e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 66 (2018), No 1
https://doi.org/10.3409/fb_66-1.05

Effects of Ultra-violet Radiation on Cellular Proteins and Lipids of Radioresistant Bacteria Isolated from Desert Soil

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Accepted April 28, 2018 *Published online June 20, 2018* *Issue online June 22, 2018*

Original article

SAJJAD W., KHAN S., AHMAD M., RAFIQ M., BADSHAH M., SAJJAD W., ZADA S., KHAN S., HASAN F., SHAH A.A. 2018. Effects of ultra-violet radiation on cellular proteins and lipids of radioresistant bacteria isolated from desert soil. Folia Biologica (Kraków) **66**: 41-52.

Organisms in hot, arid environment have to cope with adverse life conditions such as desiccation, temperature and lack of nutrients. The phylogenetic diversity of ultra-violet (UV) radiation resistant bacteria from desert soil was investigated by culture and molecular-based analysis. The bacterial strains were characterized for their tolerance to UV doses, salt concentration and heavy metals. The effect of UV radiation (UVR) on cellular lipids and proteins was studied by lipid peroxidation and protein carbonylation assay. 9 UV resistant bacteria were isolated and identified through biochemical tests and 16S rRNA sequencing. These bacterial strains were grouped into four phyla: *Firmicutes, Proteobacteria, Deinococcus-Thermus* and *Actinobacteria*. The genus *Deinococcus* was found to be resistant to high UV dosage in comparison to other genera, as indicated by maximum survival rate. The bacteria were found to grow at a wide range of temperature and pH, resistant to high salt concentration and various metal ions. The UV resistant selected strains exhibited minor damages to proteins and lipids as a result of exposure to UV radiation as compared to *Escherichia coli* (ATCC 10536), a UV sensitive bacteria. The results indicated that these microbes might harbor a sophisticated phenotypic character and molecular repair mechanism that can prolong their survival under extreme radiation.

Key words: Radio-resistant, phylogenetic analysis, *Deinococcus-Thermus*, protein carbonylation, lipid peroxidation.

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Radio-resistant is the term referred to the group of organisms that live under and can efficiently recover when exposed to radiation. These organisms can surprisingly endure both ionizing and non-ionizing radiation, which can be lethal to other species (SINGH & GABANI 2011). Ionizing radiation-resistant microbes have been isolated from a wide range of environments including dried food, irradiated meat and fish, high level nuclear wastes at Savannah River in South Carolina, hot and dry desert, and warm freshwater geothermal spring at Hanford in Washington (RAINEY *et al*. 2005). Extreme ionizing radiation resistance has been observed in several members of the domains *Bacteria* and *Archaea* (FREDRICKSON *et al*. 2004). Of the genera containing ionizing radiation-

resistant organisms, *Deinococcus* and *Rubrobacter* followed by *Kineococcus* and *Kocuria* show the highest levels of resistance (PHILLIPS *et al*. 2002).

Ultraviolet radiation (UVR) is an important stress factor for bacterial communities. It is known to induce oxidative stress in aquatic bacteria by production of reactive oxygen species (ROS) formed via photodynamic reactions involving intracellular or extracellular photosensitizers (PATTISON & DAVIES 2006; SANTOS *et al*. 2013a,b). These ROS can react with cellular constituents, most notably proteins and lipids, leading to altered membrane permeability and/or disruption of transmembrane ion gradients that eventually cause cell death (BARRERA 2012). The cellular and biological consequences of ROS are strongly influenced by metal ion homeostasis (HALLIWELL & GUTTERIDGE 2015). In bacterial cells which are exposed to UVB radiation, the potential synergistic effect of some transition metals like Cu^{+2}, Mn^{+2} and Zn^{+2} have also been reported (SANTOS *et al*. 2013a). Intercellular Cu^{+2} uptake, presence of high intracellular Mn^{2+} and Zn^{2+} uptake by UV resistant microbes are the adaptive response to peroxide stress by blocking the Fenton and Haber-Weiss reactions (BAGWELL *et al*. 2008; DALY *et al*. 2010). Production of different compatible solutes such as trehalose and ectoine in extreme environments plays a significant role in ionizing radiation protection (BEBLO-VRANESEVIC *et al*. 2017). Ionizing radiation resistance in *Halobacterium salinarum* is most likely achieved by a "metabolic route" with a combination of tightly coordinated physiological processes (ROBINSON *et al*. 2011). Therefore, it is necessary to investigate the diversity of ultraviolet resistant microorganisms in order to understand the biological mechanisms involved in survival under UVR stress.

The current study focused on determination of phylogenetic diversity of UV resistant bacteria in desert soil of Lakki Marwat and Bahawalpur deserts, Pakistan. The effect of UV radiation on intracellular lipids and proteins was also investigated by using standard oxidation assays.

Material and Methods

Sampling

Soil samples (2 different samples from 15 cm depth) were collected aseptically from Lakki Marwat and Bahawalpur deserts, Pakistan, in sterilized polyethylene zipper bags following a standard microbiological procedure, carefully transported to the laboratory and stored at 4°C for further processing.

Metal analysis of desert soil

Sample preparation

The soil samples (2 samples) were dried at room temperature for 5 days and sieved (2 mm sieve). 1 g of the soil was acidified with 0.5 ml of concentrated nitric acid and 10 ml of per-chloric acid (70% $HClO_4$). The mixture was heated till white, dense fumes of $HClO_4$ appeared (RAURET 1998). The digested samples were cooled to room temperature, filtered through Whatman # 41 and boiled to remove oxides of nitrogen and chlorine. Finally, the soil samples were subjected to Cu^{+2}, Ni^{+2}, Zn^{+2}, Mn^{+2}, Cr^{+2}, Fe^{+2}, Pb^{+2}, Cd^{+2}, Ca^{+2}, Mg^{+2} and Na^{+2} analysis using an atomic absorption spectrophotometer (AAS) on a Perkin-Elmer 460 Spectrophotometer.

Isolation of radio-resistant microorganisms

The soil samples were serially diluted and plated on TGY (tryptone glucose yeast extract) agar by the spread plate method. The plates were exposed to UV radiation in 119x69×52 cm UV chamber supplied with a 20W and 280nm UV light source (germicidal lamp) for a specified time (30-300 seconds). The UV fluence rate (energy/area/time) to the test sample was measured with He=Ee×t in units of J/m^2 (SAJJAD *et al*. 2017). The total UV dose was determined by time of exposure to UV fluence rate. All UV irradiation procedures were performed under red light to prevent photo-reactivation.

Radiant exposure (He) = the energy that reaches a surface area due to irradiance (Ee) maintained for a time duration (t).

UV radiation tolerance

The UVR resistance among bacterial isolates was determined by a method previously described by MATTIMORE and BATTISTA (1996) with some modifications in order to calculate % survivability. The UV-resistant bacteria isolated were grown in TGY broth up to OD_{600} 0.5, and then spread on TGY agar. The plates were exposed to UV-B (280nm) for the variable doses (30-180 sec) and subsequently incubated at 30ºC. The surviving fraction was calculated after 24 hrs by determining the titer of culture after irradiation divided by unirradiated control.

Identification of UV resistant microbes

Biochemical and physiological characteristics

Cellular morphology was examined by phase-contrast microscopy (Labomed Lx400). The bac-

teria were grown on TGY agar plates at wide temperature 15-45°C, pH 4.0-9.0 and NaCl (0-16%) ranges for 3 days to determine optimum growth conditions. The strains were also tested for catalase, cytochrome oxidase as well as hydrolysis of starch, casein, and gelatin by methods described in MURRAY *et al*. (1981).

Sequence alignment and phylogenetic analysis

The genomic DNA of all bacterial strains was extracted using a DNA extraction kit (QIAGEN). The 16S rRNA gene sequences were amplified using universal primers (F-27:AGAGTTTGATCMTGGCTCAG, R-1492:TACGGYTACCTTGTTACGACTT). A reaction mixture containing GoTaq Green Master Mix Promega (25 µl), primer 27F (2 µl), primer 1492R (2 µl), DNA extract (3 µl), and *Nuclease-Free* Water (50 µl) was prepared. The reaction was carried out in a MJ Mini Personal Thermal Cycler (BIO RAD). In the PCR cycle initial denaturation was completed at 95°C for 3 minutes, followed by denaturation (30 cycles) for 1 min at 95°C, annealing at 55°C for 1 min and finally extension for 1 min at 72°C and final extension at 72°C for 7 min. The PCR product was analyzed by agarose gel (1%) electrophoresis. The amplified PCR products were sequenced at Macrogen Service Center (Geunchun-gu, Seoul, South Korea). The obtained sequences were identified using the BLAST tool at the NCBI database and homologs were phylogenetically analyzed using Molecular Evolutionary Genetic Analysis (MEGA) version 6 (TAMURA *et al*. 2013). All the UV resistant isolates were compared with previously reported microorganisms submitted to NCBI.

Metal resistance

Stock solutions (1000 ppm) of various transition metals (Co^{+2}, Cu^{+2}, Cr^{+2}, Fe^{+2}, Mn^{+2} and Zn^{+2}) were prepared in deionized and filter-sterilized water from the corresponding metallic salts. The effect of metal ions on bacterial strains was determined by inoculating them on TGY agar supplemented with different metals at variable concentrations (20-400 ppm) and incubated at 30°C for 48 h.

Effect of UVB on lipids and proteins of UV resistant bacteria

Cultures grown overnight in TGY broth were harvested by centrifugation at 10,000 rmp for 10 mins. The pellets of the respective bacterial strain (10^6 cells/ml) were irradiated with UVB. The UV dose (2000 J/m^2) was calculated by a method described previously (SAJJAD *et al*. 2017). An aliquot of cell suspension was collected before and after irradiation, washed with ultrapure water, and immediately used for lipid and protein extraction.

Lipid peroxidation assay

Lipid peroxidation results in formation of malondialdehyde (MDA), a lipid peroxidation marker. The TBA (Thiobarbituric acid) assay was performed by the method described by PÉREZ *et al.* (2007) with some modification, in order to assess the MDA concentration. Total lipid extract was recovered, according to a standard protocol as previously described (BLIGH & DYER 1959). 250 µl of lipid samples from irradiated and un-irradiated culture were mixed with 125 µl of 20% trichloroacetic acid (TCA). The supernatant was collected, mixed with 0.5 ml $FeSO_4$ (0.07 M) and incubated at 37°C for 1 h. 300 µl of this solution was mixed with 0.8% TBA reagent (200 µl), 8% SDS (200 µl) and incubated at 100°C for 1 h. The absorbance of chromophore was measured at 535 nm. The MDA concentration is presented as µM of MDA produced per mg of lipids using a molar extinction coefficient of 1.56×10^5 M/cm (KONUKOĞLU *et al*. 1999). The experiment was run in triplicate and a UV sensitive *E. coli* (ATCC 10536) was used as a control.

Intracellular protein carbonylation

Protein carbonylation, an indicator of protein oxidation, was measured using the DNPH (2,4-dinitrophenyl hydrazine) method (MISRA *et al*. 2004). Both irradiated and un-irradiated cell suspensions were centrifuged and pellets were resuspended in 10 mM Tris-HCl (pH 8.0), sarkosyl (1.5% v/v) and incubated at room temperature for 20 min (OJANEN *et al*. 1993). The total protein concentration was estimated by a method previously described by LOWRY *et al*. (1951). The protein extract (2 mg/ml) in 50 mM PBS (pH 7.4) was incubated with 400 µl of 10 mM DNPH in 2 M HCl for 2hrs in the dark. Protein was precipitated, resuspended in 6 M guanidine hydrochloride. The supernatant was analyzed spectrophotometrically at 370nm. A protein control was run in parallel where DNPH was replaced with 2 M HCl. The protein carbonyl content was expressed in mM/mg of protein.

Statistical analysis

To assess the significance of the results the following tests were applied: Student's t-test for pairwise comparisons, single factor and two-way ANOVA for analysis between and within groups, and Tukey's HSD test for multiple comparisons. A P value less than 0.05 was considered significant. The effects of UV on cellular lipids and pro-

teins were estimated and all the strains were compared with a UV sensitive strain *E. coli* ATCC 10536. Bacterial cell sensitivities were studied by plotting the data between the % survivability and their respective UV doses (Jm^{-2}).

Results

Metal analysis of soil samples

The sampling areas, both selected from Lakki Marwat and Bahawalpur deserts, were characterized by high solar radiation and temperature fluctuations between day and night. Figure 1 shows the physiochemical analysis of soil samples collected from two different deserts. Both samples contained high concentrations of Mn^{+2} followed by Mg^{+2}, Fe^{+2} and Pb^{+2} but very low concentrations of Cd^{+2}, Cu^{+2} and Cr^{+2} ions. A two-way ANOVA used to check the differences between the two deserts, showed a significant difference using metal-type as blocking factor ($P<0.05$). Moreover, a pairwise t-test showed that the average characteristic of Lakki Marwat dessert is significantly higher than the Bahawalpur desert ($P=0.02$).

Isolation of ultraviolet radiation (UVR) resistant microbes

Soil samples were exposed to UV radiation from 30 to 300 sec with an energy dose of about 300-3300 J/m^2. A total of 9 representative colonies were selected after incubation at 37°C for a week, morphological characteristics (shown in Table 1) were noted and then purified.

Resistance to UV in correlation with % survivability

The bacterial strains were exposed to UVR dosage ranges from 300-3300 J/m^2 and their survival rate was determined. Following exposure to different doses of UVR, the colony-forming units (CFUs) of irradiated samples were significantly lower than un-irradiated samples ($P=0.001$) (Fig. 2). The initial dose of UVR ($2.0x10^3$ J/m^2) was found to be lethal for most of the bacterial population, hence considered as LD_{50}. A gradual decrease in CFU was observed upon an increase in UV dose up to a certain extent, a rapid decline in individual populations was observed beyond that limit. Among these 9 bacterial strains, the survival rates of strains WMA-LM9, WMA-LM30 and WMA-BD1 were noted as 79%, 68%, and 45%, respectively, even after exposure to high energy dosage (3.3×10^3) and considered as UV resistant bacteria. Strain WMA-LM19 was found to be the most sensitive that could withstand up to 1.30×10^3 J/m^2 energy. Pairwise t-test shows there is a significant affect of UV dose on the survival of isolates where $P<0.05$.

Identification of UV resistant bacterial strains

Morphology

A diverse bacterial population was observed on un-irradiated TGY plates in comparison to the irradiated TGY plates where only yellow, orange, pink, or red colonies were observed. A decrease in colony forming units (CFU) per gram of soil samples was observed upon increase in UVR dose. After UV irradiation, the UVR resistant strains were

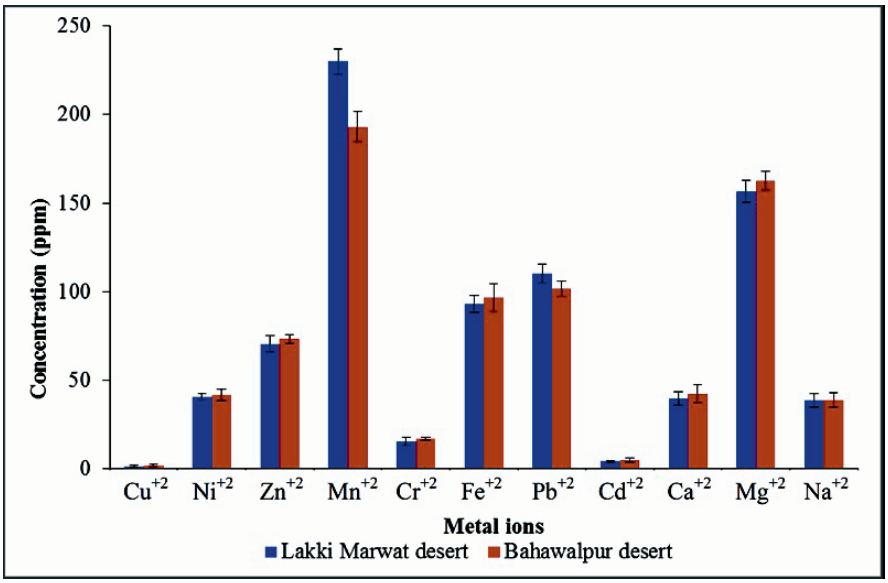

Fig. 1. Metal analysis (in ppm) of soil samples collected from Lakki Marwat and Bahawalpur deserts.

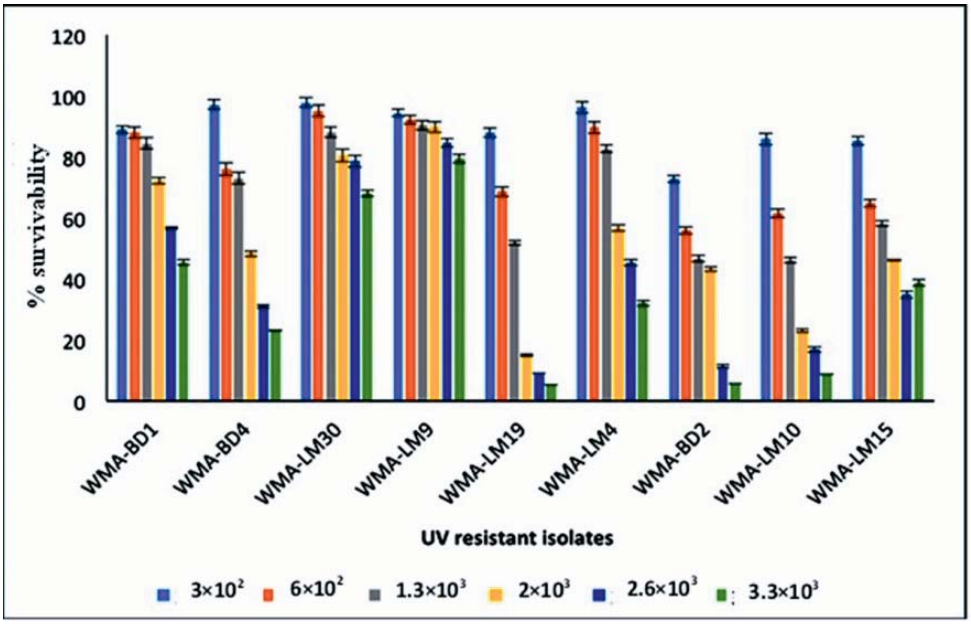

Fig. 2. Survivability of total UVR resistant isolates from desert soil at varying UV-B exposure. % survivability was measured using the formula $N_1/N_0 \times 100$. N_1 is the number of colonies after UV irradiation while N_0 number of colonies prior to exposure. The bars show mean±SD (whiskers).

Table 1

Microscopic characteristics with cultural morphology of UV resistant bacteria isolated from desert soil samples

Culture code	Sampling site	Morphology	Microscopy
WMA-BD1	Bahawalpur desert	Small to medium sized light orange colored mucoid and circular, raised colonies with entire margins	G+ve cocci
WMA-BD2	Bahawalpur desert	Large light off-white oval shaped colonies dry surface forming crystal like structure when aggregates	G+ve rods
WMA-BD4	Bahawalpur desert	Medium to large yellow colored circular raised colonies with entire margins	G+ve rods
WMA-LM4	Lakki Marwat desert	Small to medium sized off-white smooth and circular, flat colonies with entire margins	G+ve rods
WMA-LM9	Lakki Marwat desert	Medium brick red colored colonies mucoid circular with entire margins	G+ve cocci
WMA-LM10	Lakki Marwat desert	Large raised white in color with sticky surface colonies with irregular margins	G-ve rods
WMA-LM15	Lakki Marwat desert	Large flat off-white in color oval shape dry colonies with entire margins	G-ve rods
WMA-LM19	Lakki Marwat desert	Large off-white in color flat colonies with shiny surface circular with entire margins	G-ve rods
WMA-LM30	Lakki Marwat desert	Medium brick red colored colonies with dry surface circular with entire margins occur singly or tetrads	G+ve cocci

examined both morphologically and microscopically; Table 1 shows the cellular morphology and Gram's reaction of all UV resistant bacteria.

Biochemical and physiological characteristics of UV resistant microbes

Biochemical, physiological and other characteristics such as temperature, pH range and salt tolerance among all UVR resistant bacteria were also determined. Table 2 shows biochemical and other physiological characteristics of these UVR resistant strains. The results indicated that all these strains have potential to grow at a wide temperature (20-45°C) and pH (6-10) range, high salt concentration (2-16%), and also produce different hydrolytic enzymes like amylase, protease, gelatinase, and DNase as shown in the Table 2.

Table 2

Biochemical and physiological characteristics of UV resistant isolates from Lakki Marwat and Bahawalpur desert soils

Characteristics	Bacterial Strains								
	WMA-BD1	WMA-BD2	WMA-BD4	WMA-LM4	WMA-LM9	WMA-LM10	WMA-LM15	WMA-LM19	WMA-LM30
Temperature (°C)	20-35	25-40	20-37	25-37	10-30	20-45	25-45	20-45	10-30
pH	7-9	7-10	7-9	7-9	7-8	6-10	6-9	6-10	7-8
Salt tolerance	12%	10%	10%	14%	2%	10%	16%	12%	6%
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	–	–	–	–	–	+	+	+	–
Amylase	+	+	+	–	+	+	+	+	–
Protease	–	+	+	+	+	+	+	+	+
Gelatinase	+	+	–	–	+	+	+	+	+
DNase	+	–	–	–	+	+	+	+	–

Molecular characterization and phylogenetic analysis of UVR bacteria

The phylogenetic diversity was evaluated by partial sequencing of the 16S rRNA gene of 9 bacterial strains representative of each dominant morphotype cultured on plates. The sequencing results clearly divided all these strains into 4 phyla: *Actinobacteria* (1 isolate), *Proteobacteria* (3 isolates), *Firmicutes* (3 isolates) and the *Deinococccus-Thermus* group (2 isolates). *Proteobacteria* showed a distant relationship to the genera *Stenotrophomonas* with 93-99% of similarity. 2 strains were clustered near phylum *Deinococcus-Thermus* and showed 99% similarity to *Deinococcus* sp., considered to be among the most UV resistant organisms up till now. 3 *Firmicutes* clustered near the genera *Bacillus* (2) and *Staphylococcus* (1) with 99% similarity. Finally, 1 Actinobacteria was distantly related to the genus *Kocuria* with 99% similarity, as shown in Table 3. It is noteworthy that the 16S rRNA gene sequences of 9 bacterial strains were closely related to database sequences derived from arid, semi-arid environments and polluted soils (Table 3).

Nucleotide sequence and accession numbers

The 16S rRNA sequences of all pure cultures were deposited in the GenBank database under accession numbers: KT008382 (*Stenotrophomonas maltophilia* WMA-LM10); KT008383 (*Stenotrophomonas* sp. WMA-LM19); KT008384 (*Deinococcus* sp. WMA-LM30); KT008385 (*Bacillus licheniformis* WMA-BD2); KT008386

Table 3

GenBank accession number, closest related species, query coverage, 16S rRNA sequence homologues, and % survivability of Ultraviolet radiation (UV-B) resistant isolates from desert samples

Isolates	GenBank Accession Number	Closest related species	Query coverage %	Similarity score %	UVR resistance J/m^2	Survival rate (%)
WMA-BD1	KT008387	*Kocuria turfanensis*	100	99	3.3×10^3	45.45
WMA-BD2	KT008385	*Bacillus licheniformis*	100	99	2.0×10^3	43.18
WMA-BD4	KT008386	*Staphylococcus lugdunensis*	100	99	2.0×10^3	48.27
WMA-LM4	KT008388	*Bacillus pumilus*	99	99	2.60×10^3	45.28
WMA-LM9	KT008389	*Deinococcus radiopugnans*	100	99	3.30×10^3	79.47
WMA-LM10	KT008382	*Stenotrophomonas maltophilia*	100	99	1.30×10^3	46.15
WMA-LM15	KT008390	*Bacillus subtilis*	100	99	3.30×10^3	38.72
WMA-LM19	KT008383	*Stenotrophomonas* sp.	99	93	1.30×10^3	51.69
WMA-LM30	KT008384	*Deinococcus* sp.	100	100	3.30×10^3	68.03

Table 4

Effect of the metal ions (in ppm) on growth of UVR resistant selected bacteria from desert samples on TGY agar plates. The minimum metal ion concentration (ppm) that inhibits the growth of UVR resistant isolates is shown in the table

Strain code	Cd	Zn	Cr	Fe	Cu	Mn
WMA-BD1	380	160	360	360	280	300
WMA-BD2	200	200	320	360	200	260
WMA-BD4	240	240	300	360	200	240
WMA-LM4	360	280	360	300	240	200
WMA-LM9	200	80	380	280	200	220
WMA-LM10	240	200	300	360	200	300
WMA-LM15	220	280	360	360	240	340
WMA-LM19	280	120	280	360	200	280
WMA-LM30	280	200	360	200	280	340

(*Staphylococcus lugdunensis* WMA-BD4); KT008387 (*Kocuria turfanensis* WMA-BD1); KT008388 (*Bacillus pumilus* WMA-LM4); KT008389 (*Deinococcus radiopugnans* WMA-LM9); KT008390 (*Bacillus subtilis* WMA-LM15) (Table 3).

Metal resistance

The isolated radioresistant bacteria were more resistant to Mn^{+2}, Co^{+2}, Cr^{+2}, and Ni^{+2}, which could be directly correlated to UVR resistance as shown in Table 4. Some interesting changes in cultural characteristics of strains WMA-LM9, WMA-LM30 and WMA-LM19 were observed on medium supplemented with Mn^{+2}, such as an increase in colony size and bright coloration. A high capacity for intracellular Cu^{+2} ion sequestration was detected in strains WMA-BD1, WMA-LM15, WMA-LM9, WMA-LM30 (240-280ppm) that provided protection against the damaging effects of ionizing radiation as shown in Fig. 3.

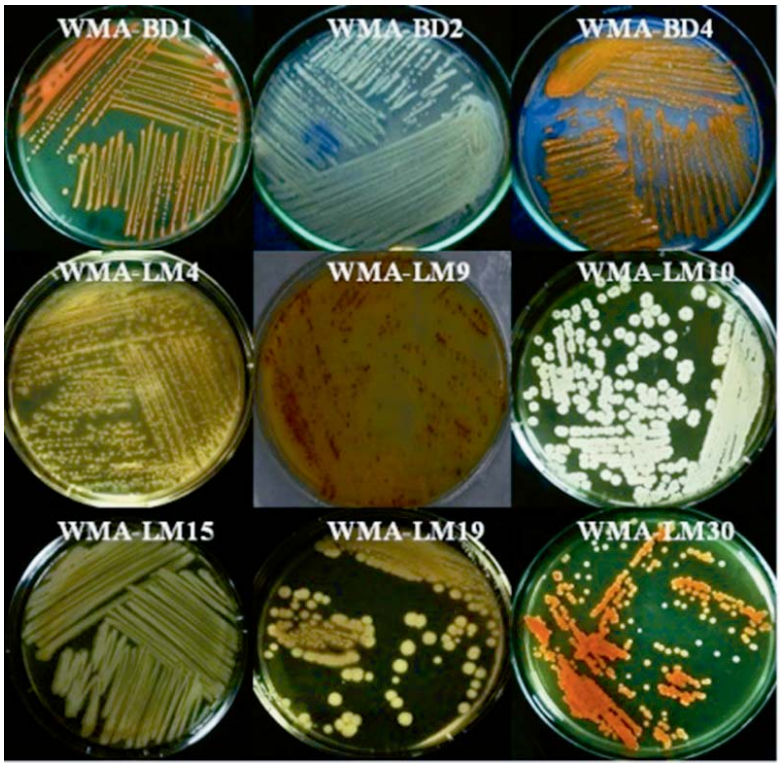

Fig. 3. Morphology of different UV resistant isolates from two desert soils on tryptone glucose yeast (TGY) agar plates supplemented with metal ions.

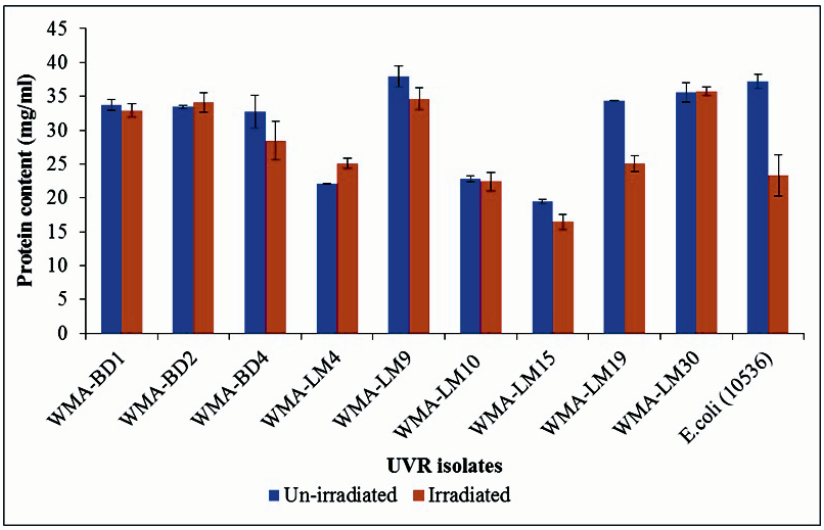

Fig. 4. Effect of UVB on total cell protein content in mg/ml. The bars show mean±SD (whiskers).

Effect of UVB on whole cell proteins

The effect of UVR on whole cell protein from radioresistant bacteria was determined. The bacteria presented strong protection to cellular protein upon exposure to UVR, in comparison to a UVR sensitive *E. coli* (ATCC 10536), used as a control. Figure 4 shows the amount of protein (mg/ml) measured from both irradiated and non-irradiated bacteria, strains WMA-LM9, WMA-LM30, WMA-BD1 and WMA-BD2 were observed for maximum amount of protein after exposure to a high UVR dose. UVB strongly damaged the whole cell protein of *E. coli* 10536, a sensitive strain. The radioresistant UV treated strains in this study showed little damage in intracellular proteins as compared to the untreated strains. The pairwise t-tests shows $P<0.05$ ($P=0.002$). Statistical analysis showed a significant difference in protein contents of sensitive strain *E. coli* and UV resistant isolates using Tukey's multiple comparisons test ($P<0.014$). The results show a better preventive system for UV in UV resistant strains than *E. coli* ATCC 10536.

Lipid and protein oxidation of UVR isolates

UV radiation-induced oxidative stress causes damage to cellular lipids and proteins that ultimately results in cell death. The effect of UV on cellular lipids and proteins was measured. A UV sensitive *E. coli* (10536) strain was used as a control. *E. coli* (10536) displayed significant damage to its cellular lipids (Fig. 5) and proteins (Fig. 6) upon UV exposure with lipid peroxidation up to 12 µM/mg and protein oxidation of 189 mM/mg. A difference between *E. coli* was observed in protein and lipids damages in comparison to the ra-

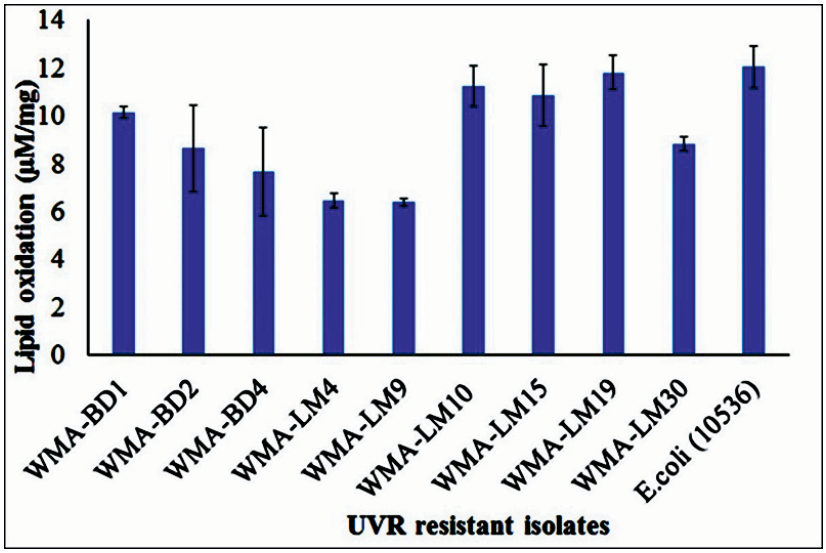

Fig. 5. Lipid peroxidation assay in UV-treated isolates from desert soil. The bars show mean±SD (whiskers).

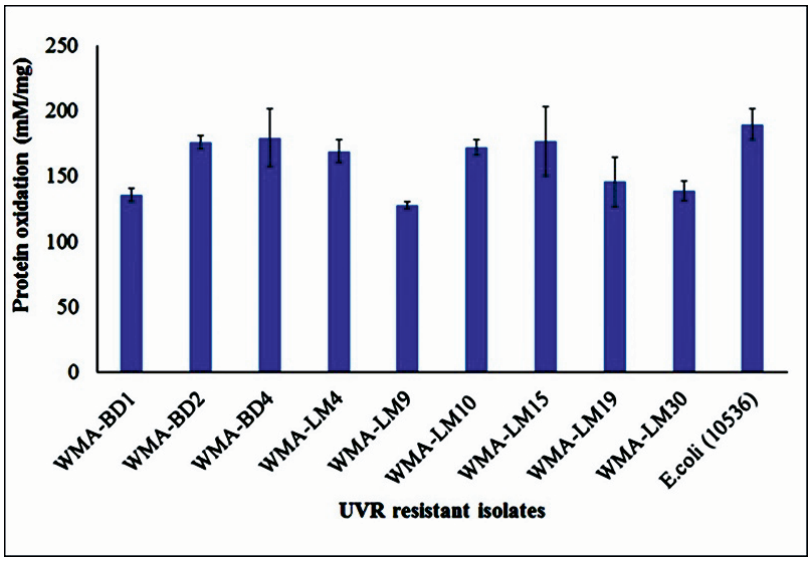

Fig. 6. Protein oxidation assay in UV-treated isolates from desert soil. The bars show mean±SD (whiskers).

dioresistant microbes, revealing their resistance to UV radiation. Figures 5 and 6 show that strains WMA-LM9, WMA-LM30 and WMA-BD1 exhibited lower protein oxidation (P=0.0099), while lipid oxidation was lower in strains WMA-LM4, WMA-LM9 and WMA-BD4 (P=0.0199) in comparison to the control strain. The data was analysed by using a two-way ANOVA and Tukey's HSD test. Moreover, a pairwise t-test shows P<0.02, and the results were considered highly significant.

Discussion

The current study was conducted to investigate the bacterial community inhabiting extreme environments including high hypersaline and high UV radiation habitats. Their survival in spite of higher UV dosage as well as resistance to salt and metal ions are some of the interesting characteristics of these microbes. The molecular mechanisms behind these phenomena need to be investigated. The soil samples were analyzed for the presence of various metal ions: Mn^{+2}, Mg^{+2}, Fe^{+2}, and Pb^{+2} were found to be the most abundant divalent cations followed by Ca^{+2}, Ni^{+2}, and Zn^{+2}. Research conducted on several biological systems has highlighted the role of transition metal ions in protection against the detrimental effects of radiation, desiccation and H_2O_2 (DALY *et al*. 2004; GHOSH *et al*. 2011). During sand formation in deserts, the manganese oxides in rock varnish very effectively blocking the transmission of ultraviolet radiation (DORN & OBERLANDER 1981). Our results demonstrated a significant role of the metal ions in two deserts and UV-resistant microbe survival in such dry and extreme environs. The microbes inhabiting the desert can synthesize different oxides of manganese on their outer surfaces which can act as a sunscreen to block the UV radiation. These manganese oxides also give a characteristic dark color to desert soil (FLEISHER *et al*. 1999).

Based on comparison of 16S rRNA gene sequences, most of the UVR resistant bacteria were Gram positive and assigned to four different clusters: *Fermicutes*, *Deinococcus-Thermus, Proteobacteria* and *Actinobacteria*. β-*Proteobacteria* and *Firmicute*s have been reported earlier with maximum survival rate at high UV dosage (BAATI *et al*. 2010; MORENO *et al*. 2012). FREDRICKSON and colleagues (2004) described *Proteobacteria*-related species from nuclear waste-contaminated sediments that exhibited resistance to 2.5 kGy of gamma radiation, with 0.0017% survival.

Most of the bacteria produced colored pigments on TGY agar, as indicated by colored colonies, that may absorb radiation in order to protect the cells from damage. The production of UVR-absorbing compounds might be induced as a result of exposure to radiation stress (DIB *et al*. 2009). We have reported a bacterium with 93% similarity to *Stenotrophomonas* sp. that showed enlargement in its colony size and pink to red coloration upon radiation exposure in the presence of Mn^{+2}. The rapid increase in cell number and size after exposure to UV needs deeper studies in order to explain the mechanism triggered by radiation that enhances cellular survival and replication (MCGLYNN & LLOYD 2002). Previously a number of researchers have reported radio-resistant bacteria from desert soil, this strategy might be the result of evolution in order to protect cells from desiccation (RAINEY *et al*. 2005). In *Deinococcus* the S-layer protein DR-2577 binds deinoxan-

thin under desiccation stress that in turn shields the bacterium from UV light and could behave as a first line of defense against radiation (FARCI *et al*. 2016). The ability of these UV resistant microbes to survive in several extreme conditions is suggested to be a result of three combined mechanisms: prevention, tolerance and repair (WHITE *et al*. 1999).

Metals like Mn^{+2}, Cu^{+2}, Zn^{+2} and Co^{+2} enhance the survivability of UVR resistant microbes because these metals block the Fenton reactions that indirectly prevent formation of several toxic oxides and by-products which can alter the cell membrane (BAATI *et al*. 2010; SANTOS 2011). In this study, all the bacteria isolated from desert soils showed high resistance to various metal ions. A high capacity for intracellular copper ion sequestration was detected in *Kineococcus* radiotolerans (*Actinobacteria*) that might be a reason for its survival against ionizing radiation (ASGARANI *et al*. 2012; PAULINO-LIMA *et al*. 2016). Research has highlighted the role of manganese (Mn^{2+}) ions in the prevention of oxidative damage to cells upon exposure to UV radiation, gamma-irradiation, heat and H_2O_2 (BARNES *et al*. 2008; MCEWAN 2009; DALY *et al.* 2010; SLADE & RADMAN 2011). Zinc (Zn^{2+}) uptake is also a key component of the adaptive response to peroxide stress (GABALLA & HELMANN 2002), protecting copper-treated *Escherichia coli* against superoxide killing (KORBASHI *et al*. 1989) and countering the effects of oxidative stress in *Lactococcus lactis* (SCOTT *et al*. 2000). The ability of these microbes in such extreme environment makes them an attractive choice for in-situ bioremediation of radioactive wastes. Bacteria are susceptible to harmful effects of UV radiation due to their small size, short generation time and absence of effective UV-protective pigmentation (GARCIA-PICHEL 1994). The effect of UV radiation on all the bacterial strains isolated from desert soil showed different survival rates as well as lipid peroxidation and protein carbonylation. *Deinococcus* was found to be the most resistant with a high survival rate and low level of lipid and protein damage in comparison to *E. coli* (10536) ATCC (Fig. 4). SANTOS *et al*. (2013b) reported that exposure to UVR causes alteration in lipids and protein structures as a result of peroxidation and carbonylation, respectively, which were confirmed by gas chromatography.

In addition to DNA damages by high UV exposure, changes in lipid membranes and protein tertiary structure also play an important role in bacterial inactivation. The targets (e.g., nucleic acids, proteins, lipids) for UV radiation inactivation may differ among the genus, species and strains and thus all these factors contribute the duration of cell survival under high UV radiation. It has also been suggested that UV-induced DNA damage in Gram-positive bacteria is lower than that of Gram-negative bacteria because of a shielding effect by the cell wall (JAGGER 1985). The presence of Mn/Fe ratio is another factor that can contribute to cell resistance under high radiation. The presence of a high concentration of Fe^{+2} in *Shewanella oneidensis* MR-1 makes it sensitive to UV radiation. The intracellular Fe^{+2} promotes the formation of ROS via Fenton type reactions (QIU *et al*. 2005). The efficiency of defence in extreme environments and highly sophisticated molecular repair mechanisms may differ among bacteria and play an important role in cellular resistance.

Conclusion

We have demonstrated that UV radiation has a clear effect on the microbes and other living cells. The resistant microbes produce compounds of great interest which can be used as sunscreen and UV protectants. Our study argues for increased exploration of the desert environment for UV resistant microbes in Pakistan. These results open exciting possibilities for investigating bacterial lenience to desiccation, radiation and survey in the deserts. The implication of the results is conferred from an environmental and industrial perspective and with admiration to potential expansion in UV-based disinfection technologies.

Acknowledgements

We highly acknowledge the Department of Microbiology, Quaid-I-Azam University, Islamabad, Pakistan for providing facilities to accomplish this study. The contribution of Biochemistry department Quaid-I-Azam University, Islamabad, Pakistan is highly appreciated.

Author Contributions

Research concept and design: A.A.S.; Collection and/or assembly of data: W.S., S.K., M.A., S.Z.; Data analysis and interpretation: W.S., M.R., W.S.; Writing the article: W.S; Critical revision of the article: M.B.

Conflict of Interest

The authors declare no conflict of interest.

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