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UV light induced DNA damages and the radiation protection effects of Lingzi mushrom extract

Vo Thi Thuong Lan[1], Dinh Ba Tuan[1], Ta Bich Thuan[1], Tran Bang Diep[2], Tran Minh Quynh[2*]

[1] *Hanoi University of Sciences, Vietnam Natural University, 334 Nguyen Trai, Thanh Xuan, Ha Noi*
[2] *Hanoi Irradiation Center, Vietnam Atomic Energy Institute, km12, Road 32, Minh Khai, Tu Liem, Hanoi*
**Email: tmqthuquynh@yahoo.com*

Abstract: UV light has strongly influenced on the growth of *E. coli* as well as caused DNA damages. Configurations of both genomic DNA and pUC 19 plasmids extracted from *E. coli* were significantly changed by the exposure to UV light of 254 nm and DLT, an extract of *Ganoderma lucidum* Lingzi mushroom. The results also revealed the radio-protective effects of DLT to UV radiation. By adding 2% DLT to its culturing suspension, the growth of *E. coli* was significantly decreased, whereas a low DLT amount of about 0.5% slightly improved its growth, indicated that the DLT extract can be used as a promising protective substance against UV radiation. At the molecular level, the radio-protective effects of DLT were observed for both UV treated DNA and protein. Thus, DLT can protect DNA *in vivo*, but not *in vitro*. This effect was also observed for Taq polymerase, suggested that the radio-protection effect of DLT may due to it accelerated the degradation of radicals or species that produced in the suspensions during UV exposure.

Keywords: *UV radiation, DNA damage, plant extract, radio-protection.*

I. INTRODUCTION

UV radiation (UVR) can attack various targets in the cell. It produces unexpected photochemical reactions that are severely damaging for the DNA, proteins and other bio-macromolecules, resulting in cellular dysfunction or even death [1]. It can also produce free radicals, which act as mutagen or carcinogenic agents. Fortunately, various living organisms and even the cells possess their own defense systems against radiation that can fix those lesions. In addition, there are many natural products with protective properties that can be used as the radio-protectors.

Within a DNA molecule, the pyrimidine nucleotides (thymine and cytosine) can link together in order to form nucleotide complexes (dimers) by the exposure to UVR. These changes prevent the normal transcription and replication of the DNA. The UVR induced mutations can also lead to serious diseases, and even cause cancers [3].

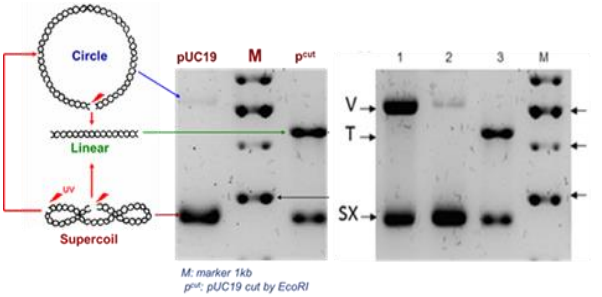

Fig. 1. Spatial configuration of pUC19 DNA plasmids: supercoil (SX), circular (V) and linear (T) DNA produced by UV radiation as mentioned in the previous study [4]

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Analysis of UVR induced DNA damages at the molecular level has been proved as an important method to determine the relationship between UV radiation doses and lesions including the changes in the DNA spatial configuration (Figure 1), as well as the damages (single strand, double strand breaks and dimerization) [5]. Investigation of the radiation damages at the molecular level for specific cells and tissues at various development and differentiation stages is essensial to determine the proper radiation dose in mutation breeding. Recently, this method has been applied to create and select new plants (cereals, legumes, fruits and flowers) with the desired traits in Vietnam [6-8].

It has been found that many natural products and compounds reveal their potential protective effects not only to radiation induced diseases, but also to several types of cancers by shielding genetic materials, activating cellular repair mechanisms or scavenging free radicals that are strong oxidants for the DNA, proteins and other biological molecules [9, 10].

In our previous study, the protective effect of the extract from *Ganoderma lucidum* mushroom against UVR has been proved by the reduction of the damages for the DNA exposure to UV [4]. The study on the Vietnam ginseng, Tran et al. found that saponin extracted from the Vietnamese ginseng can protect the liver cells against damages [11].

The extracts of red ginseng can also improve the anti-tumor activity of 5-fluorouracil, a popular anti-cancer drug [12]. Recently, the radio-protective effects of the extract of *Crinium latifolium* leave have been confirmed, this extract inhibited proliferation of tumor cells [13]. It is obviously that many plant extracts have anti-oxidant properties, which are able to scavenge several free radicals and species that may be associated to UV-oxidative stress [9]. The understanding of their protection mechanisms is very useful for formulating new safety and effective radio-protective drugs.

In this study, the extract (abbreviated as DLT) obtained from *Ganoderma lucidum* Lingzhi mushroom by normal extraction with hot water followed by precipitation with ethanol was kindly supplied by Hanoi University of Pharmacy. Its influences on the growth of *E. coli*, as well as radiation protective effects against UV radiation on the DNA and protein were investigated.

II. EXPERIMENTAL

Materials

E. coli carrying pUC19 recombinant vectors, as well as DLT extract from *Ganoderma lucidum* mushroom was prepared by the Laboratory for Medical Biology, Faculty of Biology, VNU University of Sciences, Hanoi.

E. coli was cultured in Luria - Bertani (LB) liquid medium, supplemented with or without DLT as described in the previous study, until the optical density at 600 nm reached 0.4

Plasmid and genomic DNAs were isolated by using QIA prep Spin Miniprep and WizardSV Genomic DNA Purification, respectively. EcoRI was purchased from New England BioLab (NEB).

Cell density and DNA concentration measurements

The same volume of *E. coli* was transferred and cultured in the LB liquid medium with or without supplement of DLT extract to determine the effect of DLT on bacteria growth. The cell density of *E. coli* suspension was determined by the interval measurement of its optical density at 600 nm (OD_{600}). One ml of cell suspension was taken and its OD_{600} was measured by a UV-vis spectrophotometer (UV-2450, Shimadzu, Japan).

The optical density of DNA solution was measured at 260 nm (OD_{260}), and the concentrations of both genomic and plasmid DNAs were calculated as follows:

$$[DNA] = OD_{260} \times n \times 50\ (\mu g/ml) \quad (1)$$

where OD_{260} = 1 corresponding to the DNA amount of 50 μg/ml in a test sample, and n is dilution factor.

UV radiation treatment

The genomic DNA is very sensitive to UV light in replication during the logarithmic phase in the bacteria growth, where the OD_{600} is about 0.3 to 0.6 [14]. *E. coli* cultured in the LB with or without the supplement of DLT (0.5%) were transferred to a petri dish and then the dish was exposed to UV radiation at 254 nm using BIO-LINK crosslinker (BLX 254, USA) within different periods to receive the corresponding doses of 0.5; 2.5; and 5 J/cm^2. The study on the UV effect to the DNA *in vitro*, pUC19 plasmids were isolated, purified and used as targets. Aqueous solutions of the DNA in distilled water and DLT were also exposed to UV for analyzing its UV induced damages and the protective effects of DLT for the DNA.

Estimation of DNA damage

The electrophoresis results have been used to analyze the size of the DNA fragments and the DNA damages as mentioned in our previous study [4]. Briefly, the agarose gel electrophoresis was prepared; then, the DNA samples were loaded in the agarose wells. The DNA fragments are visualized by staining with ethidium bromide, and they are taken by a specific camera. Be aware that the DNA will diffuse within the gel over time, and that the photography should be taken just after the cessation of electrophoresis [15].

Unlike the traditional PCR, the RAPD-PCR requires random primers, but not any specific DNA sequences [5]. By the RAPD-PCR method, the genetic similarity of the genome can be estimated from the patterns of DNA segments, which were amplified with the short primers (10 nucleotides) using Taq polymerase. In the present experiment, the primers amplified the DNA templates from unirradiated and UV irradiated bacteria in the RAPD-PCR to estimate DNA damages, are listed in Table I.

Enzyme activity measurement

The protease activity is its capacity to break peptide bonds. In this experiment, the protease activity was determined as its casein digestion and the liberation of amino acids and soluble peptide fragments. This was measured by the size of the hydrolysis zone produced by protease on the casein agar plate [16].

Table I. Primer sequences used for RAPD-PCR

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

III. RESULTS AND DISCUSSION

Effect of DLT on the growth of E. coli

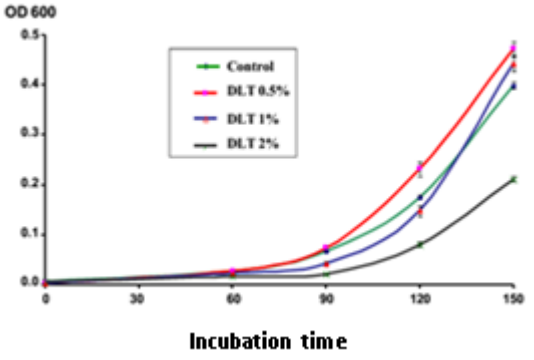

Fig. 2 Growth of *E. coli* in the media supplemented with DLT was determined by the OD_{600}

E. coli was cultured in the LB medium supplemented with various concentrations of DLT and the optical density of cultural solution was measured at the wavelength of 600 nm (OD_{600}) as a function of time intervals (see Fig. 2). At low DLT concentration of 0.5%, the bacteria growth rate was significantly increased with a higher value of OD_{600}, especially for culturing time more than 90 minutes.

However, its growth was inhibited by the presence of DLT at higher concentrations. The most obvious reduction was observed for *E. coli* culturing in the LB medium containing 2% DLT. This may be due to the effects of different bioactive substances which are present in DLT extract and their high concentration may inhibit the bacteria growth. Therefore, the DLT concentration of 0.5% was selected to investigate its radio-protective effects on UV exposed DNA.

Effect of DLT on growth and DNA demages of the UV irradiated E. coli

It is clear that UVR has much influenced the bacteria growth. Number of the irradiated cells seems to be reduced and leveled off after 90 minutes of culturing, while this value rapidly increased for the unirradiated ones. By adding DLT, the irradiated cell density was increased after 60 minutes and their OD_{600} value was nearly doubled after 90 minutes. The results also again indicated that DLT did not affect to the growth of unirradiated bacteria, though the growth of the UV exposed bacteria was recovered by adding this DLT (see Fig. 3). However, its adding points (initial and just before UV radiation) seem not to influence the growth of the irradiated bacteria, namely DLT did not protect the bacteria with the absorption of UV energy, but the radio-protective effects may be obtained by its stimulation for the repair mechanism in the cell or the degradation of radicals or species produced by UVR.

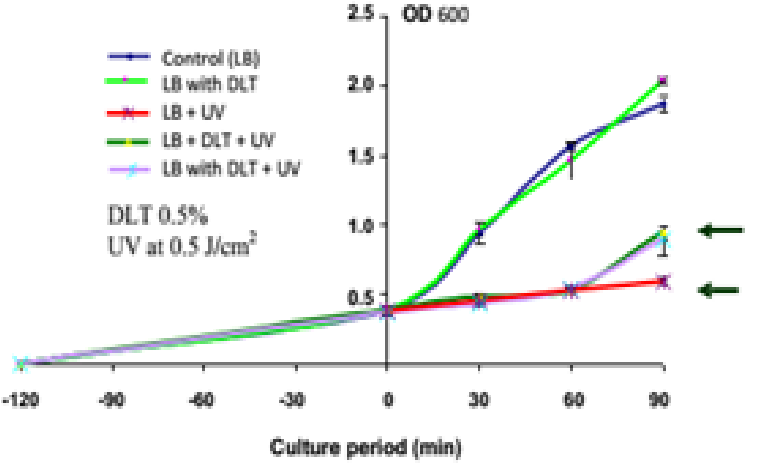

Fig. 3 The effect of 0.5% DLT on the recovery of the UV exposed *E. coli*. Bacteria at OD_{600} = 0.4 was exposed to the UV of 0.5 J/cm^2.

(♦): *E. coli* incubated in the LB media as the negative control; (■): DLT was added in the medium before culturing as the positive control; (✶): UV exposed bacteria without DLT; (✶): DLT was added immediately before and (▲) at the beginning of the incubation and exposed to UVR when the OD reached 0.4. Error bars show SD

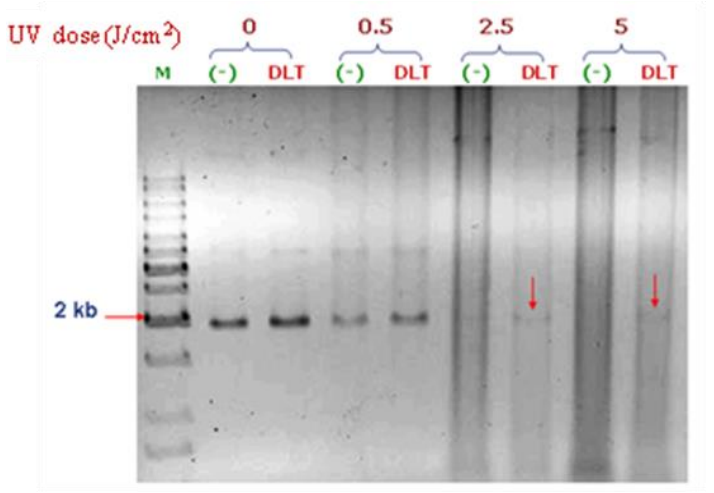

Fig. 4 Electrophoresis results of pUC19 plasmid DNA extracted from *E. coli* just after exposure to UV (-): culture and UV treated in LB. DLT: cultured and UV treated in LB supplemented with 0.5% DLT; M - marker 1kb. (Red arrow: DNA band representative for supercoil forms)

The plasmid DNA isolated from bacteria is mainly in supercoil with small amount of circular forms. By UV treatment, the number of the circular DNA increased due to increasing of the single stranded breaks, and the double stranded breaks leading to the formation of linear DNA fragments. These DNA configurations can be recognized and discriminated by electrophoresis (see Fig. 4). Therefore, changes in the plasmid DNA configuration reveal the DNA damages induced by UV treatment. The plasmid DNAs could be extracted from UV exposed bacteria, or from the bacteria, and subsequently exposed to UV.

On the other hand, the alteration of the genomic DNA caused by UV irradiation was evaluated by the RAPD-PCR using random primers. If there are differences in the genomic DNA, namely chromosomal alteration 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 the amplified DNA fragments on the gel. Therefore, different sizes in the DNA fragments yielded in RAPD-PCR reveal the changes in the bacterial DNA.

The pUC19 plasmid was also isolated from bacteria cultured in the LB medium with or without 0.5 % DLT and reached OD600 of 0.4. The same concentration of pUC19 that was diluted in either water (H2O) or 0.5% DLT was irradiated by various doses of UVR. The plasmid digested by the *Eco*RI restriction enzyme to obtain the linear form was used as the control. There are significant differences between the DNA irradiated in pure water and DLT solution as shown in Fig. 5. The changes from supercoil to circular forms were observed in DLT diluted pUC19. Both of these forms of pUC19 diluted in H_2O were decreased, suggesting that the DNA was fragmented more than that diluted in DLT. Thus, DLT did protect DNA *in vitro* against UV as it the shows radio-protective effect on the DNA *in vivo*.

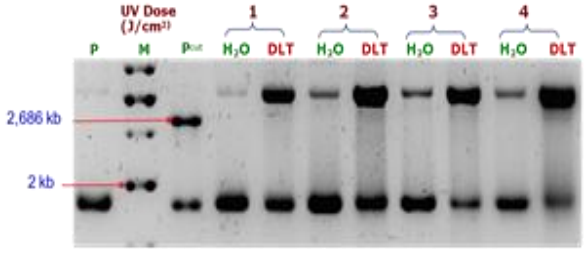

Fig. 5 Electrophoresis results of pUC 19 DNA plasmids dispensed in pure water (H_2O) and 0.5% DLT aqueous (DLT) treated by UV of dose from 1 to 4 J/cm^2. P and Pcut: PUC19 uncut and cut by *Eco*RI. M: DNA ladder 1 kb

Effect of DLT on the activity of UV treated enzymes

To study the UV protective effect of DLT on protein, commercial Taq polymerase was exposed to UVR in the presence or absence of DLT, and Taq polymerase activity was investigated by amount of PCR products.

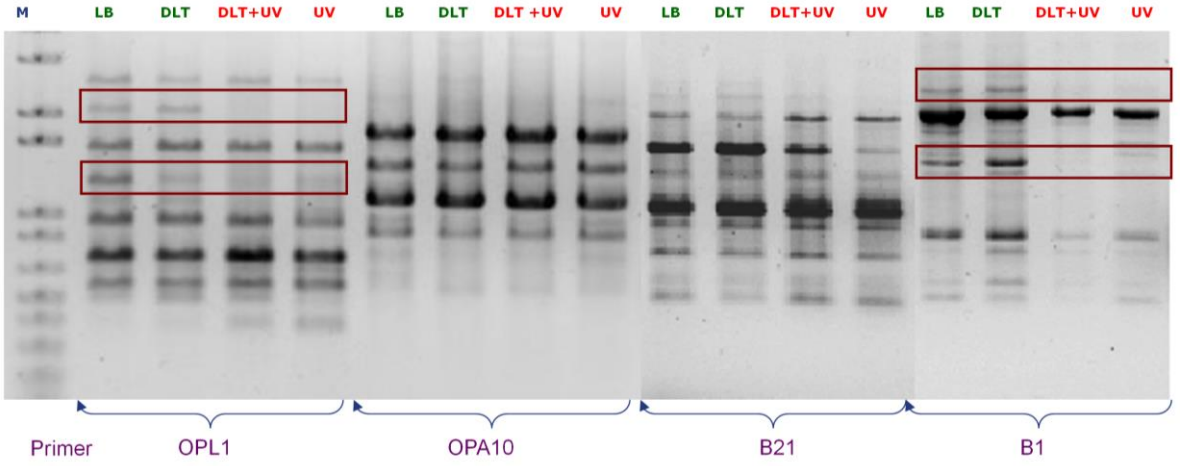

Fig. 6. Electrophoresis results of RAPD-PCR products obtained with random primers. DNA patterns amplified from 20 ng of the genomic DNA that was isolated either from non-irradiated *E. coli* cultured without (LB) and with 0.5 % DLT (DLT) or from 0.5 mJ/cm^2 of irradiated *E. coli* cultured with DLT (DLT+UV) and without DLT (UV). M: DNA ladder 1kb

After UV treatment in 1XTaq buffer supplemented with or without 0.25 % DLT, Taq polymerase was amplified using 1 ng of the DNA plasmid as the template. Electrophoresis image of the PCR products was presented in Fig. 7. It can be seen that the DNA band of the sample irradiated in the Taq buffer solution become smaller and disappear by increasing of the UV dose, but the band still observed for one irradiated in buffer solution supplemented with DLT. These results proved that DLT has a protective effect for Taq polymerase and this property may be due to the degradation of free radicals and DLT.

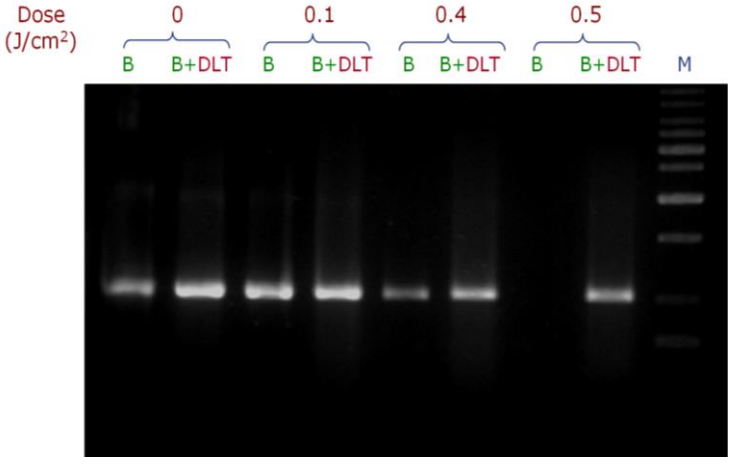

Fig. 7 PCR products amplified from 1 ng DNA plasmid by using Taq polymerase that was dispensed in Taq buffer without (B) or with 0.25 % DLT (B+DLT) and irradiated by UVR.
M: DNA ladder 1 kb

IV. CONCLUSIONS

The genomic DNA and pUC19 plasmid of *E. coli* were injured by UV exposure at some extent. The plant extract, DLT, does not affect to the growth of *E. coli*. In contrast, DLT shows positive effects against UV radiation. Bacterial growth was recovered by adding of 0.5% DLT to the cell suspension, and there are no differences in the recovery capacity among the bacteria that were cultured in the LB medium containing DLT and in LB medium, which added DLT just before UV treatment. These radio-protective effects of DLT were also recognized for the DNA and protein. DLT revealed its protective effect to the DNA *in vivo* and *in vitro*. The radio-protective effect of DLT was also observed for Taq polymerase. Thus, the plant extract DLT can be used as the radio-protector for the DNA and protein, and its protective effect may be due to its scavenging of radicals and species which are produced during UV exposure.

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