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Diversity of UV Resistance in the Halophilic Archaea

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Diversity of UV Resistance in the Halophilic Archaea

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I. Overview

Organisms in the three domains of life, Bacteria, Archaea, and Eukarya, are challenged by exposure to ultraviolet light (UV) in the environment. Short wavelength UV is a potent DNA damaging agent that causes lesions, or base modifications, within the DNA that can lead to mutations and cell death. As a result of these deleterious consequences, mechanisms have evolved that are able to protect, repair, and tolerate DNA damaged by UV. Photoprotective measures include polyploidy, a genome rich with guanine and cytosine, and pigmentation. Once the DNA is damaged by UV, the organism may perform one or more repair mechanisms including photoreactivation, nucleotide excision repair, base excision repair, or homologous recombination. An organism may otherwise tolerate the damage by inducing a coordinated cellular response that might include the upregulation of repair and recombination genes and/or lesion bypass DNA polymerases, which can give rise to UV-induced mutations in the DNA.

Archaea are a distinct evolutionary domain from the more familiar domains of Bacteria and Eukarya. Halophilic archaea proliferate in highly salt concentrated environments well above 1.0 M NaCl (1), possess adaptations like macromolecules with highly acidic surfaces to stabilize their intracellular environment (2), and thrive in shallow waters that are typically exposed to high levels of solar UV radiation. The halophilic archaea are considered among the most highly UV resistant organisms ever studied (3-7). In fact, astrobiologists are highly interested in the halophilic archaea because they see these as model organisms for considering life on other planets, such as Mars, where atmospheres are thin or absent, water is scarce, and radiation levels are high.

The intensity of UV changes as a function of altitude. At a higher altitude, the column ozone is thinner and the intensity of solar UV is stronger than at sea level (8). Halophilic archaea

residing at high altitudes, for example at 11,955ft in the Salar de Uyuni salt flats in the Andes Mountains, may have adapted to the stronger intensity of UV. My initial hypothesis for this thesis project was that halophilic archaea thriving at a higher altitude would be more resistant to UV than closely related halophilic archaea isolated at a lower altitude. The hypothesis was tested with UV sensitivity assays comparing recent isolates from the high altitude Salar de Uyuni salt flats in Bolivia with the sea level halophiles *Halobacterium NRC-1* and *Haloferax volcanii*. I cultivated these halophiles in light and dark conditions to observe the impact of DNA repair mechanisms, such as photoreactivation and nucleotide excision repair, on cell survival. While no correlation between UV resistance and altitude in these new isolates was observed, the diversity of phenotypes prompted further comparison to a number of other haloarchaeal species. With further investigation, I found a diversity of UV resistance in halophilic archaea, which defies the literature since halophilic archaea are often described as "highly or remarkably UV resistant".

My thesis consists of two portions: First, I carefully evaluate the evidence in the literature that has led many to generalize that all species of halophilic archaea are highly UV resistant. Second, I present my findings from a collaborative project with Professor David Crowley and Dr. Shiladitya DasSarma from University of Maryland Baltimore County Medical School that directly compares the UV resistance of isolates of halophilic archaea from highly saline environments at varying altitudes: the Andes Mountain, the Dead Sea, the Deep Lake in Antarctica, the Great Salt Lake and the San Francisco Salt Farm. The results of my thesis project allow us to better understand the diversity of UV responses in different species of halophilic archaea. We also gain insight on the mechanisms halophiles employ to protect and repair themselves from UV light. The project contributes information on UV resistance to the field of astrobiology, specifically to scientists researching the possibility of lifeforms in recently

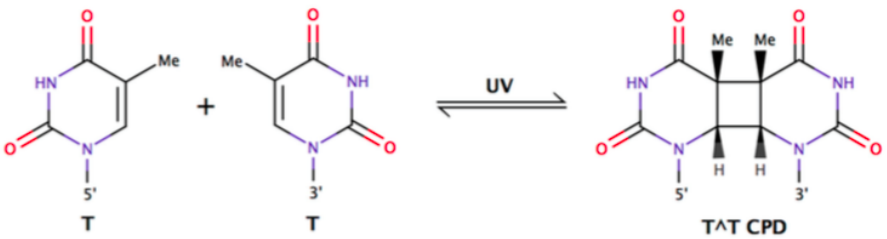
discovered liquid salt water on Mars (9). Future experiments can investigate the possible explanations for why certain halophilic archaea are more UV resistant than others.

II. Introduction

A. UV Damage & its Consequences

The three types of solar UV radiation are UV-A, UV-B, and UV-C. UV-A travels at a wavelength of 320-400 nm, UV-B at 290-320 nm, and UV-C at 190-290 nm (10). UV-A and UV-B can penetrate the Earth's atmosphere and reach the surface while UV-C, which is classified as germicidal, is the most damaging but is filtered out by the earth's atmosphere (10).

UV radiation can negatively impact the cellular DNA of an organism and can ultimately result in cancer or cell death. UV light, particularly at wavelengths at or below 320 nm, damages the DNA by changing the DNA structure in two major ways: either a cyclobutane pyrimidine

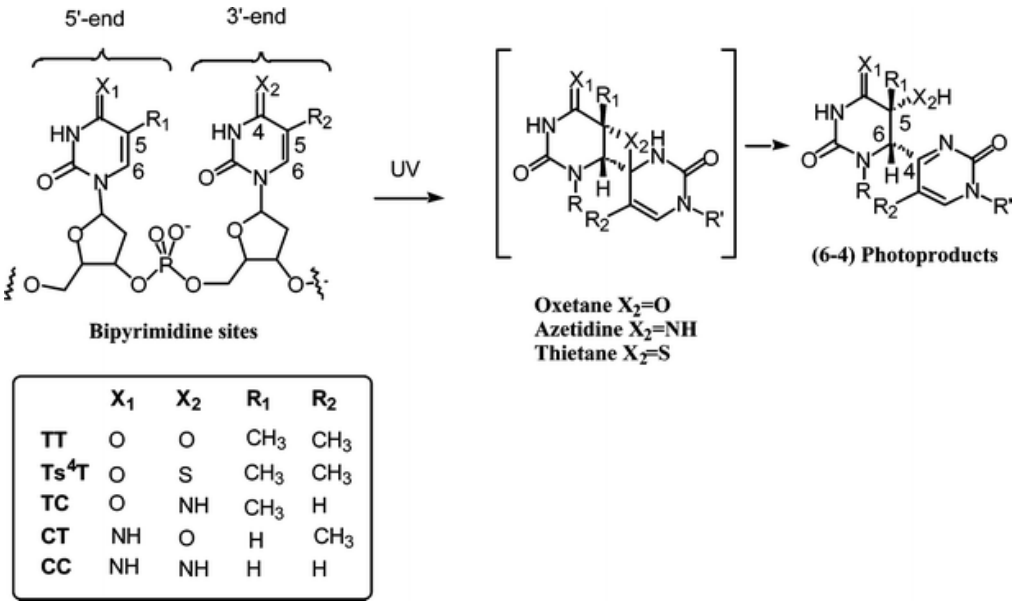


dimerization (CPD, Figure 1) or the formation of pyrimidine (6-4) pyrimidone photoproducts

Figure 1 Illustration of cyclobutane pyrimidine dimerization between adjacent thymine bases (6).

(6-4PP, Figure 2). A CPD results in the cyclization of two adjacent nitrogenous bases between either cytosine and/or thymine bases. 6-4PP formation is when two adjacent pyrimidine bases become covalently bonded between the sixth carbon of one pyrimidine and the fourth carbon of the other pyrimidine (11). Other lesions are also formed but are generally less prevalent (6, 10, 12). CPDs and 6-4PPs prevent DNA and RNA polymerases from completing a successful replication of DNA or transcription of an mRNA strand, respectively. The blockage of these

enzymes can result in mutations and disrupt genomic replication and expression of genes. The disruption of these enzymes may lead to cell death, or in multicellular organisms, aging and cancer.



	X_1	X_2	R_1	R_2
TT	O	O	CH_3	CH_3
Ts^4T	O	S	CH_3	CH_3
TC	O	NH	CH_3	H
CT	NH	O	H	CH_3
CC	NH	NH	H	H

Figure 2 Illustration of pyrimidine (6-4) pyrimidone photoproduct formation (13).

Mutagenesis, or the production of mutations, may result from an endogenous or an exogenous chemical or physical agent that damages the DNA or from spontaneous errors during DNA replication. Endogenous agents include water for hydrolysis which can lead to depurination or depyrimidination of the base, and di-oxygen molecules with free radicals for oxidation which can lead to ring saturated pyrimidines such as thymine glycol or cytosine hydrates (11). UV light is an example of an exogenous agent that can give rise to point mutations in the DNA as the polymerase attempts to pass by the lesions created in the DNA. DNA point mutations include a substitution of a base for another or the addition or deletion of a base that frameshifts the reading of the DNA strand. These point mutations may also arise from replication errors with failure of the DNA polymerase to distinguish a right from wrong nucleotide (11). A point mutation of a base pair can result in a different codon that can encode for the same amino

acid, different amino acid, or a stop codon for termination of amino acid synthesis. The function and structure of a protein is dependent upon the amino acid sequence and if the change in the sequence has a negative impact, then the protein cannot perform its necessary function for cell proliferation. The replication of a mutated strand will lead to a truly heritable change and this can signal cell death or uncontrolled cell division, known as cancer, over generations. These consequences of DNA damage make it imperative that the damage is prevented, repaired, and/or tolerated.

B. UV Protection

In order to mitigate the damage caused by UV, organisms have photoprotective measures that serve as the first line of defense. Photoprotective measures include possible light absorbing "sunscreens" like pigmentation, a high content level of guanine and cytosine in the genome, and polyploidy. Pigments absorb light and also UV-induced free radicals and can therefore prevent damage to DNA. Examples of such UV-protective pigments include melanin and carotenoids. Both melanin and carotenoids are capable of absorbing UV-B and UV-C radiation at 290-320nm and 190-290nm, respectively, and photoprotect DNA (14, 15). Melanin is synthesized by some bacteria, fungi, as well as eukaryotes, including humans. The pigment melanin gives a dark pigmentation, is synthesized by a tyrosinase and functions as an energy transducer to maintain cell integrity (15). Carotenoids are long, conjugated hydrocarbon chains with an oxygen-containing functional group, methyl groups, and alicyclic ring end groups (7, 16). The conjugated carbon-carbon backbone absorbs in the visible light spectrum (380nm-740nm) and confers a range of colors in the red-yellow region (16). Carotenoids protect against low wavelength ionizing radiation and serve as DNA repair agents for radiation damaged cells (16).

Carotenoids give cells a red-yellow pigmentation and are found in various eukaryotes, bacteria, and archaea.

Polyploidy is the state of having more than one copy of the genome. Most prokaryotes are often thought of as being monoploid, however recent evidence indicates that many prokaryotes are oligoploid (up to 9 genome copies) or polyploid (10 or more genome copies) (17). Polyploidy is advantageous because there is a decreased chance of losing function in a gene and a low mutation rate. A nongenetic advantage of polyploidy includes using genomic DNA as a phosphate storage polymer by using the phosphate in DNA for production of other phosphate-containing biomolecules, such as ATP and $NADP^+$(17). One advantage of polyploidy is gene redundancy, or the presence of many copies of a gene. With gene redundancy, mutations of some genes copies are tolerable due to the presence of information from the remaining wild-type copies (18, 19). Polyploidy also confers a high resistance against DNA double-strand breaks and resistance to desiccation and radiation (17). Presumably, these polyploid organisms are able to correct damaged sites of DNA quickly and efficiently with a nondamaged DNA strand through homologous recombination (19).

C. UV Repair

Organisms have innate mechanisms to repair DNA damaged by UV light and other physical and chemical agents. One repair mechanism that is dependent on the presence of light and is performed by most organisms (with the exception of placental mammals) is photoreactivation. CPDs and 6-4PPs can both be repaired with their respective photolyase enzymes which perform a relatively similar mechanism. The process begins with the photolyase binding to lesion sites followed by the the absorption of UV-A photons (320-400nm) and/or blue light photons (400-500nm) to activate photolyase and monomerize the pyrimidine dimers (20).

The photolyase enzymes are structurally a single polypeptide and catalyze the reaction of DNA repair in a single-step process. The pyrimidine dimer-DNA photolyase for CPD has two classes of photolyases that repair with a lesion of the CPDs. However the two classes of this photolyase are not present within all organisms; investigations of more genes related to this photoreactivation mechanism has raised questions about the evolutionary significance of the two classes (11). The pyrimidine dimer-DNA photolyases have two noncovalently bonded chromophores that absorb light, flavin adenine dinucleotide (FAD) and either a 5,10-methenyltetrahydrofolylpolyglutamate (MTHF) or 8-hydroxy-5-dezaflavin (8-HDF) chromophore compound (11, 20). The 6-4PP photolyase converts the 6-4PP into an oxetane intermediate and transfers electrons to the intermediate in order to revert the photoproduct to its native DNA conformation. The 6-4PP photolyase enzyme similarly has chromophore FAD and investigative studies are pursuing the possibility of a second chromophore that has a MTHF-like structure (11).

Organisms can also repair UV-damaged DNA with a different mechanism known as nucleotide excision repair. Nucleotide excision repair is not dependent on the presence of light and is a process in which the damaged nucleotide site, such as CPD and 6-4PP, is recognized and cleaved by multiple enzymes. An exemplary model of this repair mechanism is in bacteria which have four genes, *uvrA, uvrB, uvrC,* and *uvrD,* that encode proteins UvrA, UvrB, UvrC, and UvrD, respectively, for the repair process (21). In bacteria, the UvrA and UvrB proteins recognize the site of damage. The UvrB and UvrC proteins act as excinucleases to cleave the DNA around the lesion. Lastly, the UvrD protein, also known as DNA Helicase II, works with DNA polymerase I to assist in removing the damaged stretch of nucleotides, and DNA polymerase I synthesizes new DNA to replace the damaged site (21). The final step in the

nucleotide excision repair process is recruitment of a DNA ligase enzyme to ligate the new piece together with the parent strand. The repaired DNA then allows for DNA and RNA polymerases to continue their function, produce their respective, unaffected strands, and avoid mutation and its deleterious consequences. Analogous NER mechanisms have been well characterized in eukaryotic systems and employ *RAD/XP* genes (21). In eukaryotic organisms, such as yeast, a RAD1/RAD10 complex incises the DNA photoproduct on the 5' end and RAD2 incises on the 3' end. RAD3 and RAD25 function as DNA helicases in the opposite directions to unwind the DNA (21). In addition, some archaea exhibit both the bacterial UvrABCD system and homolog genes to eukaryotic mechanisms (7).

Homologous recombination is another mechanism employed by cells to repair UV-damaged DNA. It is the process in which a homologous DNA strand is used as a template to repair the damaged DNA strand. Homologous recombination is initiated by the recognition of double-stranded DNA breaks. The ends of the damaged site are excised which creates a recognition site for recombinase binding. Once the recombinase binds, homologous strands are paired and the homologous strand is used as a template to correct the damaged site (7). RecA is a recombinase that brings the homologous strands together and coordinates the exchange of information. Eukaryotes have homologs Rad51 to the RecA in bacteria and some archaea also have some of these homologs. Archaeal RadA are similar in function to RecA/Rad51 for recombinational repair (7).

D. Post-UV Responses

Organisms may tolerate UV-damaged DNA by upregulating repair and recombination genes and/or have DNA polymerase bypass photoproducts during replication. The bypassing of

photoproducts gives rise to UV-induced mutations and results in mutated genes that were essential for survival.

In *E. coli*, DNA repair and mutagenesis mechanisms are regulated by an SOS response which involves the upregulation of genes that encode proteins involved with the DNA repair mechanisms, among other pathways. In bacteria, the *uvrA, uvrB, and uvrD* genes of the nucleotide excision repair mechanism are regulated as part of the SOS response. When the DNA is not damaged, the SOS box operator is repressed by the LexA protein that is encoded by the $lexA^+$ gene (11). LexA represses the *uvr* genes by preventing RNA polymerase from binding to the promoters of these genes with much efficiency. When the DNA is damaged, a response is initiated and the response first begins with recognition of a damaged DNA site that signals for recruitment of the RecA protein encoded by the $recA^+$ gene (11). RecA inactivates the LexA repressor, inhibiting the repressor from binding to the SOS box and allowing the RNA polymerase to transcribe mRNA strands of the *uvr* genes more extensively. The transcribed strands can then be translated for their respective proteins and promote more efficient repair of the damaged DNA throughout the cell. Once the damaged site is repaired, there is a drop in the level of signaling for RecA and as LexA accumulates, it binds to the SOS Box and again represses upregulation of the genes (11).

In eukaryotic organisms, UV radiation can induce activation of stress proteins. UV-C and UV-B stimulate signal transduction pathways that result in activation of transcription factors jun and fos that constitute the AP-1 transcription factor, which regulates cell differentiation, proliferation, and apoptosis. UV-B may increase levels of ornithine decarboxylase, different cytokines, the p53 tumor suppressor protein and other nuclear oncogene products. UV-A may

upregulate genes such as collagenase, heme oxygenase 1, a protein phosphatase and phospholipase (22).

E. Halophilic Archaea

Archaea are prokaryotic organisms classified into a separate domain of life based on their evolutionarily distinct characteristics relative to the other two domains of Bacteria and Eukarya. As prokaryotic organisms, Archaea and Bacteria do not have membrane bound organelles such as mitochondria or a nucleus (1). Similar biological mechanisms between Bacteria and Archaea include energy transformation, metabolism, transport, nitrogen fixation, binary fission, and CRISPR-*cas* systems (23). A comparison of the lipid bilayer composition can distinguish a prokaryotic archaeal cell from a bacterial cell. Bacteria and Eukarya share a lipid bilayer composed of ester linked phospholipids with two unbranched fatty acid chains. In contrast, the lipid bilayer of Archaea is composed of an ether linked phospholipid with two branched isoprene chains (23). Although they feature distinct cell plans, Archaea and Eukarya share extensive similarities at the molecular level, including proteins involved in DNA replication, transcription, translation, and repair (23). Ultimately, the Archaea contain many genes and other features that are unique to this domain and which remain relatively understudied.

Archaea are commonly classified as either thermophiles, methanogens, or halophiles. Thermophiles reside in high temperature environments, methanogens reside in anaerobic conditions, and halophiles reside in highly salt concentrated environments. Halophilic archaea evolved to perform biology at hypersaline concentrations, importing K^+ into the cell to maintain intracellular K^+ concentrations that are isotonic with the high salt concentrated external environment (24). The intracellular proteins are not denatured by the high salt concentrations due in part to the highly acidic surface that stabilizes intracellular proteins (2). The acidic surfaces of

halophilic macromolecules allow the protein to interact with limited water in the cytoplasm (2). Examples of environments in which halophilic archaea thrive are in saltern crystallizer ponds and salt flats such as the San Francisco Salt Ponds, the Dead Sea, Solar Lake in Egypt, Vestfold Hills lake system of Eastern Antarctica, and Salar de Uyuni in Bolivia (25). The conditions of these environments differ and genetically different species of halophilic archaea have unquestionably adapted to live in each of these environments. In these environments, halophilic archaea are exposed to UV radiation and have evolved to thrive under these damaging conditions.

F. UV Protection in Halophilic Archaea

Halophilic archaea may have protective measures against DNA damage imposed by UV radiation. One protective measure is pigmentation. Carotenoid pigments are a characteristic of many halophilic archaea that give them their characteristic red-orange or pink color. Two major carotenoids are present in halophilic archaea: bacterioruberin and β-carotene (7; Figure 3). β-carotene is a precursor to retinal, a chromophore in bacteriorhodopsin, a protein complex located within some haloarchaeal membranes that uses light energy and drive a proton pump for ATP synthesis. It is has also been suggested that bacteriorhodopsin may also transfer some light energy to activate photoreactivation in haloarchaea (12).

The major carotenoid pigment in the haloarchaea, bacterioruberin, scavenges hydroxyl radicals and serves a role in resistance against oxidative DNA-damaging agents, including UV-A, due to its high number of conjugated double bonds (26). A study of bacterioruberin-deficient mutants of *Halobacterium salinarum* found them to be more sensitive to ionizing radiation, hydrogen peroxide, and UV light, than wild type cells (26), however others have not confirmed UV sensitivity (Crowley, unpublished observations; 27, 28). It remains unclear if these pigments

serve as a type of "sunscreen" for haloarchaea, protecting DNA from CPDs and/or 6-4 PPs induced by solar UV-B radiation. Carotenoid pigments absorb light in the range of 340-550 nm (29), so carotenoids do not offer complete protection from solar UV which ranges from 290-400 nm. Studies seem to suggest a role for carotenoids in UV-resistance, but it remains unclear to what extent pigmentation protects DNA from UV light.

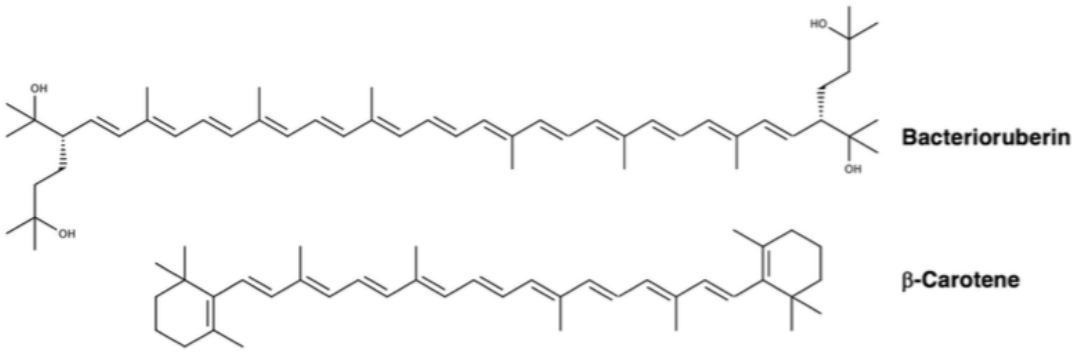


Figure 3 Carotenoid pigment structures of bacterioruberin and β-carotene (7)

Another proposed photoprotective measure in halophilic archaea is the high content of guanine and cytosine bases present in the genome. CPDs and 6-4PPs form between either two cytosines, two thymines, or a thymine and cytosine base on the same strand of DNA (6). Guanine and cytosine bases compose about 60% of the genomic DNA of most halophilic archaea. The higher content of guanine and cytosine reduces the presence of thymine bases and results in a reduction of dipyrimidine sites that include thymine, particularly longer stretches of pyrimidines that can be sites of DNA damage (6). The investigative study did not find evidence to suggest that UV exposure served a selective pressure for a photoprotective bipyrimidine signature (6). A possible explanation to the evolution of a high guanine and cytosine content in hypersaline environments may be due to the number of hydrogen bonds present. The presence of three hydrogen bonds in a guanine-cytosine base pair, in contrast to two hydrogen bonds in an adenine-thymine base pair, packs the DNA more tightly and increases DNA stability (30).

Polyploidy may also contribute as a photoprotective measure against UV in halophilic archaea. A monoploid halophilic archaea species has still not been found, therefore, polyploidy appears characteristic of all halophilic archaea (19). Halophilic archaea have approximately 15-25 copies of the genome per cell during exponential growth, however at a stationary phase, the number approximates 12-15 copies per cell (31). Evolutionary advantages of polyploidy in halophilic archaea includes low mutation rate, radiation/desiccation resistance, gene redundancy, and survival under extreme conditions (32). Halophilic archaea may be able to use their genome as a means to repair damaged areas of DNA. The presence of multiple copies of a gene reduces the chance that a function of a gene would be lost to UV damage. Studies had identified a slow rate of genome repair of CPDs in halophilic archaea, which may be due to the large amount of DNA that must be scanned and repaired by repair proteins (33). There remains no clear evidence of an direct advantageous relationship between polyploidy and UV resistance.

Another proposed mechanism of photoprotection is movement with gas vesicles or flagella. Gas vesicles are characteristic of many halophilic archaea, however not all. These proteinaceous gas-filled vesicles vary in shape and size among halophilic archaea (34). The structure of gas vesicles in halophilic archaea are largely composed of a hydrophobic protein, GvpA (34). The expression levels of gas vesicles vary at different growth phases. For example, *Halobacterium salinarum* synthesize spindle-shaped gas vesicles throughout its growth, whereas *Haloferax mediterranei* synthesize cylindrical-shaped gas vesicles only in its stationary phase (34). If exposed to UV, halophilic archaea can sense the intense UV levels and begin to move away towards a lower exposure area with gas vesicles (7).

G. UV Repair in Halophilic Archaea

Solar UV radiation can damage the DNA of halophilic archaea through the formation of CPDs and 6-4PPs. Genes which encode for repair mechanisms are different between organisms in the eukaryotic and prokaryotic domains. In order to repair the damaged DNA and prevent continued blockage of DNA and RNA polymerase enzymes, halophilic archaea perform both photoreactivation, nucleotide excision repair, and base excision repair mechanisms.

Photoreactivation is a light-dependent DNA repair process to cleave CPD and 6-4PP lesions with photolyase. The activity of photolyase is dependent on the excited state of its FAD cofactor. The genes *phr1* and *phr2* in halophilic archaea have been identified to possibly be the genes involved in photoreactivation. Experimental studies on *phr2* found the gene to be directly responsible for the transcription of the photolyase enzyme. The possibility of *phr1* coding for a second functional photolyase enzyme remains to be determined (21). Investigative studies on *phr1* have identified that the gene does not directly encode photolyase but possibly a cryptochrome; a photolyase paralog that can control circadian rhythms in response to blue light (35). However, further studies must be conducted to elucidate the responsibility of the *phr1* gene.

Halophilic archaea perform nucleotide excision repair and have been identified to have functional homologs of the bacterial genes, *uvrA, uvrB, uvrC,* and *uvrD* (36). Archaea with mutated homologs of *uvrA, uvrB,* and *uvrC* genes become hypersensitive to UV and fail to repair any UV photoproducts in the absence of visible light (36). Many Archaea, including halophiles, have been found to have homologs of the genes *RAD3* and *RAD25* from yeast, which encode DNA helicases for eukaryotic nucleotide excision repair (21). Another nucleotide excision repair homolog of a eukaryotic gene that all archaea have is *rad2/FEN-1* which codes for a flap endonuclease (21). In addition, some halophilic archaea have homologs of the gene *xpf* from the

XP system in mammals, which encode a DNA endonuclease that cleaves at a 5' site of damage (7).

Recent work from the Crowley lab has found that model species of halophilic archaea can perform transcription-coupled repair, a subpathway of nucleotide excision repair, as a mechanism to repair DNA damaged by UV (33). In this process, which has been extensively characterized in bacterial and eukaryotic systems, RNA polymerase arrests upon recognition of a DNA lesion site on the template strand and initiates nucleotide excision repair to clear the offending lesion. The RNA polymerase is removed and transcription-repair coupling factors recruit nucleotide excision repair proteins to address the damaged DNA site. The process of transcription-coupled repair allows for the organism to ensure essential proteins are being constructed after cellular damage.

Base excision repair involves DNA glycosylases that cleave damaged DNA sites from UV-induced oxidative damage. Photooxidative damage results from absorption of UV by endogenous photosensitizers, such as porphyrins and flavins, which then become activated and cause downstream effects of single strand DNA breaks and base modifications (7). The glycosylases cleave the *N*-glycosidic bond between a base and the deoxyribose ring. The DNA sugar-phosphate backbone is then cleaved by an endonuclease, the sugar is removed, and the complementary strand serves as a template for repair (7). Examples of glycosylase genes across many halophilic archaea, but not all, includes *mutY*, an adenine/guanine specific adenine glycosylase, *alkA*, an alklyadenine glycosylase, and *nth*, an endonuclease III (37).

Homologous recombination is another DNA repair mechanism from UV-induced damage. Following recognition of the damage site and excision of the damaged ends, recombinase binds, and strands pair and exchange information to correct the damaged site. RecA

is a recombinase that joins homologous strands and facilitates stand exchange. RadA protein functions similarly to RecA/Rad51 in recombinational repair. Some halophilic archaea have homologs of Mre11, a homologous recombination nuclease in yeast, and Rad50, a homologous recombination ATPase (7). When *Halobacterium salinarum* cells were exposed to UV-B and UV-C light, the *radA* gene was highly induced (3, 35, 38). Furthermore, *Haloferax volcanii radA* mutants were sensitive to UV upon exposure (39). These findings suggest that *radA* plays an important role in the response to UV damage and that homologous recombination may be UV inducible.

H. Post-UV Response in Halophilic Archaea

Halophilic archaea may tolerate UV-damaged DNA by upregulating repair and recombination genes and/or have DNA polymerase bypass photoproducts during replication. The bypassing of photoproducts confers mutations and results in mutated genes.

After irradiation with UV-C, cell cultures either exposed or not exposed to photoreactivation conditions expressed an upregulation of transcription regulatory genes (35). A downregulation of genes for metabolism was observed in post-UV irradiation responses in *Halobacterium NRC-1*, which has also been observed in other domains of life and suggests this as a possible stress-response mechanism. A downregulation of genes may then allow cells to conserve energy for DNA repair. Furthermore, the genes that encode gas vesicles are also downregulated so cells may not rise to the surface and not be exposed to UV (35).

The presence of various homologs suggests the possibility of other repair mechanisms in halophilic archaea besides the “bacterial” uvrABC system. Studies are investigating these genes and others for repair mechanisms in halophilic archaea that do not share bacterial or eukaryotic homologs. As previously mentioned, the SOS response regulates the upregulation of the genes

that transcribe proteins for the DNA repair mechanism. For example, halophilic archaea live in high salt concentrated environments under a constant exposure to UV light. Do halophilic archaea have an SOS response pathway to regulate these repair mechanisms or due to the constant exposure to UV, transcribe these genes at a consistently high rate? Or, is it possible that halophilic archaea perform other repair mechanisms in order to account for the constant UV exposure and regulate their repair mechanisms in a response system different from SOS? It would be interesting to identify how halophilic archaea have evolutionarily adapted to regulate their repair mechanisms and if there is a consistent upregulation, or constitutive expression, of the genes due to the constant exposure to UV light.

III. Thesis Narrative - Illuminating the Connection

The general consensus in the literature is that members of the halophilic archaea are highly UV resistant (3-7). For example, McCready and colleagues identified that the species *Halobacterium NRC-1* is highly UV resistant, especially as compared to human fibroblasts, and other model organisms like the bacterium *Escherichia coli*, and the budding yeast *Saccharomyces cerevisiae.* (3, Figure 4).

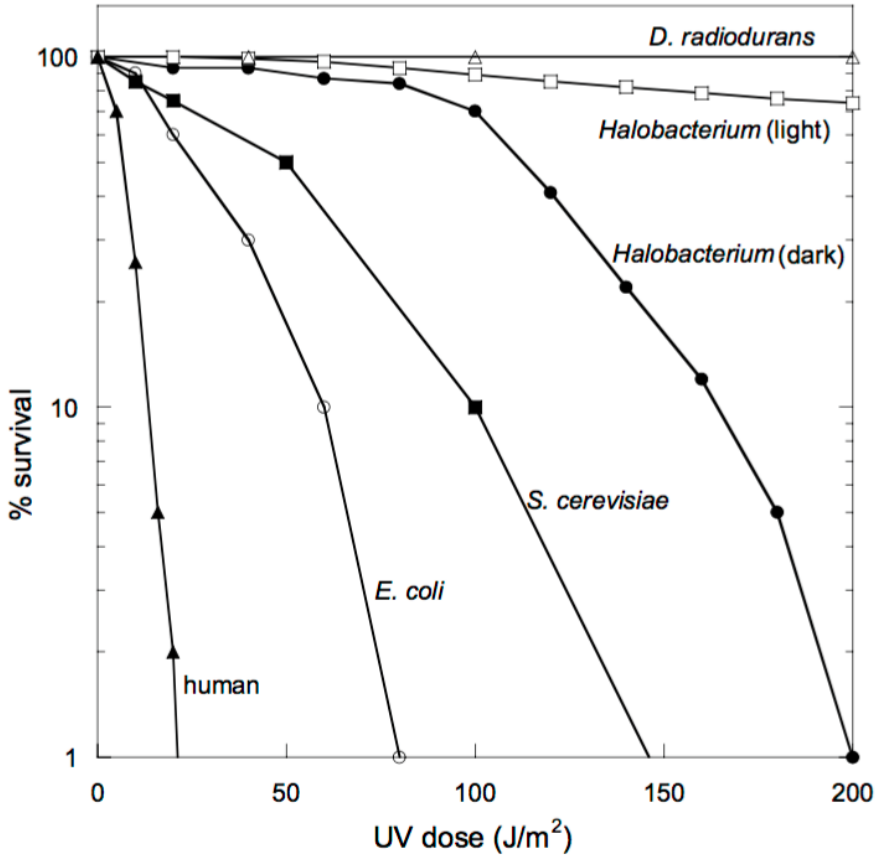


Figure 4 Survival curves of model organisms following UV-C radiation (3).

The experiment irradiated cell cultures with varying dosages of UV-C light and observed the effects on the organism three hours post-radiation. The most UV resistant organism observed was *Deinococcus radiodurans*, a renowned radiation resistant bacteria that was not affected by these doses of UV. *Halobacterium NRC-1* was also highly resistant to UV, nearly matching the resistance of *D. radiodurans*. *Halobacterium NRC-1* that were incubated after UV exposure

under visible light exhibited a higher percent survival than the *Halobacterium NRC-1* that were under dark conditions. The higher percent survival in the light conditions is the result of repair by photoreactivation. Other research studies have confirmed that *Halobacterium NRC-1* is highly UV resistant and have attempted to better understand the molecular basis for this remarkable resistance (36). While there is little question that *Halobacterium* and other species of halophilic archaea are among the most UV resistant organisms ever studied, this thesis work clearly demonstrates that not all halophilic archaea are UV resistant.

The intensity of UV light radiated from the sun varies depending on the altitude. At higher altitudes, solar UV radiation is intensified due to a thinner ozone layer in comparison to the ozone layer at sea level (8). Halophilic archaea may have evolved to withstand a stronger UV radiation if living at altitudes high above sea level, such as at 11,955ft in the Salar de Uyuni salt flats. I hypothesized that halophilic archaea at a high altitude were more UV resistant in comparison to halophilic archaea living at an altitude near sea level. The thesis project began with an investigation of five strains of halophilic archaea from the Salar de Uyuni salt flats in the Andes Mountain of Bolivia, a strain of *Halobacterium NRC-1* from the San Francisco Bay Salt Ponds, and a strain of *Haloferax volcanii* originally isolated from the Dead Sea. The five strains from Salar de Uyuni in Bolivia were characterized from ribosomal RNA sequencing to be *Natrinema 6-1, Natrinema 5-4, Haloterrigena 5-1, Halorubrum 3-1,* and *Halorubrum 4-1*. After characterizing growth conditions for these strains of halophilic archaea, I determined the relative UV resistance and observed no correlation between altitude and UV resistance and noted a diversity in resistance to UV. We then began to grow interested in the relative resistance of other strains from different environments and wanted to observe if there is an even greater diversity of UV resistance.

Our collaborator, Dr. Shiladitya DasSarma, PhD, from the University of Maryland Baltimore County Medical School gave me the opportunity to continue the investigation by providing more strains. Among these strains were *Halobacterium R-1, Halobacterium salinarum, Halobacterium GSL-19, Halorubrum sacchaorvorum*, and *Halorubrum lacusprofundi*. This work describes the diversity of UV resistance in the halophilic archaea that I have observed, which challenges the generalizations in the literature that halophilic archaea are all "highly or remarkably UV resistant"(3-7).

A typical reference used to support the above generalization is a classic paper from Dundas and Larsen in 1963 (40). They questioned whether the carotenoid pigments of *Halobacterium salinarum* serve as a protective agent against the "killing" (40) of these cells by "light". The paper builds on their 1962 publication (41), where they questioned the function of carotenoid pigments and if carotenoids protect against detrimental effects of light. It was not mentioned nor stated in the 1962 and 1963 works that halophilic archaea were highly UV resistant, but it was mentioned that cultures were exposed to light of high intensity, including sunlight and from tungsten filaments. UV-A, UV-B, and UV-C are present in sunlight. However, UV-C is absorbed by the atmosphere, UV-B would not penetrate through the glass culture vessels used in this study, and UV-A does not induce photoproducts in the DNA. Therefore, UV was not tested in these experiments nor was it claimed to be and the Dundas and Larsen publications should not be cited as support for the statement that halophilic archaea are highly UV resistant.

Works that follow the publications of Dundas and Larsen include Hescox and Carlberg in 1972, who questioned if *Halobacterium cutirubrum* photoreactivates upon UV-irradiation and if carotenoid pigments serve a role in protection from UV. While Dundas and Larsen may have

observed difficulty of growth in pigmentless strains, Sharma and Fitt as well as Hescox and Carlberg observed no increased UV sensitivity due to absence of pigment (27, 28, 40, 41). In all cases, these researchers focused all of their attention on the extremely UV resistant *Halobacterium* genus, and certainly do not provide ample evidence to support the generalization that "halophilic archaea are UV resistant".

A careful comparative study for the basis of the classification of halophilic archaea as highly UV resistant has not been performed. Halophilic archaea from the genera of *Halobacterium* and *Haloferax* are often studied since these strains grow well in the laboratory. *Halobacterium* is studied for its high resistance to UV (3, 10, 21, 36), whereas *Haloferax* is studied because of its fast growth and genetic malleability. In our lab, *Haloferax* is consistently found to be much more sensitive to UV in comparison to *Halobacterium* (unpublished observations) and we have struggled to understand why these unsupported generalizations about UV resistance persist in the literature. Other genera that I worked with in the laboratory, *Halorubrum, Haloterrigena,* and *Natrinema* are not often studied and there is little information available about their UV responses. My thesis work demonstrates a great diversity in UV resistance in the halophilic archaea, challenging the general classification of the halophilic archaea as highly UV resistant.

IV. Methods

Cell Culture

Halophilic archaea strains were inoculated in tubes with CM+ Media (250g NaCl, 20g $MgSO_4$, 2.0g KCl, 3.0g Na-citrate, 2.3mg $FeCl_2$, 440ug $ZnSO_4$, 330ug $MnSO_4$, 10ug $CuSO_4$, 5g tryptone, 3g yeast extract, and 1g casein amino acids (for 1L)) or YPC Media (8.5g Yeast Extract (Difco), 1.7g Peptone (Oxoid), 1.7g Casamino Acids, pH 8.0 with 1M KOH (for 1L)). Subcultures of strains were inoculated in side-arm flasks with a 1:1000 ratio of cells to CM or YPC Media. Growth curves were monitored with a Klett-Summerson Photoelectric Colorimeter (units of Klett).

Table 1. Halophilic Archaea Strains

Strain of Halophilic Archaea	Origin	Provided by
Halobacterium NRC-1	San Francisco Salt Farm, California	Shiladitya DasSarma
Halobacterium GSL-19	Great Salt Lake, U.S.A.	Shiladitya DasSarma
Halobacterium R-1	*	Shiladitya DasSarma
Halobacterium salinarum	*	Shiladitya DasSarma
Halorubrum 4-1	Salar de Uyuni, Bolivia	Daniel Guzman via Shiladitya DasSarma
Halorubrum 3-1	Salar de Uyuni, Bolivia	Daniel Guzman via Shiladitya DasSarma
Halorubrum saccharovorum	San Francisco Salt Farm, California*	Shiladitya DasSarma
Halorubrum lacusprofundi	Deep Lake, Antarctica	Shiladitya DasSarma
Haloferax volcanii (H26)	Dead Sea, Israel	T. Allers, U. Nottingham
Haloterrigena 5-1	Salar de Uyuni, Bolivia	Daniel Guzman via Shiladitya DasSarma
Natrinema 5-4	Salar de Uyuni, Bolivia	Daniel Guzman via Shiladitya DasSarma
Natrinema 6-1	Salar de Uyuni, Bolivia	Daniel Guzman via Shiladitya DasSarma
Haloferax volcanii RFP189	In-frame deletion of *crtB* (gene that encodes the phytoene synthase enzyme)	Ronald F. Peck, Colby College (derived from H26 from T. Allers)

R-1 is a gas-vacuole minus mutant of wild type NRC-1; origin is unknown
Highlighted strains indicate availability of genome sequence.

UV-C Survival Assays

Log phase cultures were diluted 1:100 in 2 mls of CM Salts (250g NaCl, 20g $MgSO_4$, 2.0g KCl, 3.0g Na-citrate, 2.3mg $FeCl_2$, 440ug $ZnSO_4$, 330ug $MnSO_4$, and 10ug $CuSO_4$ (per 1L)) or YPC Salts (1M Tris HCl pH 7.5, 480g NaCl, 660g $MgCl_2$, 70g $MgSO_4$, 14g KCl (per

1L)) and placed in 5 cm glass petri dishes to a depth of ~1 mm and irradiated with 254nm UV light to the doses indicated. Ten-fold serial dilutions were performed in CM Salts or YPC Salts and 20 microliter spots were pipetted in duplicate on CM+ or YPC plates. One unwrapped and one foil wrapped plate were exposed to two hours of fluorescent light (Philips F32T8 Daylight). All plates were then wrapped in aluminum foil and incubated at 40°C for 5-14 days before counting survivors.

Genomic DNA Damage Assay

One ml of a log phase culture of either *Halobacterium sp.* NRC-1, *Haloferax volcanii*, or *Natrinema 6-1* was placed in a 5 ml glass petri dish and was irradiated with UV-C for 10s. The cells were pelleted down at max speed, the media was aspirated, and the cells were lysed by the addition of dH_2O. The samples were then placed in a 70ºC heat block for inactivation of proteins for 10 minutes (42). Genomic DNA was diluted as appropriate and quantitated with a Thermo Scientific NanoDrop 2000 Spectrophotometer.

After the genomic DNA was prepped, an alkaline gel (0.75% agarose, 0.03N NaOH, 1 mM EDTA) and alkaline running buffer (0.03N NaOH, 1 mM EDTA) were prepared (43). The samples were treated with or without a *Chlorella* virus pyrimidine dimer glycosylase (gift of S. Lloyd, Oregon Health & Sciences University, (44)). The gel was run overnight at 35V, stained in neutralizing buffer (0.5M Tris base, 1.5 M NaCl, pH 7.8) with ethidium bromide, and imaged with Bio-Rad Molecular Imager® ChemiDoc™ XRS+ Imaging System with Image Lab™ Software. The intensity of the bands were measured and compared to one another.

V. Results

Strains of halophilic archaea from a variety of hypersaline habitats, including sea level (*Hfx. volcanii* and *Hbt. NRC-1*) and from the 3500m Salar de Uyuni (*Nnm. 5-4* and *6-1*; *Htg. 5-1*;

Hrr. 3-1 and *4-1*) were cultured to logarithmic phase and irradiated with 254nm UV-C at doses of 0, 24, 48, and 96 J/m^2 prior to exposure to or protection from a photoreativating light. The focus of the results is on 48 J/m^2 because it was difficult to assess the differences at 24 J/m^2 since we observed a high percent survival across almost all strains and at 96 J/m^2 since we observed a relatively high percent of cell death across almost all strains. At 48 J/m^2 we observed significant differences in percent survival and cell death between different genera of halophilic archaea (Figure 5). The bar graph depicts percent survival on a logarithmic scale and exposure to photoreactivating conditions in orange and absence from photoreactivating conditions in blue. The genera *Halobacterium* (abbv. *Hbt.*) was the most UV resistant, exhibiting over 50% survival in non-photoreactivating conditions, while all other halophiles tested showed at least 90% cell death in these conditions. *Halobacterium NRC-1*, with nearly 100% survival in both photoreactivating and non-photoreactivating conditions, was the most UV resistant organism in the study, consistent with previous findings (3, 10, 21, 36). *Haloterrigena* (abbv. *Htg.*) *5-1* was also highly resistant to UV at all doses tested, exhibiting more than 50% survival in photoreactivating and non-photoreactivating conditions (Figure 5).

Halorubrum (*Hrr.*) strains were also highly UV resistant, especially with exposure to photoreactivating conditions (Figure 5). In the absence of photoreactivating conditions, *Hrr. 3-1*, *Hrr. 4-1,* and *Hrr. saccharovorum* exhibited over one log of cell killing, consistently more sensitive than *Halobacterium* species under the same conditions. *Hrr. lacusprofundi* is a relatively UV sensitive strain of the *Hrr.* genera. *Hrr. lacusprofundi* exhibited only about 40% survival under photoreactivating conditions, however near 99% cell death in the absence of photoreactivating conditions after 48 J/m^2 UV.

Haloferax (abbv. *Hfx.*) *volcanii* was relatively UV sensitive with about 15% cell survival under photoreactivating conditions and further sensitivity to UV in the absence of photoreactivating conditions with about 99.5% cell death (Figures 5 & 6). An unpigmented *crtB* knockout strain of *Hfx. volcanii* (RFP189) showed no significant UV sensitivity compared to isogenic *crtB*+ cells under photoreactivating or non-photoreactivating conditions. Unlike the naturally unpigmented *Nnm.* 5-4, RFP189 showed a much higher UV resistance in the presence of photoreactivating light (Figure 6).

Natrinema (abbv. *Nnm.*) *5-4* is a nonpigmented strains that did not show significantly increased levels of survival under photoreactivating conditions. *Nnm 5-4* was also relatively sensitive to UV, exhibiting nearly 95% cell death in both photoreactivating and non-photoreactivating conditions (Figure 5).

Nnm. 6-1 was the most sensitive halophilic archaea strain that we tested. Under photoreactivating conditions, *Nnm. 6-1* exhibited only 0.5% cell survival and in the absence of photoreactivating conditions exhibited about 99.99% cell death. We hypothesized that perhaps this UV sensitivity was due to the absence of some sort of DNA protective mechanism. In order to test whether the high level of UV sensitivity in *Nnm. 6-1* was due to more damage being induced in the genome, a genomic DNA damage assay was devised using alkaline gel electrophoresis and a UV damage nicking enzyme, *Chlorella* pyrimidine dimer glycosylase (PDG) (Figure 7). Samples of DNA from *Hbt. NRC-1*, *Hfx. volcanii*, and *Nnm. 6-1* were prepared for this assay as outlined in the methods. The average percent of undamaged DNA after treatment with PDG for *Hbt. NRC-1* was 56.9% (SE ±10.9), for *Hfx. volcanii* was 71.9% (SE ±15.2), and for *Nnm. 6-1* was 57.2% (SE ±4.0) (Figure 7). There were no significant differences in the levels of UV damage in these genomes.

VI. Discussion

Halophilic archaea with differing phenotypic characteristics and isolated from an array of environments were assessed for their resistance to UV light (Table 1). Most, but not all, haloarchaea strains in the investigation performed photoreactivation, which was evident by higher survival when exposed to visible light after UV (compare orange survival levels to blue). *Hbt.* strains were highly UV resistant in both conditions, and most *Hrr.* strains were only slightly more sensitive than *Hbt.* strains in the dark (Figure 5). A greater sensitivity to UV was observed among the Dead Sea halophile *Hfx. volcanii*, the Antarctic isolate *Hrr. lacusprofundi,* and the Bolivian isolates *Nnm. 5-4 and Nnm. 6-1* (Figures 5). Notable strains that did not exhibit additional resistance when exposed to visible light after UV include Bolivian strains *Htg. 5-1* and *Nnm. 5-4*, both of which are nonpigmented (Figure 5). The data from the experiment suggests no correlation between high altitude and enhanced UV resistance and clearly demonstrates that a diversity of UV resistance exists between different species of halophilic archaea.

Hbt. NRC-1 was observed as the most UV resistant strain and exposure to photoreactivating conditions did not detectably enhance survival post-UV irradiation. *Hbt. NRC-1* has a rich GC content of 65.92% (6), is polyploid with 15-25 copies of its genome depending on its growth phase (31), has carotenoid pigments and gas vesicles (34) which serve as likely photoprotectors from UV damage. *Hbt. NRC-1* has been shown to be highly effective at performing light-independent DNA repair processes like nucleotide excision repair (36) and transcription-coupled repair (33). It may be that these species also possess supplementary mechanisms to repair DNA damaged by UV (35), including homologous recombination. Although a coordinated SOS response-like mechanism was not detected in *Hbt. NRC-1* (3, 35), the *radA* gene, responsible for initiating homologous recombination, is rapidly and significantly

upregulated post-UV (3, 35). Homologous recombination may contribute to UV resistance through recombinational repair with duplicate genome copies or a recombinational rescue of stalled replication forks (3). Future investigations of gene upregulation in *Hbt. NRC-1* post-UV will help to better our understanding why *Hbt. NRC-1* is notably the most UV resistant.

Similar to *Hbt. NRC-1, Htg. 5-1* was highly UV resistant with over 50% survival at 48J/m^2 in photoreactivating and non-photoreactivating conditions. Further investigation of the high level of UV resistance in *Htg.* 5-1 is merited not only for its high UV resistance but since photoreactivation was not detected. Along with *Hbt. GSL19*, which was isolated from the Great Salt Lake in Utah, *Htg.* 5-1 was the most resistant high altitude isolate we studied.

Halorubrum strains *Hrr. 3-1* and *Hrr. 4-1* exhibited near 100% survival and *Hrr. saccharovorum* exhibited 62% survival at 48J/m^2 under photoreactivating conditions. In the absence of photoreactivating conditions, *Hrr. 3-1* and *Hrr. 4-1, and Hrr. saccharovorum* exhibited over 90% of cell death. These *Halorubrum* strains are highly UV resistant under photoreactivating conditions but are slightly more sensitive to UV in the absence of photoreactivating light than *Hbt.* strains, perhaps suggesting a slightly less robust dark repair capability. Photoprotection mechanisms in these *Hrr.* strains from UV damage include carotenoid pigmentation which confers *Hrr.* their signature red color. Future works can investigate the possibility of other photoprotection mechanisms such as GC content and polyploidy in these strains. Photoreactivation appears to be a highly effective DNA repair mechanism for *Hrr.* so that damage may be corrected and the organism may continue to function properly. The sensitivity to UV in the absence of photoreactivating conditions may be due to the absence or low expression levels of genes responsible for nucleotide excision repair.

Unlike the other *Hrr.* strains, the Deep Lake Antarctic isolate *Hrr. lacusprofundi* exhibited only about 40% survival under photoreactivating conditions and near 99% cell death in the absence of photoreactivating conditions. Photoreactivation is important for *Hrr. lacusprofundi* to correct damaged DNA and prevent accumulation of damage that may lead to cell death. However, *Hrr. lacusprofundi* may remain sensitive to UV even in the presence of photoreactivating light due to possible lack of DNA protection or repair mechanisms. *Hrr. lacusprofundi* has two chromosomes and a plasmid; it has a rich GC content with 67% in its larger chromosome, 57% in its small chromosome and 55% in its plasmid (45). *Hrr. lacusprofundi* has carotenoid pigments based on phenotypic observations of its red color and is able to form biofilms, which may serve as an additional protection mechanism against UV since cells can exchange genetic information and/or aggregate for protection (46). *Hrr. lacusprofundi* may not efficiently repair its DNA and it may be possible that it may need more time to repair DNA given its slower growth rates. A whole genome sequence of *Hrr. lacusprofundi* is available and future studies can investigate the presence of genes for various DNA repair mechanisms. qRT-PCR may be also be performed for DNA repair genes post-UV irradiation to investigate possible upregulation levels and compare these to the highly UV resistant *Hbt. NRC-1*.

Hfx. volcanii is moderately UV resistant with exposure to photoreactivating light and is UV sensitive in the absence of photoreactivating light. DNA protection mechanisms are not lacking in *Hfx. volcanii* as observed in the genomic DNA damage assay. Possible protection mechanisms as discussed earlier are polyploidy and G-C content. *Hfx. volcanii* has about 12-15 copies of its genome depending on its growth phase (31) and a rich GC content of 65.46% (6). It may be that *Hfx. volcanii* then lacks DNA repair mechanisms that *Hbt. NRC-1* may have to correct damaged DNA post-UV irradiation. The genome of *Hfx. volcanii* is available and it has

been identified that *Hfx. volcanii* has homologs of the *uvrABCD* repair system and genes associated with photoreactivation *phr1* and *phr2*. *Hfx. volcanii* single mutants of *uvrA, uvrB* and *uvrC* exhibited hypersensitivity to UV-C irradiation, indicating these genes play an essential role in the nucleotide excision repair mechanism (47). A DNA repair mechanism that *Hfx. volcanii* has been found to employ upon DNA damage is compacting its nucleoid so that DNA repair proteins may locate their target and and DNA repair is accelerated (48). A qRT-PCR of the DNA repair mechanisms can assess the upregulation levels of these DNA repair mechanisms in comparison to *Hbt. NRC-1* post-UV irradiation to identify if there are differences in expression levels which may be responsible for differences in UV resistance. It would be interesting to observe if there is constitutive expression of genes for nucleotide excision repair in strains UV-irradiated and not exposed to photoreactivating conditions.

Nnm. 5-4 exhibited UV sensitivity with about 95% cell death at 48J/m^2 of UV-C. It may be possible that *Nnm. 5-4* may lack a DNA protection mechanism, which may be lack of pigmentation, or it may lack genes for a DNA repair mechanism. The role of carotenoid pigments as a means of direct photoprotection has been controversial in the literature (7). In our observations, we do not clearly observe if carotenoid pigments directly photoprotect DNA from UV damage. *Hfx. volcanii* RFP189 is an unpigmented *crtB* knockout strain that did not exhibit significant sensitivity to an isogenic *crtB*+ strain under photoreactivating or non-photoreactivating conditions. Our data suggests that carotenoid pigments are not absolutely necessary for photoreactivation. The unpigmented *Hfx. volcanii* RFP189 was able to photoreactivate in the absence of carotenoid pigments, however, naturally nonpigmented *Nnm. 5-4* did not photoreactivate and exhibited UV sensitivity. A future experiment with UV-VIS

spectroscopy can assess the presence or absence of non-colored structures that may absorb UV light in lieu of bacterioruberins and confer photoprotection.

The least UV resistant strain of halophilic archaea was *Nnm. 6-1*. It was thought that the high altitude Bolivian strain *Natrinema 6-1*, which has a rich red pigmentation, a characteristic of other notably UV resistant strains, would be highly UV resistant. However, this was not observed and *Nnm. 6-1* was the most sensitive strain we studied, even in the presence of photoreactivating conditions. Due to the presence of pigmentation yet sensitivity to UV in *Nnm. 6-1*, we wondered what may be the possible reason for its sensitivity. To assess if *Nnm. 6-1* possibly lacked some DNA protection mechanisms, I developed a genomic DNA damage assay with an alkaline gel electrophoresis. When treated with a *Chlorella* pyrimidine dimer glycosylase (PDG), *Nnm. 6-1* was predicted to have more damage than a strain with a more protected genome because a less protected genome would have more lesion sites for PDG to nick. *Hbt. NRC-1* and *Hfx. volcanii* were used for comparison since *Hbt. NRC-1* is highly UV resistant and *Hfx. volcanii* was moderately UV resistant. It was predicted that *Hbt. NRC-1* would have less damage induced, *Hfx. volcanii* would have moderate levels of damage induced, and *Nnm. 6-1* would have the most damage induced.

Data from the genomic DNA protection assay suggests UV-C damages the genomes of *Hbt. NRC-1*, *Hfx. volcanii*, and *Nnm. 6-1* to similar extents and we conclude that there is no relationship between DNA protection and UV resistance, at least in these three isolates (Figure 7). This result makes it more likely that *Nnm.* 6-1 lacks the repertoire of DNA repair mechanisms found in more UV resistant organisms. We are planning whole genome sequencing of *Natrinema 6-1* to determine which DNA repair and tolerance homologs are present in the genome.. The genome sequence will allow for qRT-PCR to identify if there is an upregulation or

a decreased expression of these genes post-UV irradiation. Furthermore, whole genome sequencing will provide further insight into the presence or absence of possible protection mechanisms such as polyploidy, G-C content, and genes for gas vesicle biogenesis in *Nnm. 6-1*.

We have found a remarkable diversity in UV resistance among the halophilic archaea both in photoreactivating and non-photoreactivating conditions that does not correlate with culture pigmentation and which suggests that undiscovered species-specific mechanisms may be involved in promoting enhanced UV resistance, such as DNA protective, repair and tolerance mechanisms. Our findings suggests that there is no correlation between altitude and UV resistance. There is a diversity in UV resistance among the halophilic archaea, which challenges the generalized classification in the literature that all halophilic archaea are highly UV resistant. *Nnm. 6-1* is remarkably UV sensitive, however it does not lack a genomic DNA protection ability but possibly a repair deficiency. Future experiments with the genomic DNA damage assay will include nonpigmented strains to identify if nonpigmented strains have less protected genomes than pigmented strains. This experiment can then examine the role of pigments and address if pigments play a direct role in photoprotection due to their presence. *Hbt. NRC-1* serves as a great model organism for environmental simulations of Mars due to the presence of high UV irradiation and little to no water on Mars. The simulations can further develop our understanding about the biology on other planets.

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VII. Figures

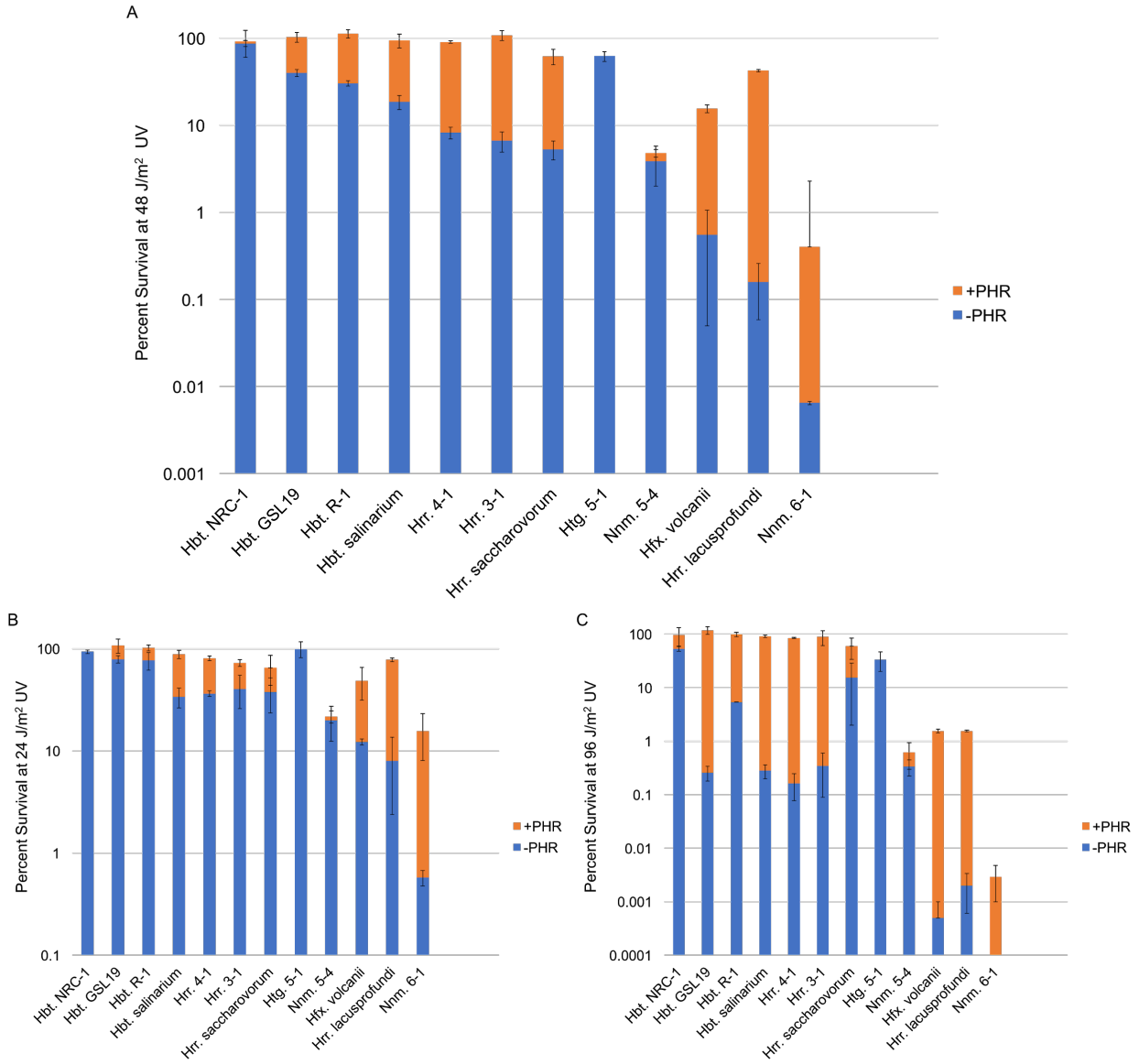

Figure 5. Survival of halophilic archaea irradiated with 254nm UV-C light. Strains of halophilic archaea cultured at logarithmic phase were irradiated with (A) 48J/m^2, (B) 24 J/m^2, or (C) 96J/m^2 of UV-C light. Strains were either exposed to (+PHR) or shielded from (No PHR) photoreactivating light. Data are averages of at least two independent experiments. Error bars depict standard error.

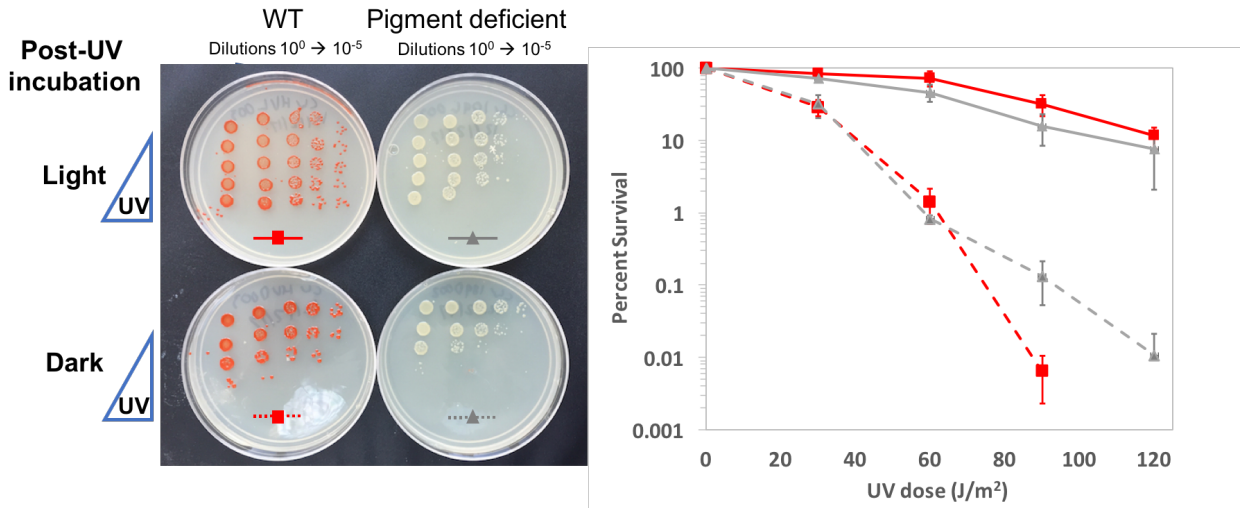

Figure 6. Photoreactivation in wildtype and pigment-deficient mutants of *Hfx. volcanii*. Left panel depicts representative data. Cells were treated with increasing doses of UV, diluted and spotted on YPC plates. Plates were either wrapped immediately and incubated in the dark at 42°C (bottom two plates) or treated with two hours of photoreactivating light prior to being wrapped and incubated (top two plates). Wildtype cells on left (WT) and pigment-deficient mutants are on the right. Right panel quantitates UV survival of wildtype (squares) and pigment-deficient (triangles) cells treated with (solid line) or without (dashed line) visible light after UV. Data shown are averages of three independent experiments.

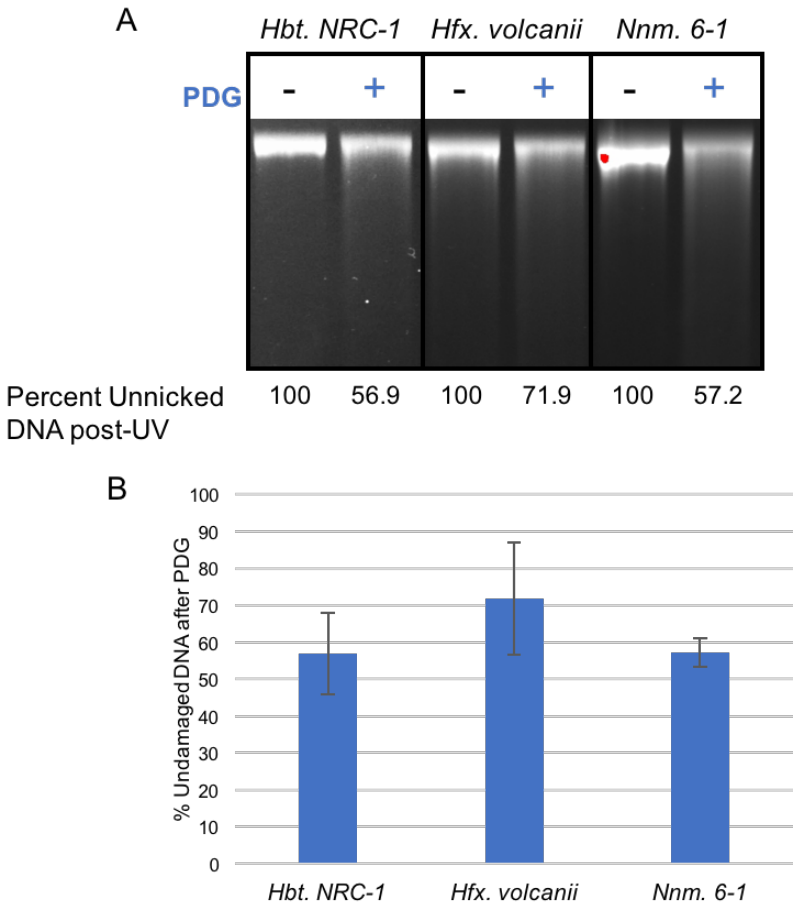

Figure 7. UV-induced genomic DNA damage in halophilic archaea. (A) Genomic DNA of *Halobacterium NRC-1, Haloferax volcanii,* and *Natrinema 6-1* was isolated after 10 seconds of UV irradiation. The DNA samples were either untreated or treated with a UV Damage nicking enzyme, *Chlorella* pyrimidine dimer glycosylase (PDG). Alkaline gel electrophoresis was performed on the DNA samples and the percent of genomic DNA maintained was determined from measured band intensities. (B) Graphed data of UV-induced genomic DNA damage in halophilic archaea. (A&B) Data averages and standard error values are from two independent experiments that were each repeated twice.

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