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Study on DNA damages induced by UV radiation

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Abstract: DNA damages in Escherichia coli (*E. coli*) exposed to UV radiation have been investigated. After 30 min of exposure to UV radiation of 5 mJ/cm^2, the growth of *E. coli* in LB broth medium was about only 10% in compared with non-irradiated one. This results suggested that the UV radiation caused the damages for *E. coli* genome resulted in reduction in its growth and survival, and those lesions can be somewhat recovered. For both solutions of plasmid DNAs and *E. coli* cells containing plasmid DNA, this dose also caused the breakages on single and double strands of DNA, shifted the morphology of DNA plasmid from supercoiled to circular and linear forms. The formation of pyrimidine dimers upon UV radiation significantly reduced when the DNA was irradiated in the presence of *Ganoderma lucidum* extract. Thus, studies on UV-induced DNA damage at molecular level are very essential to determine the UV radiation doses corresponding to the DNA damages, especially for creation and selection of useful radiation-induced mutants, as well as elucidation the protective effects of the specific compounds against UV light.

Keywords: *UV radiation, DNA damage, single strand break, double strand break, dimer.*

I. INTRODUCTION

Similar to ionizing radiation, ultraviolet radiation (UVR) can cause severely damages to DNA, result in different mutations in the cells and organisms [1]. UV light induces single or double strand breaks on DNA molecules, leads to the changes in DNA conformation and structure. As a consequence, some potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis, can be occurred. While most mutations are harmful or even lethal, some mutations are useful for evolution. Under UV radiation, the pyrimidine nucleotides (thymine and cytosine) can link together in order to form nucleotide complexes (dimers), which the most common are cyclobutane-pyrimidine dimers (CPDs), 6-4 photoproducts (6-4 PPs) and their Dewar valence isomers [2]. These changes prevent the normal transcription of DNA and replication, but only few dimers cause mutations. These UVR induced mutations can lead to serious diseases, and even cause cancers [3].

Analysis of UVR induced damages at the molecular level have been proved as an important method to determine the relationship between the UVR doses and lesions include the changes in DNA spatial configuration (linear, circle and supercoiled forms), as well as the damages (single strand, double strand breaks and dimerization). If the damages are not fully recovered, the integrity and accessibility of essential genetic information in the damaged cell may corrupt to form various mutations [2]. Estimation of the DNA damages at molecular level for the specific cells and tissues (plant, animal and bacteria) at various development

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and differentiation stages can be applied to calculate the suitable doses for wide spectrum of mutation using UVR in particular, or other ionizing radiations in general [4]. Fortunately, cells possess several DNA repair systems that can fix the lesions. For example, damage in DNA molecule alters the spatial configuration of the helix, and such alterations can be detected by the cell. Once damage is localized, specific DNA repair molecules bind at or near the site of damage, and form a complex that enables the actual repair to take place. The repair ability of a cell is vital to the integrity of its genome, namely to the normal functionality of that organism.

Fortunately, the DNA repair process is constantly active as it responds to damage in DNA structure. It found that some compounds reveal their potential in reduction the harmful effects of radiation and protection the living organism against radiation. These findings are essential for further studies on mutation breeding, DNA repair mechanism, radiation protection effect… In Vietnam, the radiation mutation has been applied to produce new plants (cereals, legumes, fruits and flowers) for long time, but it still limited in creating and screening new microbial strains. In this study, the UVR dose required to cause 90% mortality of *E. coli* cells (D_{10}) was determined, and the changes in the spatial configuration of DNA, their break and dimerization caused by UVR at that dose were investigated at that dose *in vitro* (separated DNA plasmids) and *in vivo* (*E. coli* cells).

II. EXPERIMENTAL

Materials

E. coli strain, DH5α carrying pUC19 or pJET1.1 (1.0 kb insert) recombinant vectors, *Gal* extract from *Ganoderma lucidum* Lingzhi mushroom were kindly supported by Laboratory for Medical Biology, Faculty of Biology, VNU University of Sciences, Hanoi. The *E. coli* was cultured in LB to prepare the cell suspension.

Plasmid DNA extraction (QIA prep Spin Miniprep), Total DNA extraction kits (WizardSV Genomic DNA Purification) were purchased from QIAGEN and PROMEGA, respectively. Eco RI and T4 endonuclease V enzymes were bought from New England BioLab (NEB).

Taq DNA polymerase, dNTPs for PCR; EDTA, tris buffer solution for electrophoresis; other chemicals and solvents were purchased from Merck and Promega Corp.

Characterizations

UVR treatment and absorbance of *E. coli*

5 ml solution of *E. coli* cell's suspension in LB medium at logarithmic phase with optical density of about 0.4 (~ 5×10^8 CFU/ml), as measured at 600 nm (OD600) by a UV-vis spectrophotometer (Shimadzu, Japan), was poured in plastic petri dish for UV radiation at 254 nm using BLX 254 (USA). For DNA plasmid, 20 µl of 1 µg/µl DNA solution was pipetted into a sterile device for UV treatment with various dose, ranging from 1-8 mJ/cm^2, as determined by the intensity and the exposure time of treatment, according to manufacturer's instructions [3].

After treatment, the irradiated *E. coli* cells were continuously cultured with the same medium in thermostat shaker for further 30 min, and their OD (OD600/30) were measured. The recovery abilities of *E. coli* after exposure to UV light were estimated by the differences in its turbidity before and after 30 min treatment by UVR, as the following equation:

$$\Delta OD600 = OD600/30 - OD600 \quad (1)$$

Agarose gel electrophoresis of DNA

Agarose powder of 1% was mixed with electrophoresis buffer (Tris/Borax/EDTA – TBE 1X) to the desired concentration, heated until completely melted, then cooled down and poured into a casting tray containing a sample comb. After the gel had solidified, the comb was removed in order to form the well, and

inserted horizontally into the electrophoresis chamber with buffer. Samples containing DNA mixed with loading buffer were then pipetted into the sample wells. DNA will migrate towards the positive electrode by the applied voltage; DNA fragments are visualized by staining with ethidium bromide, and are taken by a specific camera. Be aware that DNA will diffuse within the gel over time, and photography should take place just after cessation of electrophoresis.

Alkaline denaturing electrophoresis

The denaturing electrophoresis was carried out in an alkaline agarose gel makes the nucleotide thymine (T) and guanine (G) lost proton should not create links with adenine (A) and cytosine (C), as reported by Sambrook and Russell [6]. Therefore, DNA was separated and maintained in the form of single strands. The electrophoresis results have been used to analyze single DNA fragment size and estimate the DNA breaks.

DNA amount are determined by their absorbance at 260 nm (OD260), where those of purine and pyrimidine base are maximum. The quantity of DNA is calculated as follow [7]:

$$[DNA] = OD260 \times n \times 50\ (\mu g/ml) \quad (2)$$

where OD260 = 1 corresponding to the DNA amount of 50 μg/ml in a test sample, and n is dilution factor.

Random amplified polymorphic DNA (RAPD)

Unlike traditional PCR analysis, RAPD bases on PCR with random primers, but not requires any specific DNA sequence. By RAPD-PCR method, the genetic similarity of genome can estimate from the pattern of amplified DNA segments on the gel with short primers (10 nucleotides) using Taq polymerase as. Table I showed the sequences of the primers (IDT, US) have been used for the present study [8].

Table I: The primers and primer sequences used for RAPD-PCR

Primer	**Sequences** (5' - 3')	**Primer**	**Sequence** (5' - 3')	**Primer**	**Sequence** (5' - 3')
OPL1	AAGAGCCCGT	S216	TTAGCAATTG	B1	CCCAGCTGTG
OPL12	GGGCGGTACT	S256	ATCCGCGTGT	B21	AAGCCTCGTC
OPK19	CACAGGCGGA	S285	TGGACACTGA	OPA10	GTGATCGCAG
OLG2	GGACCACTAC	AD2	GCAAGTAGCT	OP15	CCTGGGTTCC
S201	GCCAACGAGA	AD3	TCACGATGCA	OPK8	GCCAACGAGA

III. RESULTS AND DISCUSSION

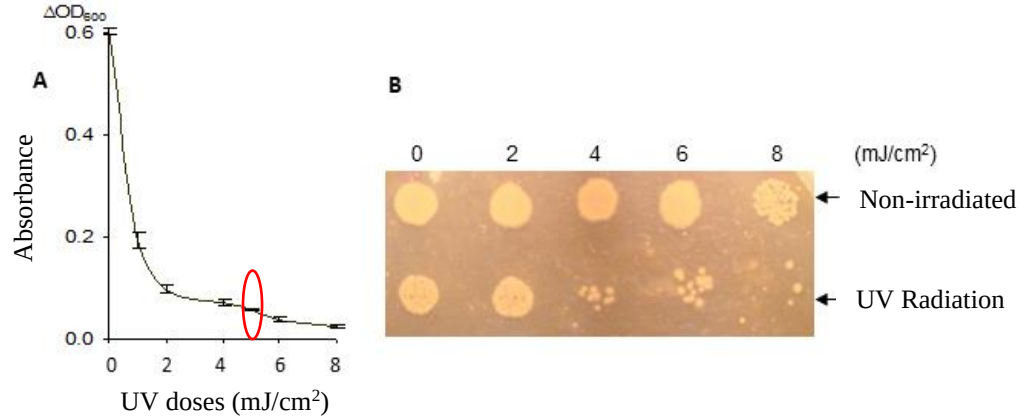

Fig. 1. (A) Recovery abilities of the irradiated *E. coli* after 30 min exposure to UV light with UV dose; (B) The colonies of non-irradiated and irradiated *E. coli* grown in LB agar plates at 37°C for 4 h.

The UVR effects on E. coli

Though *E. coli* cells were almost killed by UVR at dose higher than 2 mJ/cm^2, survival ones can continuously grow and multiple. Their recovery abilities were estimated to be the differences or increases in the optical density (OD) of the viable cells after 30 min exposure to UV light, with the dose in mJ/cm^2. Each measurement was repeated at least 5 times and the data were the average values as showed in Fig. 1A.

A 10 µl aliquot of the irradiated solution of cell suspension was immediately transferred to LB agar just after UV radiation, incubated at 37°C for 4 h, and colonies were observed as presented in Fig. 1B. From these results, it can be concluded that the recovery of the irradiated *E. coli* as well as its survival exponentially reduced by the increase of UVR dose. Thus, the bacteria are severely damaged by UVR at dose of 5 mJ/cm^2, then they hard to be recovered as revealed by low ΔOD600 value of about 10% compared to that of non-irradiated ones. For further studies, the UV dose of 5 mJ/cm^2 has been chosen due to the effects of the protective substances against radiation can be easily observed with the bacteria irradiated.

Configuration of the UV irradiated DNA

DNA extraction is a routine procedure used to isolate DNA from the nucleus of cells, and the extracted DNA products are mainly in supercoil with small amount of circular forms. During irradiation, the number of the circular DNA increased due to increasing of the single stranded breaks, and the double stranded breaks leading to formation of linear DNA fragments. These DNA configurations can be recognized and discriminated by agarose gel electrophoresis technique. The Fig. 2 showed that UVR caused transformation of pUC19 plasmid from supercoil to circular DNA fragments. For pJET1.1 plasmid, the ratio of DNA plasmid without breaks was determined by PCR amplified with 1.0 kb segment. 0.1 µg of the irradiated pJET1.1 was used as a template for PCR with the specific primers (M13F/R). The electrophoresis results of PCR products suggested that UVR caused the breaks in DNA template, resulted in reduction of the products in compared to non-irradiated ones.

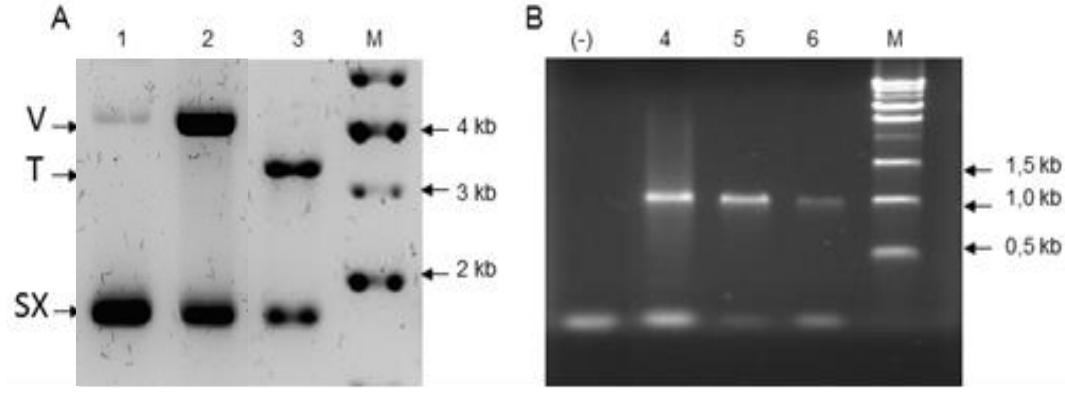

Fig. 2. (A) Electrophoresis results of pUC19 DNA plasmids: 1) Non-irradiated with main supercoil (SX) and small amount of circular (V) DNA fragments; 2) Some SX transferred to circular (V) due to the single-strand breaks in DNA by UVR at dose 5 mJ/cm^2 and 3) Formation of linear (T) DNA because of formation of the double-stranded breaks caused by Eco RI enzymes. (B) Electrophoresis results of the DNA products from the irradiated pJET1.1 plasmids as template: 4) non-irradiated; 5) and 6) irradiated by UVR with doses of 1 and 5 mJ/cm^2, respectively. (-): negative control without DNA template; M: DNA standard ladder.

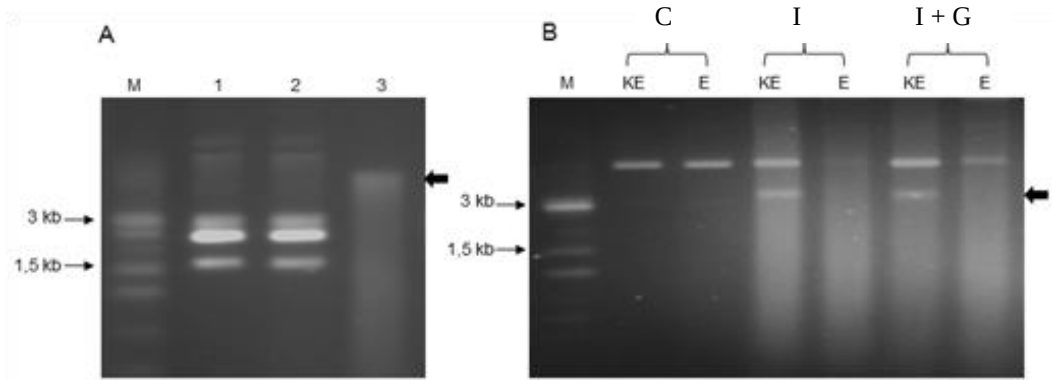

Fig. 3. (A) Alkaline denaturing electrophoresis results: 1, 2 Non-irradiated pUC19 and pJET1.1 plasmids and 3) pDNAs exposed to UV of 5 mJ/cm^2 (DNA damages were mainly single stranded breaks (prolonged streak of light) and some double-stranded breaks produced linear DNA fragments (solid black arrow). (B) Dimer formation in the DNA plasmids: C) non-irradiated bacteria; I) irradiated with UVR of 5 mJ/cm^2 and G) irradiated in the medium supplemented with Gal extract; KE and E are intact and cut by T4 endonuclease V enzyme, respectively, M: DNA standard ladder

DNA breaks and dimerization

UV radiation caused transformation in DNA plasmid's configuration and formation of dimers. While the DNA transformation can be easily observed by agarose gel electrophoresis, the dimerization between its nucleotides is difficult to determine. In this study, the DNA plasmids were mainly broken in their single strands, therefore the alkaline denaturing electrophoresis was applied to analyze the breaks and damages in the DNA exposed to UVR. And the T4 endonuclease V enzyme was used to cut the dimerization bonds at specific site in order to accurately evaluate the UV induced dimerization [9]. As presented in the Fig 3A, pUC19 plasmid was mainly broken in single strand, and the breaks were occurred at many various sites resulting in the prolonged streak of light. There are few double strand breaks formed by UV radiation at dose of 5 mJ/cm^2 as indicated by the band corresponds to the circular DNA. Just after UVR treatment, the pJET1.1 plasmids were restricted with T4 endonuclease V, and their alkaline denaturing electrophoresis results revealed that the UV-induced damages occurred on both single and double strands as observed by both prolonged streak of light and band of circular DNA in the Fig. 3B.

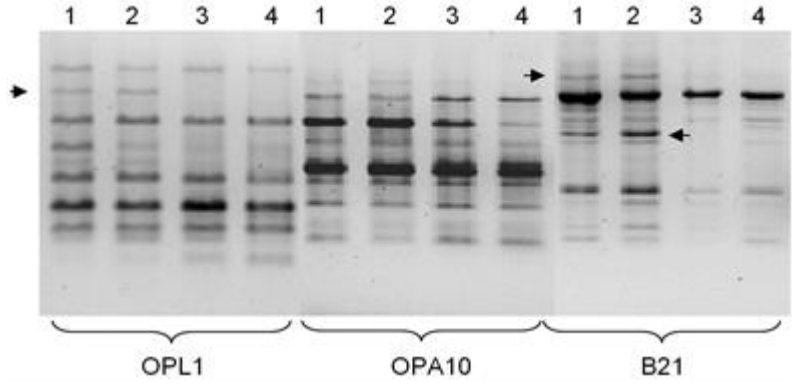

Fig. 4. Electrophoresis results of RAPD-PCR products obtained with random primers of OPL1, OPA10 and B21: 1, 2) non-irradiated; 3.4) exposed to UVR of 5 mJ/cm^2.
The differences in DNA bands were indicated by black arrows

The results also revealed the similar breaks with a smaller dimerization in the plasmids extracted from the irradiated E. coli cells, which cultured in LB medium added 0.5% Gal extract from Lingzhi mushroom. This proved that the DNA damages and dimerization caused by UVR were different mechanisms and Gal extract can add to reduce the DNA dimerization.

The UVR effects on genome

Diversity in DNA genome of E. coli was evaluated by the RAPD - PCR using random primers. If there are differences in DNA genome, namely a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel. Therefore, RAPD-PCR was applied to analyze the changes in the bacteria DNA genome. In this study, E. coli cells were also treated by UVR at dose of 5 mJ/cm2, then DNA genome has immediately isolated and used as DNA template for RAPD-PCR. The electrophoresis results of the PCR-products proved that DNA genome was significantly changed by UVR treatment, as presented in Figure 4.

IV. DISCUSSIONS AND CONCLUSIONS

To be a model organism possesses large numbers of replicate DNA plasmids, *E. coli* can easily survive in variable conditions. The culture media containing simple and inexpensive ingredients and nutrients can successfully spur *E. coli* to grow and divide. Its growth rate may be monitored continuously using simple methods, for example, the cell density of *E. coli* suspension can be easily determined by its optical density measured at 600 nm (OD600). This value is ranging from 0.3 to 0.6 during the logarithmic phase in its growth, when genomic DNA is continuously replicated and very sensitive to UV light [10]. Therefore, *E. coli* and DNA plasmid have been used as the targets for the study. The same *E. coli* suspensions with OD600 equal 0.4 were treated by UVR at different doses. Our results indicated the survivals and recovery abilities of the irradiated bacteria were much reduced with UVR dose, and their recovery was only 10% after exposure to UVR at 254 nm with dose of 5 mJ/cm^2. These results are consistent with literatures [10,11], then this UV dose was applied to analyze and evaluate the UV induced DNA damages *in vitro* and *in vivo*. The electrophoresis results with agarose gel, alkaline agarose gel denaturing for the PCR products of DNA plasmids treated with or without restriction enzyme revealed that the UVR mainly generated single stranded breaks (point mutations) and dimerization in the DNA plasmid. These lesions were observed in both DNA exposed directly to UVR and the DNA isolated and extracted from the irradiated *E. coli* cells.

Thus, from the results, basic methods to evaluate the *in vitro* and *in vivo* damages for the DNA exposed to UV have been established. These methods are essential to study on the effects of dose not only for UV, but also for other ionizing radiation; evaluate DNA repair and the radiation protection of the biomaterials. Our preliminary results also suggested that Gal extract from *Ganoderma lucidum* Lingzhi mushroom can be added to the LB culture in order to reduce DNA breaks and dimerization. This result is consistent with several previous reports on the radiation protection and bio-activities of Lingzhi mushroom [12,13].

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