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Characterization of Growth and Ultraviolet Light Resistance in Four Novel Halophilic Archaea Isolates

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Abstract:

The goal of this project was to characterize the growth and UV resistance of four unique halophilic strains, JOR-1, BOL 4-2, BOL 6-1 and BOL 5-4. Halophilic archaea are interesting to study in part because of their notably high UV resistance. Four previously uncharacterized halophilic archaea, JOR-1, a *Salararchaeum* from the Dead Sea sediments, BOL 4-2, a high altitude *Halorubrum* from the Salar de Uyuni, and BOL 6-1 and BOL 5-4, both *Natrinema* from a salt mine in Bolivia, were cultured, their growth rates were measured, and their relative resistance to ultraviolet light exposure was determined. It was found that JOR-1, BOL 4-2, and BOL 6-1 had very similar doubling times (~3.5-5 hours) while BOL 5-4 grew significantly slower with a doubling time of 5.2 days. JOR-1 and BOL 4-2 each exhibited the high UV resistance typical of halophiles despite their different origins and genetic backgrounds. However, BOL 6-1 and BOL 5-4, isolated from the same location and found to be members of the same genus, showed unusual UV resistance profiles. BOL 6-1 is a relatively UV sensitive halophile while BOL 5-4, which lacks the typical halophilic red/orange pigmentation, showed a notable defect in its ability to perform photoreactivation, a key DNA repair process active only in the presence of blue light wavelengths. This work helps us better understand these four previously uncharacterized strains and adds to our understanding of the natural diversity of halophilic archaea, particularly pertaining to pigmentation and UV resistance.

Introduction:

UV Light

Ultraviolet light (UV) is a type of electromagnetic radiation that can have numerous negative effects on organisms that are exposed to it. The sun emits radiation at different wavelengths, including the three types of ultraviolet light: UV-A (320 to 400 nm), UV-B (295 to 320 nm), and UV-C (100 to 295 nm). When organisms are exposed to UV radiation, their cells absorb this energy and it effects the bonding of pyrimidines (thymine and cytosine) in the DNA structure, causing lesions called pyrimidine dimers. These lesions block replication and transcription, cause mutations if not removed, and can lead to cell death. To avoid these consequences, cells use means of protection, including pigmentation, as well as various DNA repair mechanisms. Photoreactivation is a repair mechanism used by many organisms in which a photolyase enzyme recognizes UV damage in DNA, absorbs blue light, and reverses the chemistry to restore the DNA to its normal structure. (Friedberg, 2006).

UV Damage

Ultraviolet light (UV) is a type of electromagnetic radiation. This type of radiation is given off by the sun and transferred in the form of waves or particles. These waves or particles are transmitted at a variety of wavelengths and frequencies that are measured according to what's known as the electromagnetic spectrum. UV light can be dangerous to cells and their DNA in proportion to wavelength. The shorter the wavelength, the higher the energy, thus more damage (Lucus, 2017). The sun's ultraviolet radiation is divided into three different ranges of wavelengths: UV-A (320 to 400nm), UV-B (295 to 320nm), and UV-C (100 to 295nm). UV-A and UV-B make up the majority of the solar radiation that cells encounter because they have the

ability to penetrate the ozone layer and reach the earth's surface. Wavelengths under 300nm (such as UV-C) are scattered and absorbed by the earth's atmosphere (Friedberg, 2006). Although cells don't experience exposure to UV-C radiation in nature, it is utilized in a lab setting for two reasons. First, it primarily effects DNA, yet isn't yet absorbed by proteins (Friedberg, 2006). Second, it creates the same types of lesions in DNA as longer UV-B wavelengths but in less time, making it more convenient. Both UV-B and UV-C wavelengths are absorbed by the double bonds in pyrimidine bases, most commonly between thymine and cytosine (Friedberg, Errol C. 2006). This causes damage to DNA, such as cyclobutane pyrimidine dimers (CPDs) and (6-4) pyrimidine-pyrimidone photoproducts (6-4 PP), which eventually lead to mutations and cell death if not repaired (Pulschen, et al., 2015). This damage interferes with transcription and DNA replication, which are vital mechanisms for the cell's viability. Breaks in DNA can consist of a single or double stranded break. Double-stranded breaks are specifically detrimental and mutagenic because they can interfere with the replication or expression of a gene (Goosen, N, and G F Moolenaar, 2008). This radiation exposes the bonds between bases in the DNA which allows them to react with other or adjacent molecules. The most common occurrence of this is when two new bonds are created between adjacent bases which form a membered ring (CPD). On other occasions, two carbon atoms form a single bond on a ring which form a (6-4) photoproduct (Goodsell, 2001). These various photoproducts are illustrated in Figure 1. These consequences from UV radiation are detrimental to the cells it corrupts. These lesions must be repaired due to their high potential to result in mutations, cellular transformation, and cell death. Cells uses a variety of repair mechanisms to relieve this tremendous stress. The most important mechanisms in an organism are photoreactivation and nucleotide excision repair, mentioned in the introduction.

DNA repair

DNA is essentially a blueprint that is utilized by the cell for all cellular functions – growth, metabolism, and reproduction. Due to the fact that DNA is such an important aspect of the cell, any damage to this molecule can have severe repercussions. DNA damage can lead to problems in the cell's ability to perform other important chemical and biological reactions such as the synthesis of proteins. DNA damage can either be caused by outside forces, such as chemicals or UV radiation, or can occur spontaneously. Direct reversal is a type of DNA repair that involves undoing the lesions. This process reverses the damaged bases and replace it with a correct DNA sequence and known as excision repair. Other indirect DNA repair mechanisms eliminate the potential of mutagens and other destructive consequences that come out of a result of environmental exposure to UV light. These mechanisms such as photoreactivation and nucleotide excision repair are therefore present in almost all organisms (Boron, 2012).

Photoreactivation

Photoreactivation (PHR) is a DNA repair mechanism that uses visible light (blue light) to directly reverse UV-induced damage in DNA such as CPD or (6-4) PP. PHR rearranges the nitrogenous bases that had been misbonded due to exposure to UV light, figure 1, (Jones and Baxter, 2017). This process evolved in prokaryotes, but was lost in placental mammals (Friedberg, Errol C. 2006). The enzyme that is utilized to combat a CPD dimer is known as pyrimidine dimer-DNA photolyase and the one used for (6-4) PP is known as (6-4) photoproduct-DNA photolyase (Friedberg, 2006). Photolyase enzymes can be found in bacteria, archaea and eukaryotes (Jones and Baxter, 2017). This process of PHR begins when a photolyase enzyme recognizes the lesion in the DNA (ex: CPD or (6-4) PP). The enzyme binds to the site of

damage on the DNA and is activated by absorbing blue light from the sun. Once DNA is repaired to its correct structure, the enzyme is recycled. This process is a light dependent reaction (Jones and Baxter, 2017).

There are two main genes that are involved in photoreactivation, *phr1* and *phr2*. In some species or photoreactive organisms, *phr2* is found to aid in the PHR repair mechanism but only in repairing CPD's and not (6-4) PP's. The function of *phr1* remains unclear, but it is believed it might have a role in encoding for a blue light receptor that is necessary for the activation of the photolyase enzyme (Jones and Baxter, 2017). A presence, or lack of these, specific gene might have a role in why some organisms more UV resistant then others.

Nucleotide Excision Repair

Nucleotide excision repair (NER) is the most prevalent mechanism to repair damaged DNA in humans. Unlike PHR, NER is a general repair mechanism, removing lesions non-specifically (such as CPD and (6-4) PP). This mechanism cuts out discrepancies found in the base pairing, caused by UV exposure, or other agents, and restores the DNA sequence (Friedberg, Errol C. 2006). This process is another mechanism that can repair lesions such as CPD and (6-4) PP but doesn't require light unlike photoreactivation (Jones and Baxter, 2017). Studies have been done using bacteria (*E. coli*) and eukaryotes (yeast, mice, humans) to better understand NER mechanisms. It was then determined that NER works similar in all organisms as well as using the same enzymes or homologs of them. The specific enzymes that are key components of this process are known as the UvrABC excinuclease and UvrD (also called Helicase II). These enzymes (UvrABC excinuclease and UvrD) were first discovered in the studies done *E. coli*. UvrABC excinucleases is composed of 3 polypeptide protein subunits

encoded by the genes *uvr*A, *uvrB,* and *uvr*C (Friedberg, Errol C. 2006). These different proteins each have a specific role in this multi-step process. UvrA is involved in recognizing the damaged segment of DNA, UvrB and UvrC are in charge of cleaving on both sides of the damaged DNA, lastly UvrD is responsible for removing the damaged strand (Jones and Baxter, 2017). The UvrABC complex begins this process by identifying the damaged region of DNA (such as pyrimidine dimers). The Helicase II enzyme then unwinds the DNA and discards the damages segment. This gap created in the DNA is resolved by a DNA polymerase that repairs the missing portion, by utilizing the unharmed single strand of DNA as a template – DNA is reverted to its normal state as illustrated in Figure 2. This repair mechanism is important because it restores correct coding in the genome and ensures normal cellular function. It was found that some species of halophilic archaea contain some eukaryotic homologs of the NER system as well as contain the bacterial UvrABC system (Crowley, 2006). In the archaea domain both the XP system (mammalian) and the Rad system (yeast) have also been described as homologs (Jones and Baxter, 2017).

Archaea

Eukarya, Bacteria, and Archaea are the three main domains of life. Archaea fall into the category of prokaryotes, which are single celled organisms that lack true organelles and a nucleus. Archaea can live habitats of extreme conditions either of high salinity, high and low pH, hydrothermal vents, hot springs, or anaerobic environments. Archaea have been found to be genetically more similar to eukaryotes then bacteria when comparing their genomes but appear to resemble the morphology of bacteria in size and shape (Eme and Doolittle, 2015). Along with Bacteria, they also contain a single circular chromosome of DNA, and sometimes a flagellum.

Archaea have similar membranes to bacteria and eukaryotic cells in that they contain phospholipids (double layer of lipids) but differ in the fact that they have lipids that are bound by glycerol-ether bonds rather than glycerol-ester links. This branch lipid membrane leads to a change in membrane structure in the archaea (Eme and Doolittle, 2015). Most prokaryotes contain cell walls but differ in the substances that make them up. Bacterial walls are made of peptidoglycan (proteins and sugars), while archaeal cell walls are composed of polysaccharides (sugars). These changes in the materials that compose the membranes and cell walls of an archaea allow it to survive in the extreme environments that it inhabits (Kerr, 2018).

Halophilic Archaea

There are many types of Archaea that inhabit many different places on earth. In my opinion, one of the most interesting types is called halophilic archaea. These organisms have been found exclusively in hypersaline environments (2-5M NaCl), such as salt lakes and evaporation ponds (Hamawi, R, 2018). These organisms have evolved, enabling them to counteract environmentally stressful situations such as harsh levels of ultraviolet (UV) radiation, high salt concentrations, and desiccation.

Haloarchaea have developed many characteristics to help them succeed in these environments, such as protection and repair. Halophilic archaea display resistance to ultraviolet light at high levels as well as the ability to protect themselves against harsh solar radiation. Most species have different components that allow them to have this ability. They contain DNA repair mechanisms such as nucleotide excision and photoprotection including photoreactivation to fix any lesions produced through exposure to UV radiation (reviewed in Jones and Baxter, 2017). These organisms exhibit phototaxis, chemotaxis, movability and also gas vesicles the allow for

flotation. Some haloarchaea even have the ability to perform phototrophic growth and are facultative anaerobes (Berquist, et al. 2006). Being phototactic or a chemotactic means that haloarchaea can move according to light or chemicals (towards or away). Some halophilic archaea possess genes used for the formation of gas vehicles that are regulated through the exposure to visible light. These gas vesicles provide the ability for the cell to increase its buoyancy in water. This buoyancy enables the haloarchaea to vertically move within the water traveling to regions which have conditions allowing for optimal growth (Offner, S., et al, 1998).

Another important characteristic is the pigmentation found in most haloarchaea. Pigmentation has been hypothesized to play a key role in providing protection from UV radiation for these organisms. One of the major pigments that Halophilic archaea contain is a C_{50} carotenoid known as bacterioruberin (BR), which causes it to appear as a red color. (Squillaci, et al., 2017). These pigments can be located within the cell's membrane and might have to do with a photoprotection process that makes haloarchaea so UV resistant (Jones and Baxter, 2017), although data to support this hypothesis are lacking. The halophilic archaea have become good model organisms for study of DNA damage and repair because of their ability to survive and thrive in the extreme UV exposure environment. In fact, members of the Haloarchaea are considered some of the most UV resistant organisms ever discovered (Berquist, et al, 2006).

Natrinema 6-1 and 5-4

BOL 6-1 and BOL 5-4 are uncharacterized strains of *Natrinema*, a relatively understudied genus of the haloarchaea that are chemoorganotrophs, can be aerobes, and require at least 1-7M of NaCl to grow (Mcgenity, T. J., et al., 1998). Both of these strains were isolated from a salt mine in Bolivia. This location has an elevation of 1,230 meters above sea level. BOL

6-1 exhibits a red/ orange pigment in late stationary cultures and on plates and BOL 5-4 exhibits no detectable pigmentation. Sequencing of ribosomal RNA indicated that both are *Natrinema* however BOL 6-1 has 3,785 genes in a 3.8 Mbp and BOL 5-4 has 4,589 genes in a 4.7 Mbp genome. BOL 6-1 has a GC content of 64.3% and BOL 5-4 has a GC content of 63.4% (DasSarma, P. et al, 2019).

JOR-1 and BOL 4-2:

JOR-1 and BOL 4-2 are mostly uncharacterized strains of halophilic archaea that were isolated from different locations around the world. JOR-1 was isolated from the sediment 30cm below the Dead Sea in Jordan. This location has an elevation of -415 m below sea level. Sequencing of ribosomal RNA indicates that JOR-1 belongs to the *Salarchaeum* species and has a red/pink pigment. JOR-1 has been sequenced and its genome is predicted to contain 2,633 genes in a 2.5 Mbp genome with a GC content of 66%. It was also found to contain a circular chromosome and a megaplasmid. (Anton et al., 2019).

BOL 4-2 was isolated from the Salar de Uyuni in Bolivia. This location has very high salt concentration (10X more than sea water) and high elevation (3,647 m above sea level). Ribosomal RNA analysis suggests that BOL 4-2 is a member of the *Halorubrum* species (DasSarma, P., personal communication). This strains also has a red/pink pigment color. Its genome sequence is not yet published.

Methodology

Halophilic Archaea:

All of the halophilic archaea used in this experiment were obtained from the DasSarma Lab at the University of Maryland. Table 1 displays specific characteristics about each strain and where they were originally isolated.

Growth Curves

A growth curve is a graphical representation of how the specific strain grows overtime. There are four phases of growth: lag phase, log phase, stationary phase, and death phase. A growth curve of each strain, BOL 6-1, BOL 5-4, JOR-1, and BOL 4-2 was constructed in order to better understand each of them. Cultures were made by inoculating 10 ml of CM^+ broth (250g NaCl, 20g $MgSO_4$, 2.0g KCl, 3.0g Na-citrate, 2.3mg $FeCl_2$, 440ug $ZnSO_4$, 330ug $MnSO_4$, 10ug $CuSO_4$, 10 g peptone (Oxoid)) with 10 µl of a stationary phase culture in 100 ml side arm flask. The cultures were placed in a shaking water bath at 40^o C and growth was monitored with a Klett-Summerson Photoelectric Colorimeter, an instrument designed to measure density of a liquid culture through the use of light. Readings were taken hourly during active phases of growth and cultures were photographed to monitor pigmentation. Klett readings were natural log transformed and plotted as a function of time in order to be visualized graphically and to determine the slope of the log phase and culture doubling time.

Survival curves

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)

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 and 20 microliter spots were pipetted in duplicate on CM+ plates (CM+ broth + 20g Difco agar (per 1L)). 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 5-15 days before counting survivors. Figure 4 depicts a visual representation of our method.

Results

Growth and Pigmentation

It was found that JOR-1, BOL 4-2, and BOL 6-1 have very similar growth rates with doubling times of approximately 3.5-5 hours (Figure 5). However, BOL 5-4 grows at a drastically slower rate compared to the other strains with a doubling time in CM+ over 5 days. During the growth process, JOR-1 and BOL 4-2 exhibit a pink/orange color very early in log phase which is different from BOL 6-1. BOL 6-1 pigmentation does not start to develop until the cells have reached late log or early stationary phase. There is a complete lack of pigmentation in BOL 5-4 cultures (data not shown).

The various phases in a growth curve are lag phase, log phase, and stationary phase. JOR-1, BOL 4-2, and BOL 6-1 have a lag phase of about 24 hours (Figure 5). JOR-1, BOL 4-2, and BOL 6-1 are in log phase from approximately hour 24 hours to 40 hours, doubling every 3.5-5 hours. These strains are in transition between log and stationary phase around 20-80 hours. JOR-1, BOL 4-2, and BOL 6-1 reach stationary phase after approximately 72 hours. At stationary phase these strains reach densities in excess of 250 Klett units. The extremely slow growth of BOL 5-4 made it difficult to determine growth stages accurately. Certainly, the lag and log

growth phases were much longer than the other strains. It is also important to note that BOL 5-4 never reached the same densities as the other strains, with a maximum observed density of 125 Klett units (data not shown).

Pigmentation changes were observed in the growth process of each of these strains (left panels, Figure 5). For JOR-1 and BOL 4-2, pigmentation was observed as soon as 24 hours and is clear in images taken during log phase growth (left panels, Figure 5). BOL 6-1 cultures did not have significant pigmentation until late log phase and developed more prominent culture pigmentation in stationary phase. BOL5-4 never showed any detectable pigmentation in culture or on plates.

UV Responses

JOR-1 and BOL 4-2 showed similar UV survival profiles (Figure 6). JOR-1 and BOL 4-2 are extremely resistant in the light, demonstrated by greater than 50% cell survival out to 300 J/m^2. In the light JOR-1 and BOL 4-2 are more resistant compared to in the dark. JOR-1 and BOL 4-2 are resistant at low doses (below 50 J/m^2), but their resistance decreases as the dose does. They are more than three logs more sensitive in the dark than in the light. There is minimal killing out to 50 J/m^2 of UV but by 300 J/m^2 99.99% of cells are killed. At high doses of UV there is a plateau of BOL 4-2 cells that have not been killed. Overall both strains exhibit similar UV responses.

BOL 6-1 and BOL 5-4 are both *Natrinema* species and were both isolated the same Bolivian salt mine yet showed different UV survival profiles from each other as well as from JOR-1 and BOL 4-2. In order to characterize BOL 6-1, much lower UV doses were necessary because of its relative UV sensitivity. BOL 6-1 is slightly but significantly more UV resistant in

the light then in the dark (Figure 7). Even in the presence of light, there is less than 50% cell survival when exposed to only 30 J/m^2. At about 60 J/m^2 there is over 99% of cell death. In the dark, there were no cells able to be counted at 96J/m^2 because all were killed off by the UV. Overall, BOL 6-1 is quite sensitive to UV for a halophilic archaea.

BOL 5-4 demonstrates a different profile compared to BOL 6-1. BOL 5-4 is somewhat less resistant in both the light and the dark compared to JOR-1 and BOL 4-2. Most notably however, BOL5-4 showed no enhanced UV resistance in the light, a unique phenotype that to our knowledge has not previously been observed in a natural isolate of halophilic archaea (Figure 7).

Discussion:

The photos presented allowed us to get a better understanding of the way pigment changes in the growth process in each of these strains (Figure 5, right panels). In these strains, pigment development occurs differently for JOR-1, BOL 4-2 and BOL 6-1. JOR-1 and BOL 4-2 develop pigment early compared around hour 34 in early log phase. BOL 6-1 does not have detectible pigmentation until hour 144 and in late log phase. A reason for this lack of detectable pigment in the early log phase could be that BOL 6-1 is losing its pigment. Pigmentation develops slow in BOL 6-1 which means it might not have a purpose in growth and is not needed. This could be the first signs of a future loss of pigmentation altogether. JOR-1 and BOL 4-2's pigment develops in log phase which might indicated that it has a function in nutrients absorbance and growth. 5-4 has a lack of pigment which could contribute to its slow growth, perhaps because the pigments assist in harvesting energy or protecting the cells from oxidative damage (Jones and Baxter, 2017). Finding pigmentless mutants in each of the wildtype strains

could help determine if pigmentation has a purpose in growth. If pigmentless mutants were found of both JOR-1 and BOL 4-2, then growth curves could be developed for them. These growth curves of the wild types and mutants could be compared to see if pigmentation has an effect on growth rate.

The growth curves illustrate important characteristics of the four strains. As stated above JOR-1, BOL 4-2, and BOL 6-1 have relatively fast growth rates compared to BOL 5-4. A reason why BOL 5-4 grows differently from the others could be due to the environment. Although BOL 5-4 and BOL 6-1 are from the same location they could have slightly different exposures of nutrients need to grow. BOL 5-4 might favor another type of media other than CM+. A future experiment might be to change the type of media and see how that effects growth rate. This media could contain different nutrients other than amino acids such as sugars. BOL 5-4 has a lack of pigmentation compared to the other strains that have a pink/orange pigment (bacteriorubin). Further experiments, focusing on pigmentation, could help to determine if it is tied to BOL 5-4's slow growth. It would also be interesting to change the environment (temperature, light exposure, different media, etc). One way could be to use a different media, rather than CM+, and determine if this effects growth rate and other phenotypes, perhaps even including pigmentation.

A reason that could account for the high resistance of JOR-1 and BOL 4-2 in the light could be that they could be utilizing both their photoreactive processes and their NER processes. Both of these mechanisms combined would allow the survival to be greater. They also might have more enzymes expressed constitutively for these processes which could allow them to perform repair more efficiently. However, in the dark that are unable to photoreactivate which means they solely rely on their NER processes. Because the cells are still remarkably UV

resistant in the dark, studying the levels of NER proteins in these cells, along with investigating other protection, repair and tolerance mechanisms, is vital.

BOL 4-2 had very similar results to JOR-1 but did have a plateau of cell death at high doses of UV. The plateau that is seen in BOL 4-2 could be the result of cells that have become resistant to high levels of UV. Mutant cells could have developed extreme resistance to UV compared to other cells in the culture. This could explain why these cells start to plateau and not die off. We would like to culture these "resistors" and study them further in the future.

Although BOL 6-1 and BOL 5-4 are from that same light limited salt mine and the same species they show very different UV survival characteristics. BOL 6-1 might be in the process of losing its pigment as well as it PHR abilities, in part due to the lack of direct exposure in the salt mine. This could explain why in the light BOL 6-1 is only slightly resistant than in the dark. There is not a significance difference between survival in that light and the dark which could be contributed to a less efficiency of NER processes. Overall BOL 6-1 was exposed to lower doses compared to JOR-1 and BOL 4-2. This means that BOL 6-1 was overall more sensitive than JOR-1 and BOL 4-2. In the dark BOL 6-1 is more sensitive compared to that other strains (JOR-1, BOL 4-2, and BOL 5-4). This is of course attributed to the lack of PHR as well as weaker NER processes. With both of these processes lacking or absent it would cause a decrease resistance to UV. BOL 5-4 lacks pigment and the ability to photoreactivate. Interestingly the recently completed BOL 5-4 genome sequence reveals no homology to known halophilic *phr* genes (DasSarma, S., personal communication). This fact explains why there is no difference in survival in the light compared to the dark. It clearly performs NER well because it still more UV resistant than BOL 6-1. Finding a non-pigmented BOL 6-1 strains could help rule out any

possibilities that pigment benefits UV survival. Then we would could also test the *phr* genes expression levels to see if they have any effect on the UV survival and to what extent.

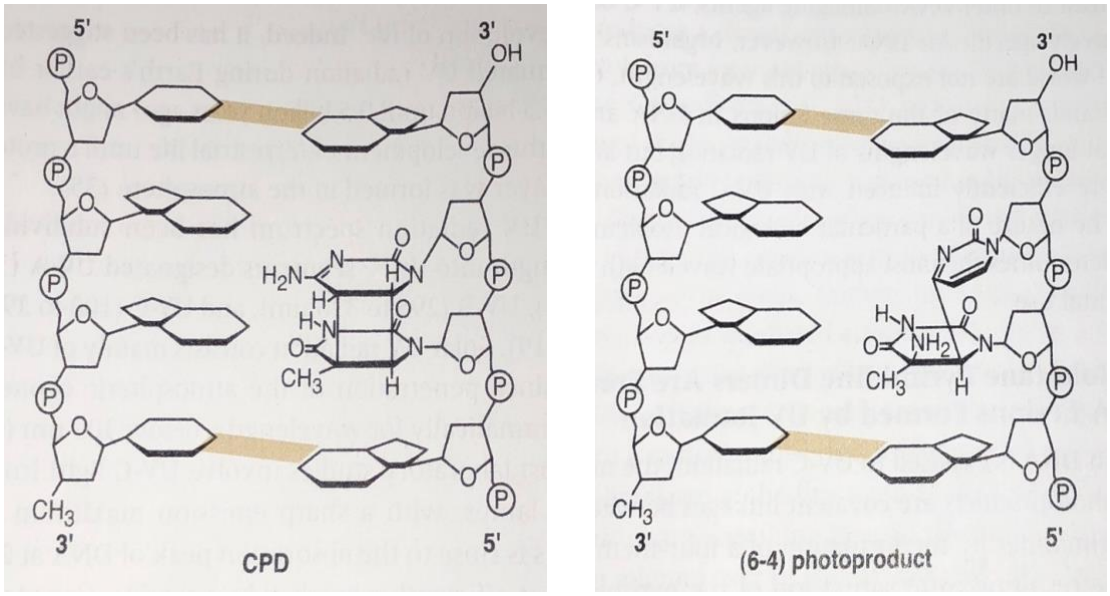


Figure 1. A CPD (left) and (6-4) PP which are two types of lesions that occur in DNA when exposed to UV-C or UV-B radiation. This example shows dimerization between adjacent cytosine and thymine bases. (Friedberg, 2006)

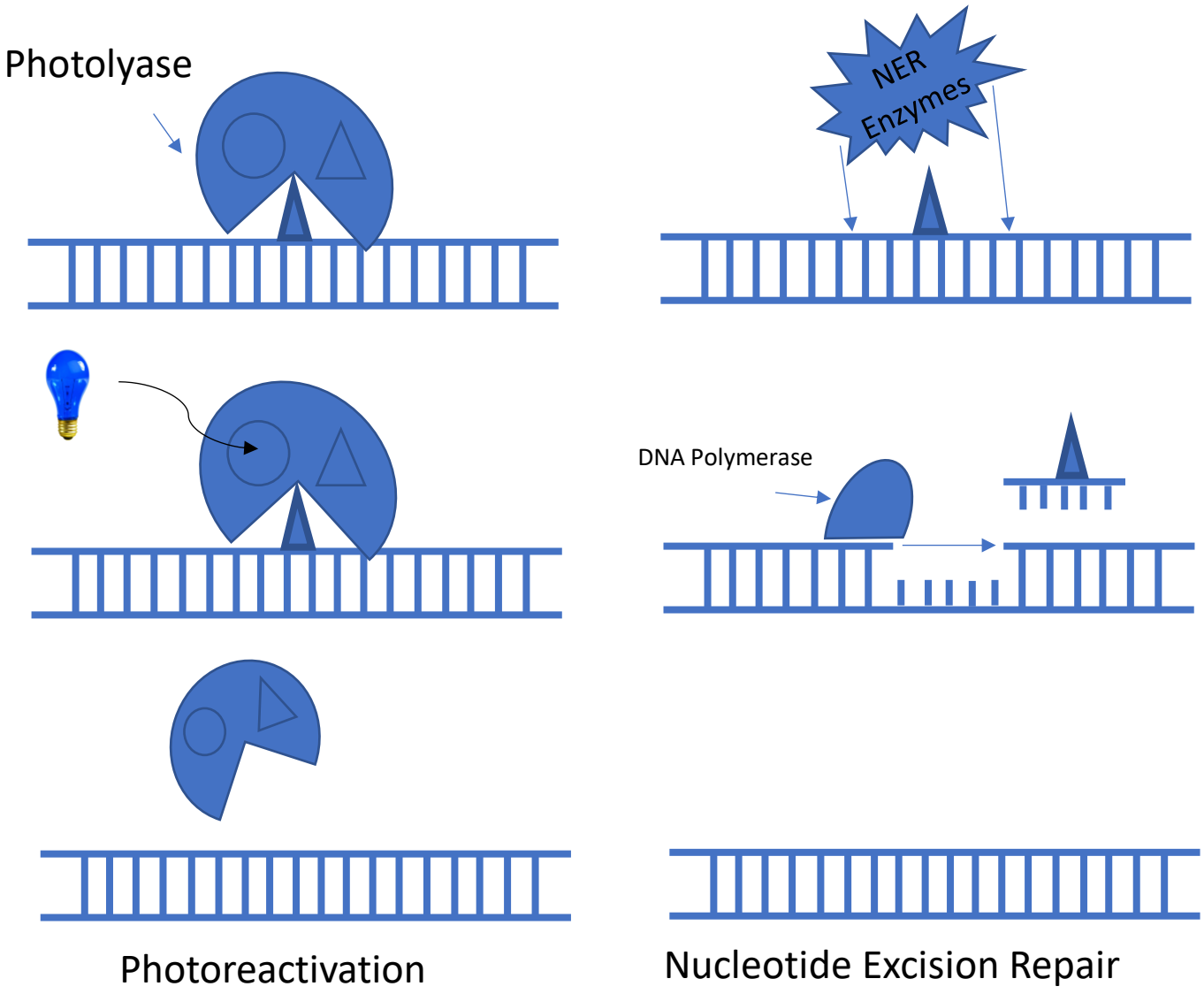


Figure 2. (Left image) Illustration of the photoreactivation mechanism. First, the photolyase identifies the pyrimidine dimer in the DNA, binds to it and then utilizes blue light to reverse the chemical reaction that caused this lesion. The DNA is then restored to its original state. (Right image) Illustration of a complex of NER proteins locating that lesion, cutting around the damage, then leaving the DNA. A DNA polymerase and ligase seal the gap in the strand.

A

B

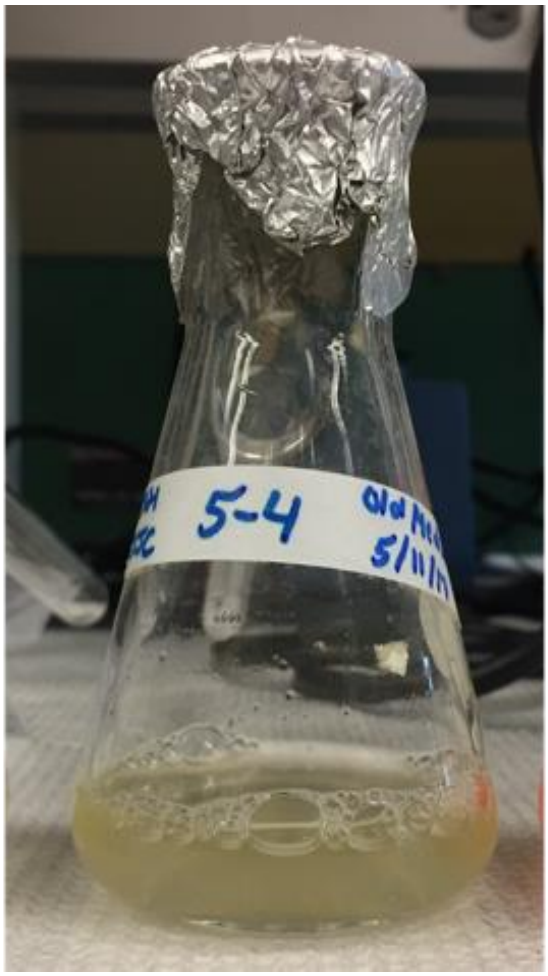


Figure 3. Representation of stationary phase liquid cultures. (A). BOL 6-2, Pink pigmentation. (B). BOL 5-4, unpigmented

Table 1. Characteristics of Novel Halophilic Archaea

Strain	Place of origin	Elevation at origin (meters above sea level)	Temperature at origin (°C)	Genus (based on 16S rRNA)	Culture pigmented	Genome size (Mbp)	Predicted number of genes	GC (%)	Cell Morphology
BOL 6-1	Pink salt from Bolivian salt mine	1,230	-10 to 37	*Natrinema*	Yes, late onset	3.8	3,785	64.3	Rod
BOL 5-4	Pink salt from Bolivian salt mine	1,230	-10 to 37	*Natrinema*	No	4.7	4,589	63.4	Rod
JOR-1	Dead Sea sediment (30 cm depth)	- 415	34	*Salarchaeum*	Yes	2.5	2,633	66	Cocci
BOL 4-2	Salar de Uyuni salt crust	3,647	-15 to 22	*Halorubrum*	Yes	N.D.	N.D.	N.D.	Rod

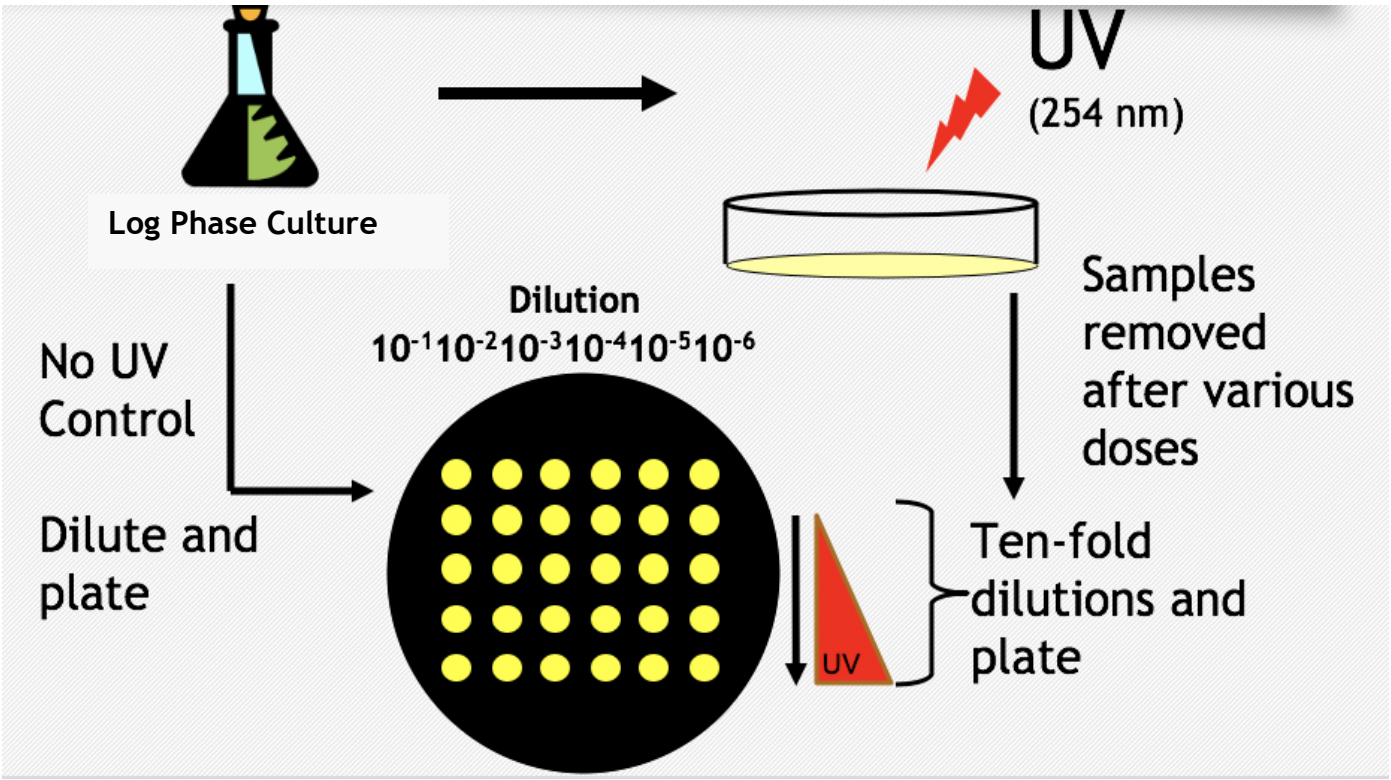
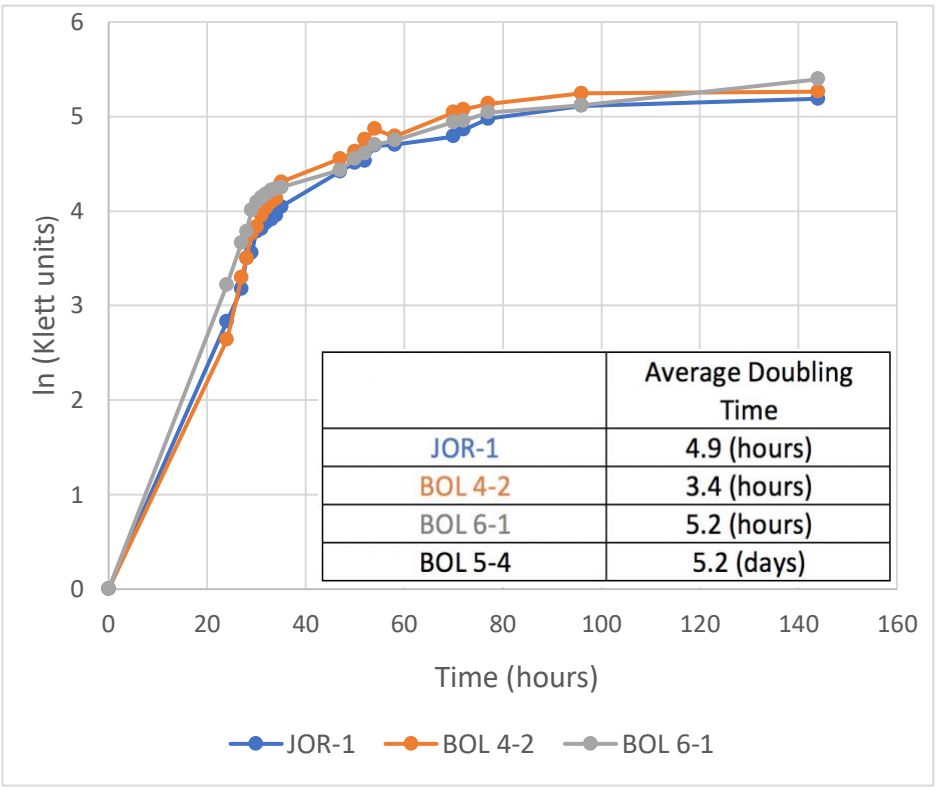


Figure 4. Graphical depiction of UV survival methodology.



	Average Doubling Time
JOR-1	4.9 (hours)
BOL 4-2	3.4 (hours)
BOL 6-1	5.2 (hours)
BOL 5-4	5.2 (days)

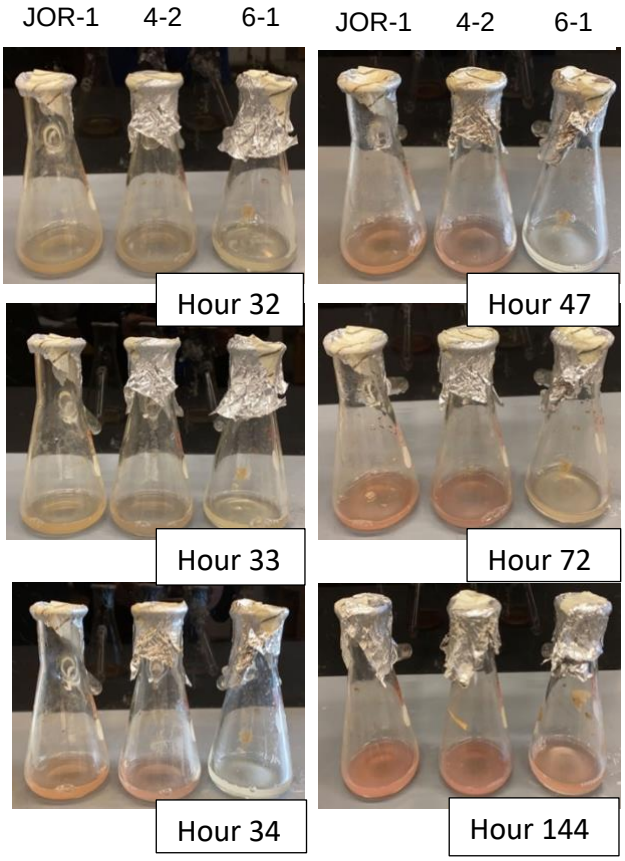


Figure 5. Representative Growth curves of JOR-1, BOL 4-2, and BOL 6-1. Each culture was inoculated with 10 µl of stationary phase cells in 10ml of CM^{+} and culture density was monitored with a Klett meter. (Right) Pictures of JOR-1, BOL 4-2, and BOL 6-1 cultures throughout their growth phases. (inset) Average doubling time of each culture. Averages are from two independent experiments. Growth data for 5-4 not shown.

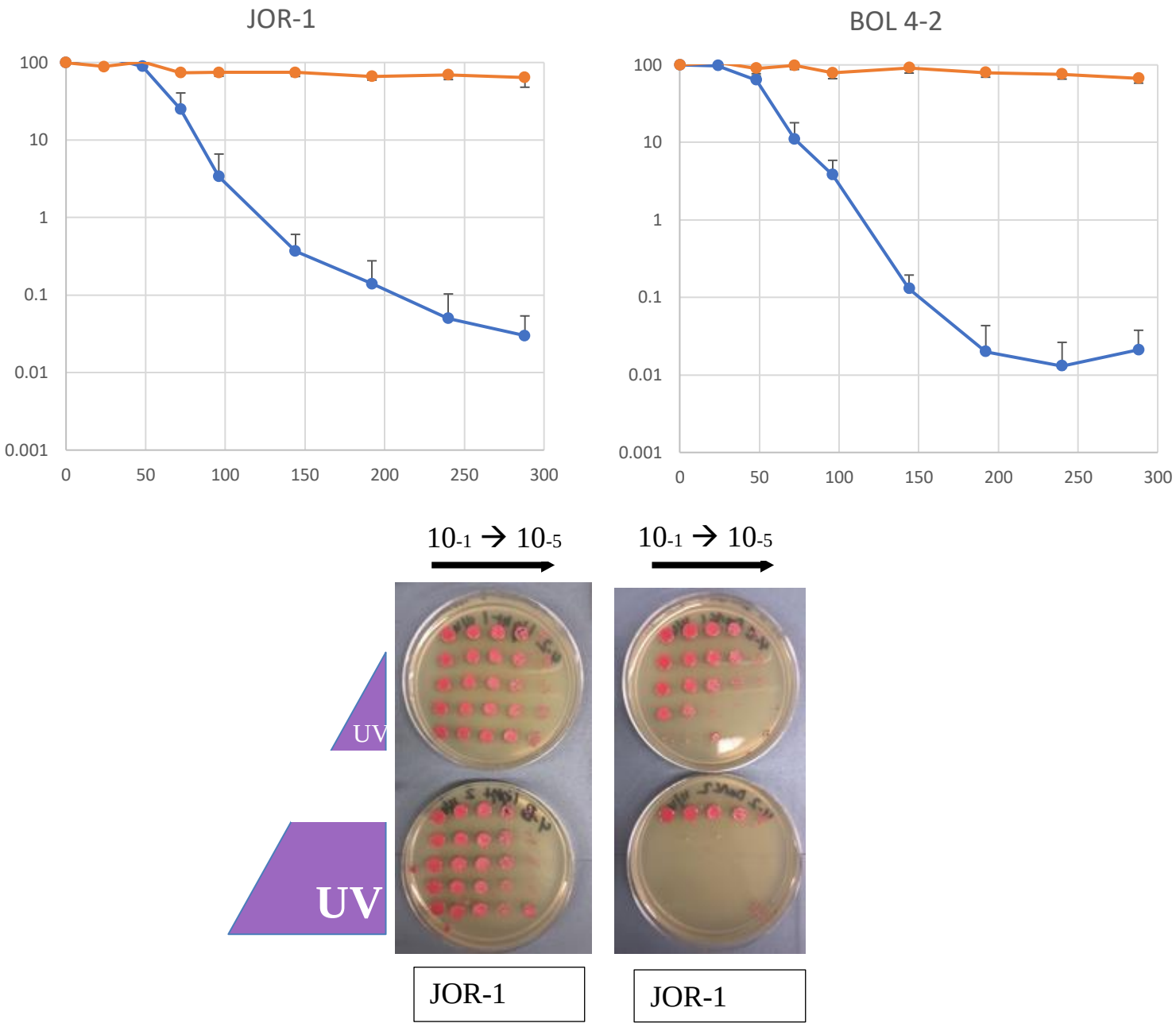


Figure 6. UV survival of log phase cultures of JOR-1 (left) and BOL 4-2 (right). Orange line represents survival after UV-C radiation (254nm) and subsequent exposure to 2 hours of fluorescent light. Blue represents survival of same cells in the absence of the 2-hour post-UV fluorescent light treatment. These graphs represent the average of at least 3 experiments. Error bars represent the standard error. (inset) Representative pictures of plates from one JOR-1 survival experiment. Purple triangle: increase in UV exposure, black arrow: increase in dilution.

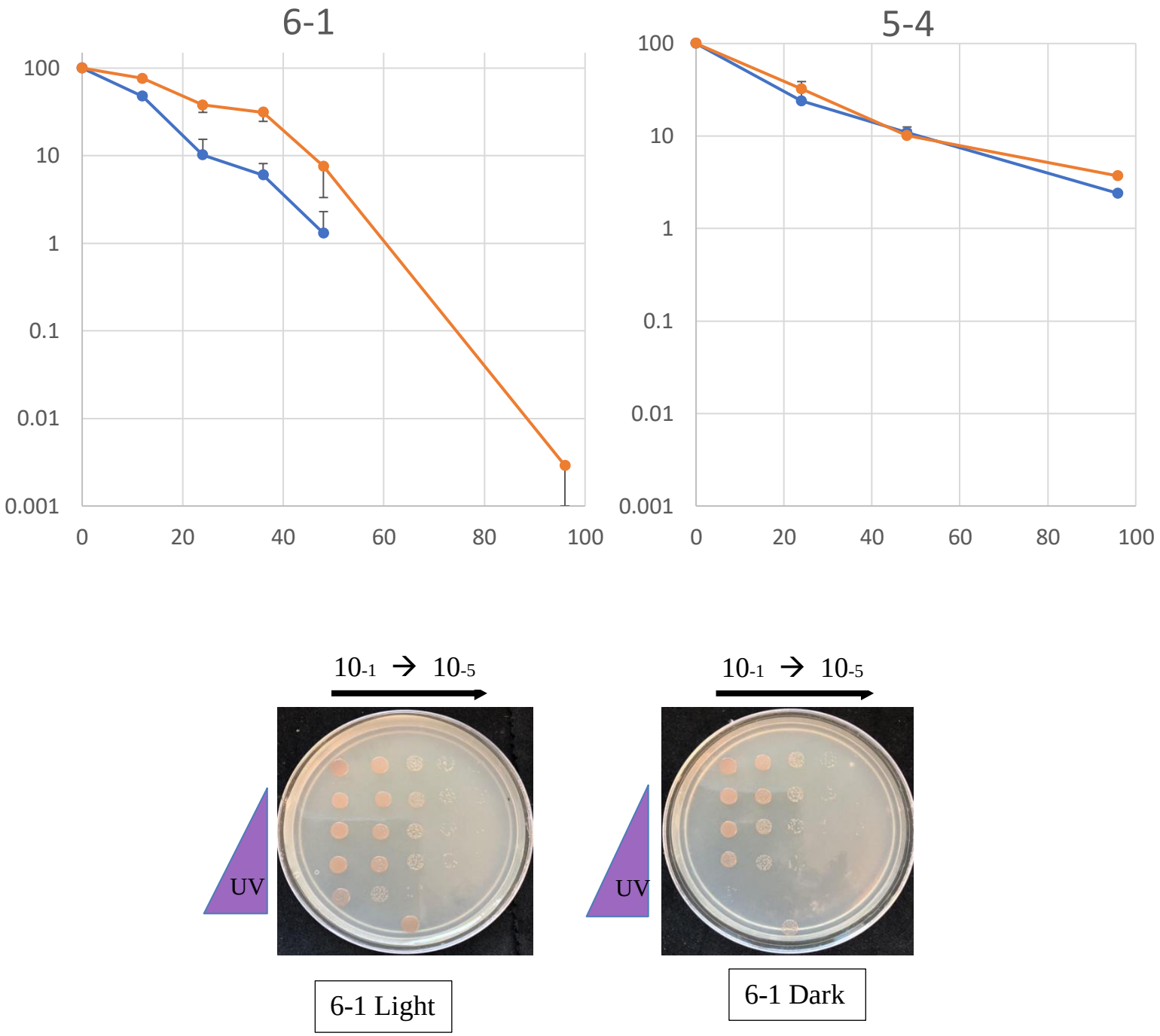


Figure 7: UV survival of log phase cultures of BOL 6-1 (left) and BOL 5-4 (right). Orange line represents survival after UV-C radiation (254nm) and subsequent exposure to 2 hours of fluorescent light. Blue represents survival of same cells in the absence of the 2-hour post-UV fluorescent light treatment. These graphs represent the average of at least 3 experiments. Error bars represent the standard error. (inset) Representative pictures of plates from one BOL 6-1 experiment. Purple triangle: increase in UV exposure, black arrow: increase in dilution.

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