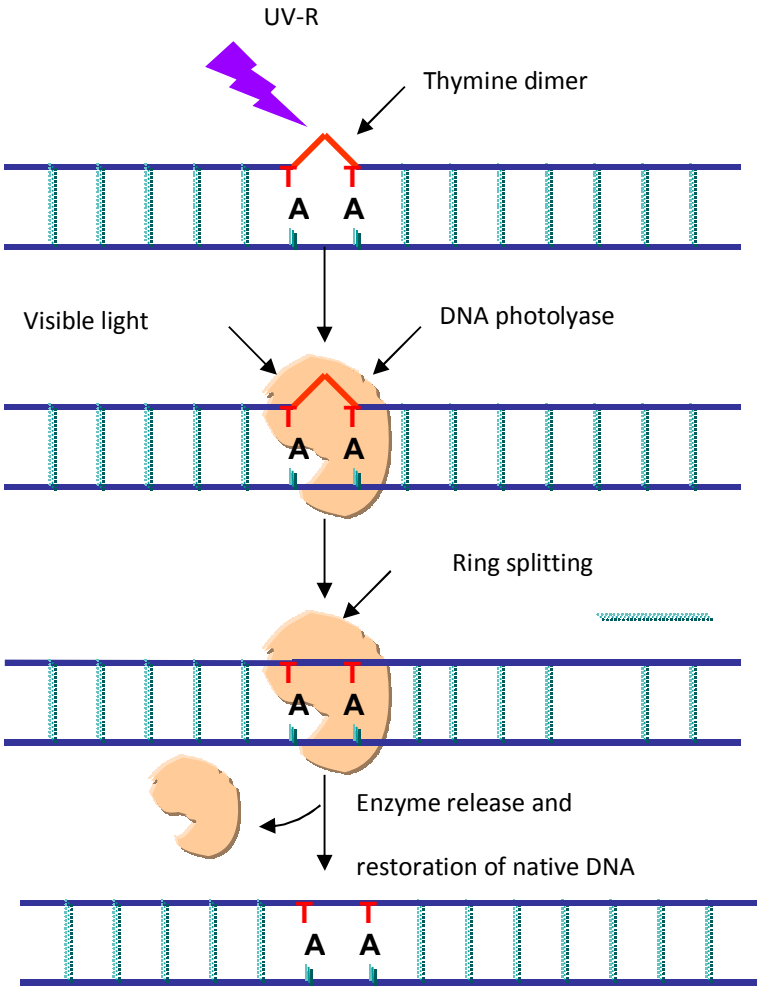

Figure 1 Schematic representation of the pathway showing photo-reactivation

Base excision repair (BER)

BER is also important in repairing DNA damage by ionizing radiation and strong alkylating agents. DNA glycosylases are the key enzymes involved in BER which remove different types of modified bases by cleavage of the N-glycosidic bond between the base and the 2-deoxyribose moiety of the nucleotide residues. After the base is removed, the apurinic/apyrimidinic (AP) site is excised by an AP-endonuclease or an AP lyase, which nicks the DNA strand 5′ or 3′ to the AP site respectively followed by the excision of the remaining deoxyribose phosphate residue by a phosphodiesterase. Subsequently, the gap is filled by a DNA ligase (Fig. 2) .

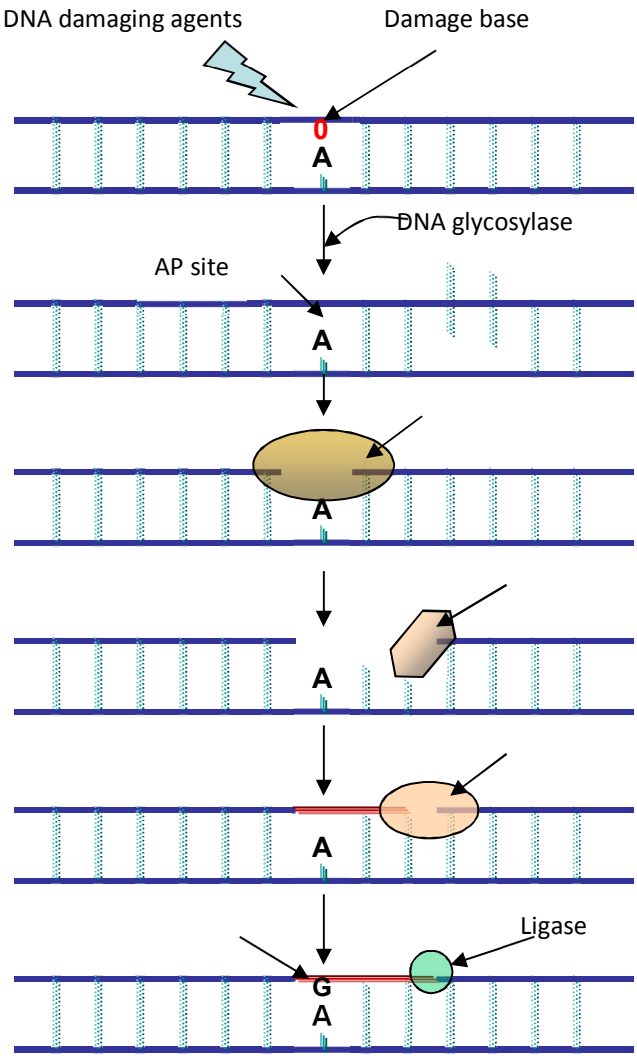

Figure 2 Schematic representation of BER

Nucleotide excision repair (NER)

NER is a complex pathway, which removes a wide variety of DNA distorting lesions including CPDs and 6-4PPs. This mechanism is present in most organisms and is highly conserved in eukaryotes. A total of some 30 gene products are involved in removing damaged nucleotides. NER can be subdivided into transcription coupled (TC-NER) repair and global genome (GG-NER) repair. In contrast to eukaryotes, the prokaryotic NER requires only three proteins such as UvrA, UvrB and UvrC.

Recombinational repair

Recombinational repair is one of the most widespread processes in DNA repair. Double strand breaks (DSBs) and single-strand breaks (SSBs) in damaged DNA are efficiently repaired by complex biochemical reactions. Recombinational repair fills the daughter strand gap by moving a complementary strand from a homologous region of DNA to the site opposite the damage (Fig. 3).

MATERIAL AND METHODS

Biological material

The cyanobacterium *Scytonema* sp. was used for the present study that was routinely grown in Chu 10 (without N supplement) liquid medium at a temperature of 20°C and a white light illumination of 20 W/m^2. The organism belongs to the order Nostocales and family Scytonemataceae, having distinct morphological features. *Scytonema* sp. is filamentous and the filaments interwine to form a scum of bluish green colour. Each filament, made up of spherical, oval or squarish cells, is surrounded by an individual mucilaginous sheath. The sheath may be hyaline or coloured, smooth, homogeneous or lamellated. The species, in particular, possess pigmented sheaths due to the presence of a pigment, scytonemin. A characteristic feature of *Scytonema* sp. is the presence of false branches.

Culture medium

Table 1 shown the chemical compostion of of Chu 10 cultural medium.

Table 1 Chemical composition of Chu 10- medium

Chemicals	Quantity (g/l)
MgSO4.7H2O	0.025
CaCl2.2H2O	0.027
Na2SiO3.5H2O	0.044
Na2CO3	0.020
K2HPO4	0.010
Fe-citrate	0.005
Citric acid	0.005
H3BO3	0.500
ZnSO4.7H2O	0.050
MnCl2.4 H2O	0.500
CuSO4.5 H2O	0.020
Na2MoO4.2 H2O	0.010
CoCl2	0.040
pH=8.0	

Autoclaving

Solutions of inorganic sources i.e phosphate, Fe-citrate and citric acids were autoclaved separately and added to cool sterile medium. Organic chemicals and their analogues and other compounds were sterilized by filtration through Millipore membrane filters (0.22 µl) before use.

Isolation of DNA

The pellet obtained were washed twice with 1 ml of STE buffer (50 mM NaCl + 50 mM Tris-HCl, pH-8.0 + 5 mM EDTA) and re-suspended in a 500 µl TE (wash) buffer (50 mM Tris-HCl, pH-8.0 + 50 mM EDTA). Thereafter the cells were broken by sonification for 3 min on ice. Subsequently the cells were treated with 100 µg/ml of proteinase K. Thereafter 750 µl of prewarmed (55 ^{0}C) extraction buffer[3% (w/v) CTAB + 1% (w/v) sarkosyle + 20 mM EDTA + 1.4 M NaCl + 0.1 M TrisHCl, pH-8.0 + 1% (w/v) 2-mercaptoethanol] were added and incubate at 55 ^{0}C for one hour in a water bath with mixing by gentle inversion every 10 minute. The resulting suspension was allowed to cool for 1-2 minute and thereafter 750 ml Chloroform:isoamyl alcohol (24:1, v/v) were added and mixed by gentle inversion until an emulsion was formed. After centrifugation (10,000x g for 8 min at room temperature) the supernatant was transferred to sterile microcentrifuge tubes. Thereafter 2 volumes of ethanol and 0.1 volume of 3 M sodium acetate (pH-5.2) were added and kept at 0 ^{0}C overnight for the precipitation of DNA. Next day the precipitated DNA was centrifuged at 10,000 x g for 15 minute. The pellet obtained was briefly rinsed once with ice-cold 70 % ethanol, dried and re-hydrated with 30 ml TE buffer (10mM Tris-HCl, pH-8.0 + 1mM EDTA.

Purification of DNA

The purity of DNA was determined by calculating for ratio of O.D. at 260 and 280 (Table 2).

Table 2 Purity of DNA

Ratio between 260/280nm	Results
1.8-2.0	Pure DNA
<1.8	Protein contamination
>2.0	RNA contamination

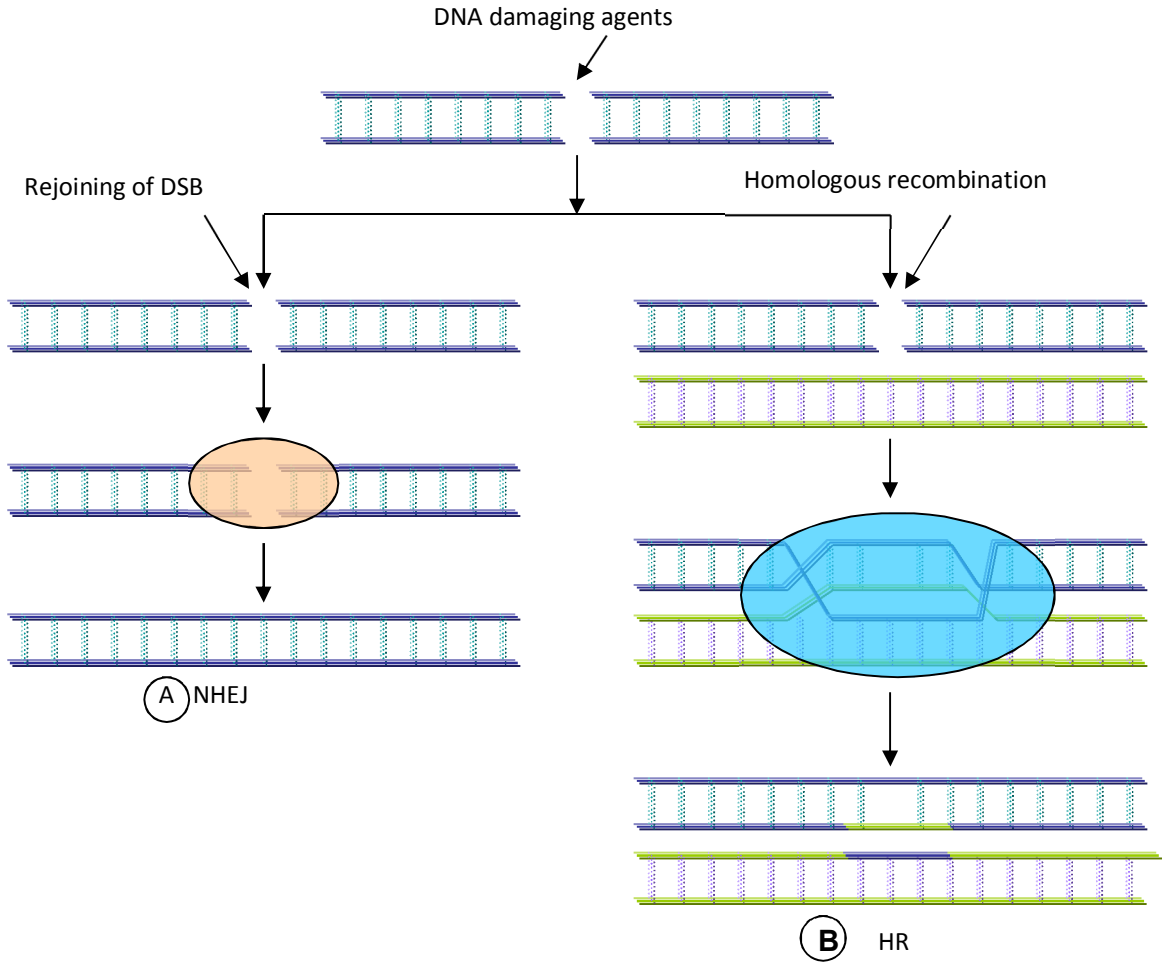

Figure 3 Diagrammatic view of double strand break (DSB) repair as a result of exposure of ionizing radiation, oxidative damage and spontaneous cleavage of the sugar phosphate backbone of the DNA molecule. A- Nonhomologous end joining and B- Homologous recombination. Both processes involves different multiprotein complex

Electrophoresis

5 µl of control as well as UV-treated DNA samples + 5 µl of BPB (1X) were mixed and added into the well in 0.8 % agarose gel (contaning 0.5 µg/ml EtBr) immersed in electrophoresis buffer in a horizontal tank. Electrophorosis was run at 20 mA for the 1st 30 minute, than the current was increased to 120 mA for further 90-120 minute. After electrophorosis the gel was placed on a molecular imager system (Gel Doc, Bio-Rad) and photographs were taken for further analysis.

Immuno-dot-blot Assay

DNA samples were transferred to the membrane and washed once with TE buffer. The membrane was dried for one hour at 80 ^{0}C to immobilize the DNA. Subsequently, the membrane was incubated for 1 h in PBS-T with 5 % (w/v) skimmed milk powder to block the non-specific sites. Thereafter, the membrane was incubated with the primary antibody (anti-thymine dimer; diluted 1:10000 in PBS-T) for 2 h at room temperature and then washed (3 x 10 min) with PBS-T. Afterwards the membrane was incubated with the secondary antibody [(anti-mouse IgG (Fab specific) peroxydase conjugate; diluted 1:10000 in PBS-T with 5 % skimmed milk powder] for 1h at room temperature and washed with PBS-T. Finally, the membrane was placed in a detection reagent for 1 min before scanning.

RESULTS AND DISCUSSION

Before assessing the impact of UV-B radiation on DNA, we observed its effects on growth and survival of the test organism *Scytonema* sp. It was observed that growth and survival were severely affected by UV-B radiation for different durations. DNA is one of the key targets for UV-B radiation in a number of organisms including cyanobacteria. The effect of UV-B radiation specifically on genomic DNA of the test organism was studied. Accordingly genomic DNA isolated from the exponentially grown culture was exposed to UV-B radiation for varying time periods and absorption spectra were determined at 260 nm both in control as well as UV-B irradiated DNA. It was found that there was a progressive increase in absorbance (at 260 nm) with increasing duration of UV-B treatment to DNA (data not shown). The increase in absorbance may be due to separation of two complementary strands of DNA (denaturation), a fact generally observed during the estimation of melting curve of DNA with temperature treatment. However, the denaturation of DNA caused by UV-B treatment seems irreversible since there was no decrease in absorbance of UV-B- exposed DNA following transfer to fluorescent light or in the dark. In this work it was observed that unirradiated cultures showed more distinct bands whereas DNA from cultures exposed to UV-B radiation showed loss of various fragments with increasing duration of UV-B (Fig. 4) radiation.

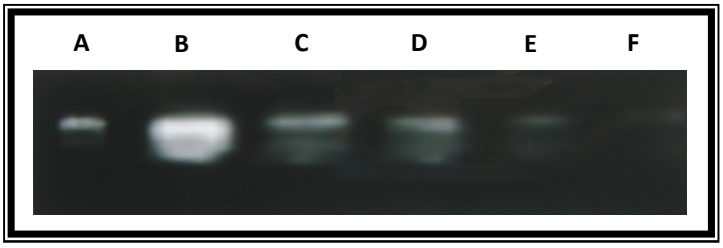

Figure 4 UV-B-induced damage of genomic DNA in the cyanobacterium *Scytonema* sp.
(A) control, (B) 60 min, (C) 90 min, (D) 120 min, (E) 150 min, (F) 180 min

To detect the DNA lesions, equal amount of DNA from test organisms were loaded onto the nylon membrane. Figure 5 represents the blotting pattern of DNA from the cyanobacterium Scytonema sp. after increasing duration of UV radiation. There was a gradual increase in the integrity of the luminescence with increasing UV irradiation time.

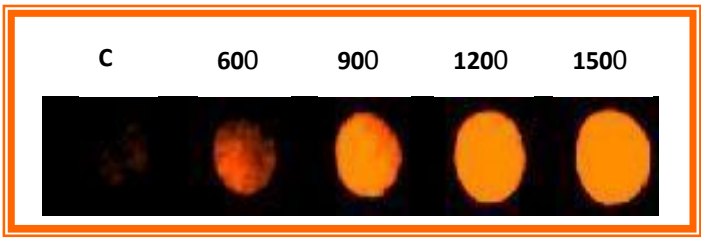

Figure 5 Dot-blot of DNA after different durations (min) of UV radiation. C-control

Transmission of the genetic information from one cell to its daughter is must for survival of any organisms, which requires not only extreme accuracy in replication of DNA and precision in chromosome distribution, but also in the ability to survive spontaneous and induced DNA damage while minimizing the number of heritable mutations. Increase in the level of UV-B radiation is likely to induce changes in cyanobacterial community composition since there are great differences in the susceptibility of species to UV-induced damage. Species having the ability to accumulate UV screening substances or with more effective repair mechanisms will likely be favored.

CONCLUSION

UV-B induced DNA damage has also been reported in other cyanobacteria such as *Anabaena* sp., *Nostoc* sp. and in several other aquatic organisms (**Kumar *et al*., 2004; Sinha *et al*., 2001**). UV-B treatment causes loss in the cooperative binding property of DNA which is evident from the failure of complementary strands of DNA. **Kumar *et al*., (2004)** has observed UV-induced DNA damage by increase in absorbance of DNA following UV-B exposure and also by PCR assay. A number of methods are in use to determine the DNA damage in a variety of organisms (**Sinha *et al*., 2001; Sommaruga and Buma, 2000; Pakker *et al*., 2000; Perdiz *et al*., 2000**). UV-induced DNA degradation has been reported in the cyanobacterium Synechocystis by using radioactive methods and showing percentage radioactivity lost from DNA as a measure for DNA degradation. **Buma *et al*., (1995)** developed an immunofluorescent thymine dimer detection method by labeling dimers with antibody followed by a secondary antibody (Fluorescein isothiocyanate) staining and finally visualization of DNA damage with flow cytometry or fluorescence microscopy. **Sinha *et al*. (2001)** has presented a simple and efficient quantitative method to determine the frequency of thymine dimers in a variety of organisms in relatively short period of time by using blotting and chemiluminescence methods. This method permits the measurement of low as well as high levels of DNA lesions in nanogram quantities of DNA.

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REFERENCES

ALSCHER, R.G., DANAHUE, J. L., CRAMER, C.L. 1997. Reactive oxygen species and antioxidants: relationship in green cells. *Physiol. Plant*., 100, 224-233.

BECKER, M. M., WANG, Z. 1989. Origin of ultraviolet damage in DNA. *J. Mol. Biol*., 210, 429-438.

BLUMTHALER, M., AMBACH, W. 1990. Indication of increasing solar ultraviolet-B radiation flux in alpine regions. *Science*, 248, 206-208.

BRITT, A. B. 1999. Molecular genetics of DNA repair in higher plants. *Trends Plant Sci*., 4, 20-25.

BUMA, A. G. J., DE BOER, M. K., BOELEN, P. 2001. Death distribution of DNA damage in Antarctic marine phyto- and bacterioplankton exposed to summertime UV radiation. *J. Phycol*., 37, 200-208.

BUMA, A. G. J., VAN HANNEN, E. J., ROZA, L., VELDHUIS, M. J. W., GIESKES, W. W. C. 1995. Monitoring ultraviolet-B induced DNA damage in individual diatom cells by immunofluorescence thymine dimer detection. *J. Phycol*., 51, 314-321.

CADET, J., SAGE, E., DOUKI, T. 2005. Ultraviolet radiation-mediated damage to cellular DNA. *Mut. Res*., 571, 3-17.

CARELL, T., EPPLE, R. 1998. Repair of UV light induced DNA lesions: a comparative study with model compounds. *Eur. J. Org. Chem*., 1245-1258.

COURDAVAULT, S., BAUDOUIN, C., CHARVERON, M., CANGUILHEM, B., FAVIER, A., CADET, J., DOUKI, T. 2005. Repair of the three main types of bipyrimidine DNA photoproducts in human keratinocytes exposed to UVB and UVA radiations. *DNA Repair*, 4, 836-844.

CRUTZEN, P. J. 1992. Ultraviolet on the increase. *Nature*, 356, 104-105.

DIZDAROGLU, M. 1992. Oxidative damage to DNA in mammalian chromatin. *Mut. Res*. 275, 331-342.

FRIEDBERG, E. C., WALKER, G. C., SIEDE, W., WOOD, R. D., SCHULTZ, R. A., ELLENBERGER, T. 2005. DNA repair and mutagenesis, 2nd ed., ASM press.

HALLIWELL, B., GUTTERIDGE, J. M. C. 1999. Free radicals in biology and medicine (3rd ed.). Oxford University Press.

JANS, J., SCHUL, W., SERT, Y.-G., RIJKSEN, Y., REBEL, H., EKER, A. P. M., NAKAJIMA, S., VAN STEEG, H., DE GRUIJL, F.R., YASUI, A., HOEIJMAKERS, J. H. J., VAN DER HORST, G. T. J. 2005. Powerful Skin Cancer Protection by a CPD-Photolyase Transgene. *Curr. Biol.*, 15, 105-115.

KIM, S.-T., MALHOTRA, K., SMITH, C. A., TAYLOR, J.-S., SANCAR, A. 1994. Characterization of (6-4) photoproduct DNA photolyase. *J. Biol. Chem*., 269, 8535-8540.

KLISCH, M., SINHA, R. P., HELBLING, E.W., HÄDER, D. P. 2005. Induction of thymine dimers by solar radiation in natural freshwater phytoplankton assemblages in Patagonia, Argentina. *Aquat. Sci*. 67, 72-78.

KRIPKE, M. L., COX, P. A., ALAS, L. G., YAROSH, D. B. 1992. Pyrimidine dimers in DNA initiate systemic suppression in UV-irradiated mice. *Proc. Natl. Acad. Sci. USA*., 89, 7516-7520.

KUMAR, A., TYAGI, M. B., JHA, P.N. 2004. Evidences showing ultraviolet-B radiation-induced damage of DNA in cyanobacteria and its detection by PCR assay. *Biochem. Biophy. Res. Commun.*, 318, 1025-1030.

LINDAHL, T. 1993. Instability and decay of the primary structure of DNA. *Nature*, 362, 709-715.

LYAMICHEV, V. 1991. Unusual conformation of (dA)n.(dT)n-tracts as revealed by cyclobutane thymine-thymine dimer formation. *Nucleic Acids Res*., 19, 4491-4496.

MACKERNESS, S. A. H., JORDAN, B. R., THOMAS, B. 1999. Reactive oxygen species in the regulation of photosynthetic genes by ultraviolet-B radiation (UV-B: 280-320 nm) in green and etiolated buds of pea (*Pisum sativum* L.). *J. Photochem. Photobiol. B: Biol*., 48, 180-188.

MADRONICH, S., MCKENZIE, R. L., BJÖRN, L. O., CALDWELL, M. M. 1998. Changes in biologically active ultraviolet radiation reaching the earth's surface. *J. Photochem. Photobiol. B: Biol*.,46, 5-19.

MCKENZIE, R. L., BJÖRN, L. O., BAIS, A., LLYAS, M. 2003. Changes in biologically active ultraviolet radiation reaching the earth's surface. Photochem. *Photobiol. Sci*. 2, 5-15.

PACE, N. R. 1997. A molecular view of microbial diversity and the biosphere. *Science*, 276, 734-740.

PAKKER, H., BECKMAN, C. A. C., BREEMAN, A. M. 2000. Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhodymenia pseudopalmata* (Rhodophyta). *Eur. J. Phycol*., 35, 109-114.

PEAK, M. J., PEAK, J. G., MOEHRING, P., WEBB, R. B. 1984. Ultraviolet action spectra for DNA dimer induction, lethality and mutagenesis in *Escherichia coli* with emphasis on the UV-B region. *Photochem. Photobiol*., 40, 613-620.

PERDIZ, D., GRÖF, P., MEZZINA, M., NIKAIDO, O., MOUSTACCHI, E., SAGE, E. 2000. Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. *J. Biol. Chem*., 275, 26732-26742.

PRASAD, S. M., ZEESHAN, M. 2004. Effect of UV-B and monocrotophos, singly and in combination, on photosynthetic activity and growth of non-heterocystous cyanobacterium *Plectonema boryanum*. *Environ. Exp. Bot.*, 52, 175-184.

QUAITE, F. E., SUTHERLAND, B. M., SUTHERLAND, J. C. 1992. Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature*, 358, 577-578.

QUESADA, A., VINCENT, W. F. 1997. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *Eur. J. Phycol*., 32, 335-342.

SANCAR, A. 1994. Structure and function of DNA photolyase. *Biochemistry*, 33, 2-9.

SANCAR, A. 1996. No "end of history" for photolyases. *Science*, 272, 48-49.

SCHOPF, J. W. 1993. Microfossils of the early archaean apex chert: new evidence of the antiquity of life. *Science*, 260, 640-646.

SINHA, R. P., DAUTZ, M., HÄDER, D.-P. 2001. A simple and efficient method for the quantitative analysis of thymine dimers in cyanobacteria, phytoplankton and macroalgae. *Acta Protozool*., 40, 187-195.

SINHA, R. P., KUMAR, A., KUMAR, H. D., HÄDER, M., HÄDER D.-P. 1996. Effect of UV radiation on certain physiological processes in cyanobacteria. *J. Photochem. Photobiol. B: Biol*., 32, 107-113.

SINHA, R. P., KUMAR, H. D., KUMAR, A., HÄDER D.-P. 1995. Effects of UV-B irradiation on growth, survival, pigmentation and nitrogen metabolism enzymes in cyanobacteria. *Acta Protozool*., 34, 187-192.

SINHA, R.P., DAUTZ, M., HÄDER, D. P. 2001. A simple and efficient method for the quantitative analysis of thymine dimers in cyanobacteria, phytoplankton and macroalgae. *Acta Protozool,* 40, 187-195.

SINHA, R. P., HÄDER, D. P. 2002. UV induced DNA damage and repair: a review. Photochem. *Photobiol. Sci*.,1, 225-236.

SINHA, R. P., KRYWULT, M., HÄDER, D. P. 1998. Effects of ultraviolet, monochromatic and PAR waveband on nitrate reductase activity and pigmentation in a rice field cyanobacterium, *Anabaena* sp. *Acta Hydrobiol,* 2, 105-112.

SOMMARUGA, R., BUMA, A. G. J. 2000. UV-induced cell-damage is species-specific among aquatic phagotrophic protests. *J. Eukaryot. Microbiol*., 47, 450-455.

THOMA, F. 1999. Light and dark in chromatin repair: repair of UV- induced DNA lesions by photolyase and nucleotide excision repair. *EMBO J*., 18, 6585-6598.

TYAGI, R., SRINIVAS, G., VYAS, D., KUMAR, A., KUMAR, H. D. 1992. Differential effects of ultraviolet-B radiation on certain metabolic processes in a chromatically adapting *Nostoc. Photochem. Photobiol*., 55, 401-407.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M. T. D., MAZUR M., TELSER, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *The Internat. J. Biochem. Cell Biol*., 39, 44-84.

VALKO, M., RHODES, C. J., MONCOL, J., IZAKOVIC, M., MAZUR, M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact*., 160, 1-40.

VAN STEEG, H., KRAEMER, K. H. 1999. Xeroderma pigmentosum and the role of UV-induced DNA damage in skin cancer. *Mol. Med. Today*, 5, 86-94.

YOU, H. Y., SZABO, P. E., PFEIFER, G. P. 2000. Cyclobutane pyrimidine dimers form preferentially at the major p^{53} mutational hotspot in UVB-induced mouse skin tumors. *Carcinogenesis*, 21, 2113-2117.