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Molecular Classification and UV Tolerance of Pigmented Antarctic Extremophiles

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Undergraduate Thesis

By

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Abstract

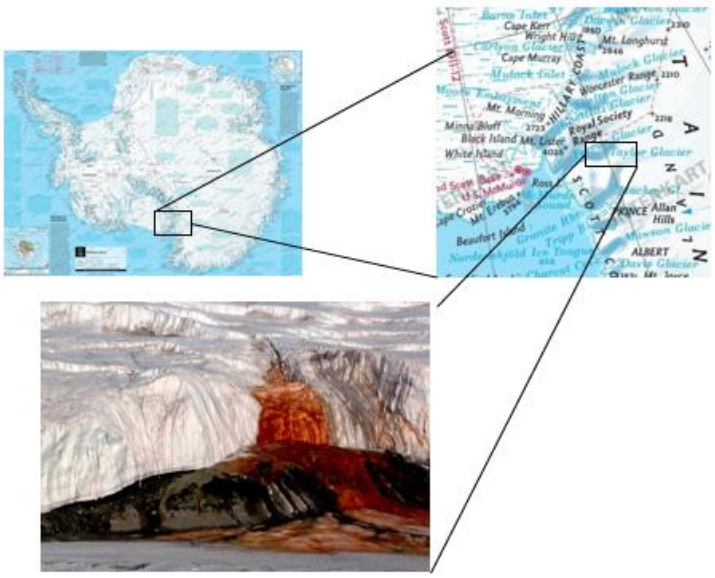
Microbes living on the Antarctic continent must withstand a cold and barren environemt. Understanding the genomic content of these organisms can provide important information on how they have adapted to extreme environments. Microbes that inhabit Antarctica are subject to varying amounts of UV radiation, with – high intensities on the surface of glaciers and ice sheets during summer, and low to absent in sub-ice environments and during winter. In this study, five microbial isolates were selected because of their colorful pigmentation or lack of pigmentation and subjected to UV challenge experiments. All microbes tested, despite where they were originally isolated from or amount of color pigmentation, showed some level of resistance to shortwave UV (254 nm) light. While results confirm that microbes with pigments are able to withstand UV stress, these findings suggest other mechanisms may also be important for extremophile survival and growth in Antarctica.

Introduction

Life on the Antarctic Continent is dominated by microbes. Despite low temperature and limited nutrients, microbes can survive the extreme cold on the surface of, within, and underneath the Antarctic Ice Sheet using novel adaptations to icy life (Mikucki and Priscu 2007). Due to the often slow microbial growth rates and the inherent difficulty in obtaining samples from remote Antarctica, these extremophilic microbes are largely under-represented in scientific research. Therefore, studying these microbes presents an opportunity to discover new microbial cellular adaptations and functions and to better understand the extreme limits of life and the diversity of life on Earth.

While the microbial isolates studied for this thesis were all collected from Antarctica, they were isolated from a range of locales, and survived under diverse environmental conditions. These isolates were collected from, in, and around glaciers, from desiccated soils and brine lakes, and therefore would experience distinct ranges in exposure to UV light. Intense or prolonged exposure to ultraviolet light can damage genetic material within the cell. Microbes have evolved a range of methods for counteracting exposure to UV including UV-stress avoidance, UV-stress defense, and active repair mechanisms (Ehling-Schulz and Scherer, 1999). A majority of the Antarctic isolates collected for this study are pigmented, suggesting that pigments provide protection from UV damage as well as low temperatures (Mojib 2011). These pigments are produced as secondary metabolites and may vary in occurrence depending on light exposure and dissolved oxygen content (Mojib 2011). These factors could potentially enhance or limit secondary metabolite production as light and available oxygen shape the rate and mode of metabolism in some organisms. Well studied in both plants and microorganisms are carotenoids, a naturally occurring pigment that has been shown to be effective at absorbing a wide range of light and also combating oxidative stress within a cell (Órdenes-Aenishanslins et al., 2016). Another example is of UV-absorbing compounds found in microbes is mycosporine amino acids that protect cyanobacteria by absorbing UV radiation, thus, preventing damage to cell membranes and organelles (Ehling-Schulz and Scherer, 1999). This study focuses on UV defense mechanisms, specifically pigmentation, of Antarctic isolates and how well they withstand UV exposure. Five microbial isolates, four from the exposed surface of Blood Falls located in the Taylor Valley and one from 22m depth with the water column West Lake Bonney (Figure 1), with distinct pigmentation were selected to observe their response to UV challenge experiments.

Figure 1 Visual representation of the location of the Taylor Glacier, Lake Bonney, and Blood Falls.



National Geographic

Materials and Methods

Cultivation

The isolates BF11_BFM03, DTM06_05, BF02_S3, WLB02_22m8, and BF02_Schw were previously isolated and stored at -80°C in a glycerol stock. To confirm purity, these cultures were streaked for isolation on Marine Agar (Difco) using aseptic techniques. Plates were then placed in a 10°C incubator and grown until isolated colonies reached 1-2mm in diameter, which took approximately 2-3 weeks. Single colonies from marine agar plates were picked using a sterile loop and inoculated into 5 mL Marine Broth (Difco) in 15mL centrifuge tubes and grown at 10°C. Before use, both marine broth and marine agar were autoclaved at 121°C for 25 minutes. Marine broth was also run through 0.22 μm filters to remove undissolved broth residue. Marine broth and marine agar were transferred aseptically into centrifuge tubes and petri dish plates, respectively, within a biological safety cabinet (Labconco). The liquid cultures were incubated until there was noticeable turbidity indicating dense growth which took approximately

2 weeks. The 5 mL cultures were then sub inoculated into 20 mL cultures in 50mL centrifuge tubes of a 1 to 9 ratio of 5 mL culture to marine broth, and incubated at 10°C. For DNA extractions, the 20mL liquid cultures were allowed to grow until stationary phase was reached and cultures were turbid which took approximately 2 weeks. For UV exposure experiments, cultures were grown for 2 weeks following exposure to UV. To ensure the purity of each isolate liquid culture, negative control tubes containing no inoculum were created.

CTAB DNA Extraction

The CTAB DNA extraction was used as described previously (Campen 2015). Briefly, liquid cultures (20 mL) were placed on ice for approximately 3 hours to slow cell growth. After pelleting the cell contents by centrifugation at 2700 xg for 20 minutes and resuspending the cells in 400 μL TE buffer, the cells were heat killed in an 80°C water bath. Next, the cells were incubated with 10 μL 50 mg/mL lysozyme for 1 hour at 37°C and followed with 4.1 μL 10 mg/mL proteinase K and 61.5 μL 10%SDS treatment. 95.1 μL 5M NaCl and 95.1 μL cetyltrimethylammonium bromide (CTAB) buffer were then used to precipitate cell debris. 660 μL Chloroform was added and the samples were then centrifuged at 12,000 xg for 8 minutes, separating the mixture into aqueous and organic phases. The upper aqueous phase was removed and treated with isopropanol. The organic phase containing cell debris was discarded. After addition of 360 μL 99% isopropanol, the samples were centrifuged at 12,000 xg for 15 minutes, creating a pellet of DNA. The isopropanol was removed, and the DNA was then washed with 1 mL 70% ethanol. The sample was again centrifuged at 12,000 xg for 5 min, further pelleting the DNA. After removal of ethanol, the extracted DNA was resuspended in 50 μL of PRC grade water. Samples were stored at -20°C.

Polymerase Chain Reaction (PCR)

To classify each microbe, the 16S rRNA gene was amplified for DNA sequencing. To amplify the 16S rRNA gene of each isolate, a Polymerase Chain Reaction (PCR) was ran using the universal 27F (AGAGTTTGATCCTGGCTCAG) and 1492R primers (GGTTACCTTGTTACGACTT). A mix of PCR components was created containing 12.5 µL AmpliTaq Gold 360 Master Mix DNA polymerase (Applied Biosystems), 0.5 µL 27F primer, 0.5 µL 1492R primer, and 10.5 µL PCR grade water per each reaction. 24 µL of the mix was then transferred to 200 µL PCR tubes for each reaction. 1 µL of sample genomic DNA was added to the PCR tube in addition to the mix.

The PCR protocol for this amplification included the following cycle:

1) Incubate at 95.0°C for 10 minutes

2) Incubate at 95.0°C for 45 seconds

3) Incubate at 50.0°C for 60 seconds

4) Incubate at 72.0°C for 90 seconds

5) Repeat steps 2-4 35 times

6) Incubate at 72°C for 10 minutes

Gel Electrophoresis

PCR products and the extracted genomic DNA were separated by gel electrophoresis on a 1% agarose gel. Gelred Nucleic Acid Gel Stain (Biotium) was added to the agarose gel so that the genetic material would fluoresce when exposed to UV light. Samples were mixed with a

ThermoFisher Scientific DNA Gel Loading Dye, which allows visualization of the DNA migration during electrophoresis. A 23 kilobase ladder (Invitrogen) was used to validate the length of the genomic DNA while a 1 kilobase ladder (Invitrogen) was used to validate the length of the PCR products.

DNA Quantification

Both genomic DNA concentration and PCR product concentration were measured using the Qubit 3.0 Fluorometer (Invitrogen) using the Qubit dsDNA BR Assay Kit (Invitrogen). The Qubit utilizes a binding dye that only fluoresces when bound to genetic material, allowing the measurement of DNA concentration.

Sequencing Preparation

All PCR products were cleaned using the DNA Clean and Concentrator kit (Zymo Research) according to the manufacturer's protocol without modification. Sequencing was performed in Walters Life Science Building room A211 using 27 F primer (AGAGTTTGATCCTGGCTCAG) and 1492R primer (GGTTACCTTGTTACGACTT).

Sequencing Analysis

Geneious version 11.0.4 (Geneious, Kearse et al., 2012) was used to analyze 16s rRNA gene sequencing results for each isolate. The forward and reverse sequences were trimmed and aligned to form a consensus sequence. The NCBI web BLAST (BLAST, Altschul et al., 1990) program was used to search for close relatives of each isolate using their 16S rRNA sequences. Closest hits were determined by running BLAST against the non-redundant nucleotide collection

(nr/nt) database, while closest type strains were determined by running BLAST against the 16S Ribosomal RNA sequence database, limiting results to type material. Results were used to confirm the identity of each isolate. Nucleotide collection (nr/nt) and 16S ribosomal RNA sequence type strain hits were downloaded and saved to Geneious. The Silva incremental aligner (SINA, Pruesse et al., 2012) was used to align the isolate's consensus 16S rRNA sequences with the BLAST hits. Aligned sequences were imported into Geneious and trimmed to the length of the shortest sequence at both ends. The trimmed sequence length was 1248 bases. The aligned sequences were exported to Mega 7 to build a maximum likelihood tree using the Tamura-Nei model with gamma distribution and 500 bootstraps replicates (MEGA, Sudhir et al., 2015).

AntiSMASH

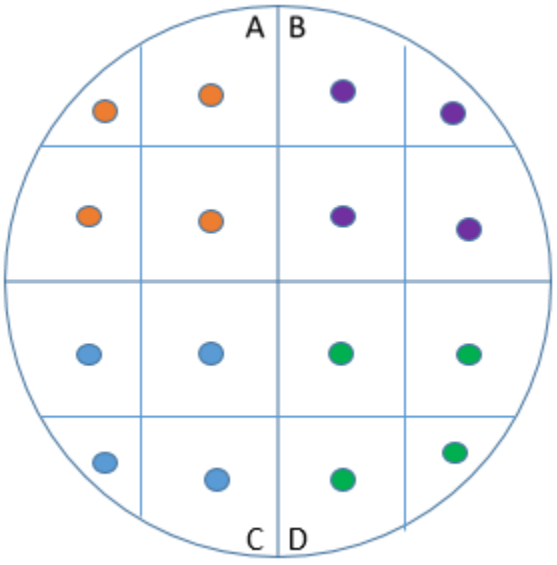
AntiSMASH (version 4.0.2) web server allows analysis of secondary metabolites through the input of genomic DNA sequences. AntiSMASH identifies key gene clusters which allows examination of morphological structures within and between organisms. The previously annotated genome of BF02_Schw was uploaded to antiSMASH and analyzed using the default settings.

UV Exposure Experiment

Isolates BF11_BFM03, DTM06_05, BF02_S3, WLB02_22m8, and BF02_Schw were selected for further examination to their pigmentations. To investigate if the pigmentation of these microbes played a role in UV protection, cells were challenged with increasing UV exposure by a 254 nm UV lamp within a biological safety cabinet. 254 nm light, which is within the UV-C range of ultraviolet light, is more energetic and detrimental to life than light within the

UV-A and UV-B range that is typically experienced in nature. Deletion of the protective ozone layer has led to increases in UV-B (280-320nm) radiation in Antarctica, light that can penetrate substantial depth of waters systems (Karentz et al, 1991). While 254 nm UV light might not be a common occurrence in nature, this light exposure still provides valuable insight to resiliency of each isolate. Marine agar plates were sectioned into 16 parts (Figure 2). Each isolate was transferred from liquid marine broth and placed as four 10μL drops of each isolate on the plates. Eight different marine agar plates were made with this organization. Immediately after placing the aliquots of isolates on the marine agar plates, each plate was exposed to a different time length of UV exposure: no exposure, 10 seconds, 30 seconds, one minute, five minutes, ten minutes, and twenty minutes. Following exposure to UV light, the isolates were incubated at 10°C and grown for 2 weeks.

Figure 2 Example of UV experiment plate setup. Each dot represents a 10 μL aliquot marine broth containing an individual isolate.



Results and Discussion

Isolate Classification

Following DNA extraction, the DNA concentration results were: 232 ng/µL BF02_S3, 21.2 ng/µL WLB02_22m8, 31.3 ng/µL DTM06_05, 61.2 ng/µL BF11_BFM03, and 6.13 ng/µL BF02_Schw. Differences in the amount of DNA concentration can be attributed to a variety of factors including the initial cell density and strength of cell wall. Resistance to lysozyme and proteinase K can prevent successful cell lysis, preventing downstream steps of DNA extraction from working as efficiently. Sufficient DNA for each isolate, however, was obtained to successfully run a PCR to amplify the 16S rRNA gene. Results from the BLAST analysis of the sequenced 16S rRNA genes returned as follows: BF11_BFM03 as *Planococcus* sp., DTM06_05 as *Arthrobacter* sp., BF02_S3 as *Psychrobacter* sp., WLB02_22m8 as *Marinobacter* sp., and BF02_Schw as *Shewanella* sp (Table 1).

The isolate BF02_S3's closest type strain is *Psychrobacter glacincola*, as indicated in Figure 3. BF02_S3 and *P. glacincola* have 99.5% sequence identity. Also, as seen in BF02_S3, *P. glacincola* is described as having an off-white color and originates from Antarctic sea ice (Bowman et al., 1997). Other BF02_S3 related clones originate from a variety of environments, such as extreme acidic pit lakes of the Iberian Pyrite Belt, permafrost soil from the Kunlun Mountain Pass in the Tibet Plateau of China, and coalbeds located in Eerduosi Basin China. While these locations are diverse, they all have a common theme of cold, exposed conditions that make life challenging.

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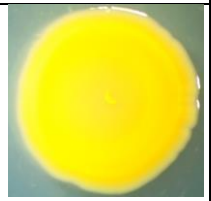
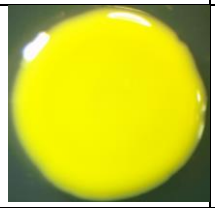
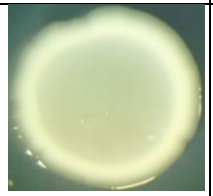
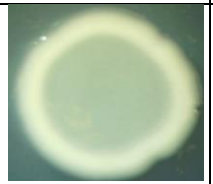
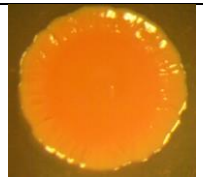
Table 1: Identification of isolates used in study. Pictures provided were representative of each isolate when grown on Marine Agar at 10°C.

Strain Name	Putative Classification	Location	Color	Year Collected	Characteristics	Picture	References
BF11_BFM03	*Planococcus* sp.	Taylor Glacier Moraine	Orange	2011	Circular, Entire, Smooth, Raised		This study
DTM06_05	*Arthrobacter* sp.	Taylor Glacier Moraine	Yellow	2006	Circular, Entire, Smooth, Raised		This study
BF02_S3	*Psychrobacter* sp.	Blood Falls Shoreline	White	2002	Circular, Entire, Concentric, Raised		This study
WLB02_22m8	*Marinobacter* sp.	West Lake Bonney water column (22m)	Tan	2002	Circular, Entire, Concentric, Raised		This study
BF02_Schw	*Shewanella* sp.	Blood Falls Surface	Pink/ Orange	2002	Circular, Serrate, Rugose, Raised		Mikucki and Priscu 2007

The isolate WLB02_22m8's closest known relative is *Marinobacter antarcticus* with 98.0% identity. *M. antarcticus* is described as having a brown pigmentation while WLB02_22m8 had a light tan pigmentation (Liu et al., 2012). Clones closely related to WLB02_22m8 originate from Polar Regions like the Supraglacial spring system in Canadian high arctic, Lake Vida in Antarctica, and Antarctic sandy intertidal sediments. These locations share the common characteristic of extreme cold and high salinity, indicating that WLB02_22m8 and related clones prefer, and have adapted to, sparse and cold environments. Also, as indicated in Figure 3, WLB02_22m8 is closely related to previously isolated BF14_3D isolate from the BF brine collected in 2014.

The isolate BF02_Schw's closest known relative is *Shewanella arctica* with 99.6% sequence identity. Similar to BF02_Schw distinct pink/orange pigmentation, S. *arctica* has been identified as having a tanned orange pigmentation (Kim et al., 2012). BF02_Schw has many closely related clones that originate from Antarctica and similar environments such as Baltic Sea ice and a subarctic fjord. Similar to what was observed with the relative of WLB02_22m8, the closely related clones of BF02_Schw share a common theme of cold and icy environments.

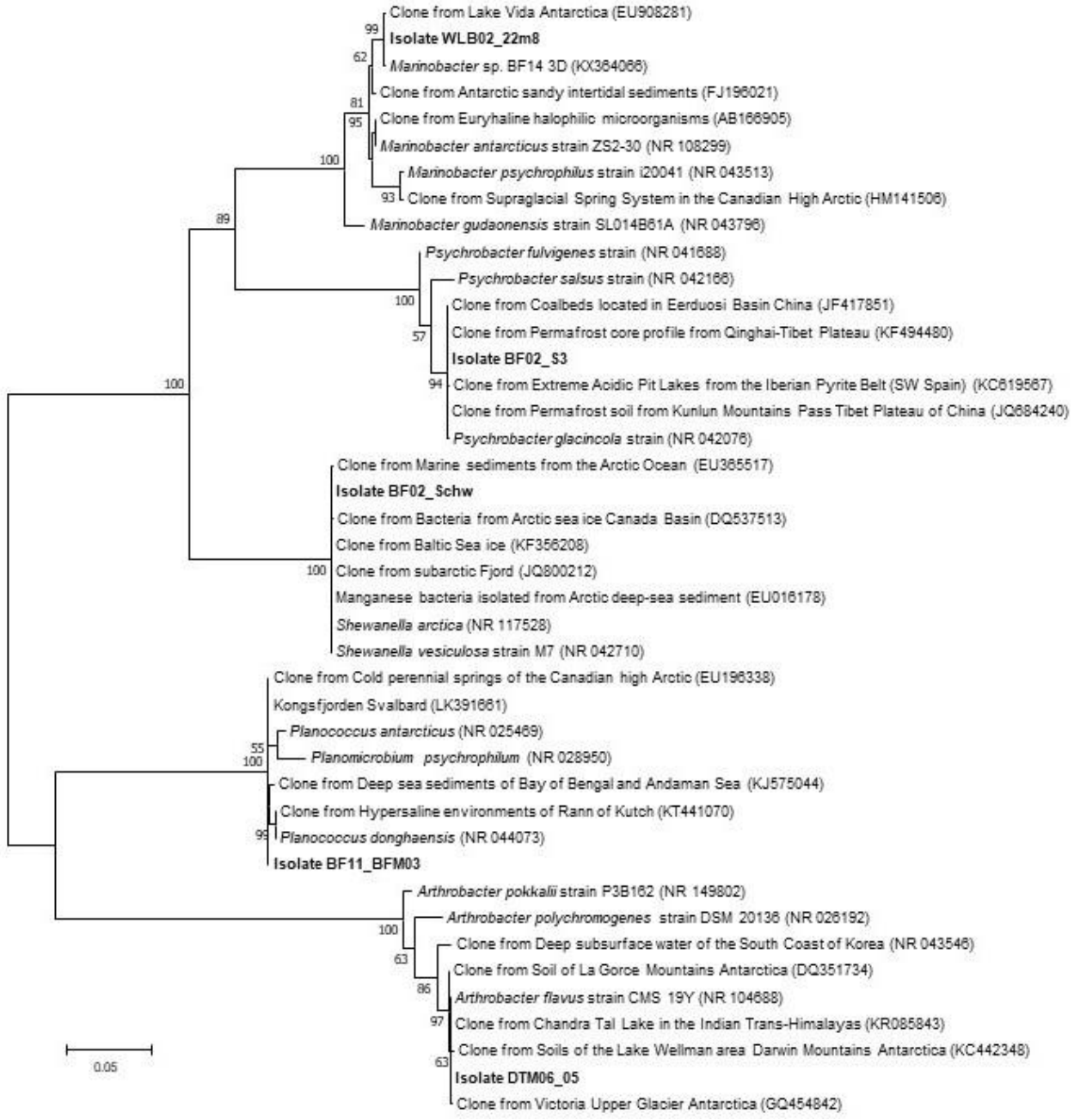
The isolate BF11_BFM03's closest known relative is *Planococcus donghaensis* with 99.5% sequence identity. *P. donghaensis* is described as having orange pigmentation as seen in BF11_BFM03 (Choi et al., 2007). The clone relatives of BF11_BFM03 are geographically diverse ranging from spring of the Canadian high arctic to the Andaman Sea and Rann of Kutch. However, these locations all have high saline content, indicating that BF11_BFM03 and its relatives thrive under conditions of high salt content.

The isolate DTM06_05's closest known relative is *Arthrobacter flavus* with 100% sequence identity. *A. flavus* has a yellow pigmentation like DTM06_05 (Reddy et al., 2000).

DTM06_05 has close clone relatives from Lake Wellman area in the Darwin Mountains of Antarctica, Chandra Tal Lake in the Indian Trans-Himalayas, and deep subsurface water of the South Coast of Korea. Similar to BF11_BFM03, the location of these closely related clones originate from diverse geographic locations. Each clone location, though, is a body of water, indicating that DTM06_05 and its relative may grow best in a wet or aquatic environment.

The classification of each isolate through the sequence of their 16S rRNA gene allows the comparison to closely related organisms, helping to confirm the identity of each organism while learning more about the possible characteristics they may contain. While each isolate had a related clone that originated from Antarctica or a related region, many also had relatives located geographically far away. Having relatives from distinct and distant locations can provide insight to environmental features at which each isolate can thrive. For instance, while the relatives of BF11_BMF03 prefer a salty environment, DTM06_05 relatives thrive in a wet environment. Comparisons between these isolates indicate that each have developed many adaptations to live in these cold, salty, exposed environments, which, in the context of this study, calls into question what adaptations they may have to survive UV light exposure.

Figure 3 Phylogenetic tree of BF11_BFM03, DTM06_05, BF02_S3, WLB02_22m8, and BF02_Schw with closely related clones and type strains. Isolates from this study are indicated in bold.



Pigments

Each isolates identified in this study had pigmentation matching, or very close to their respective closest known relative. Orange pigmentation, as seen in BF11_BFM03 and BF02_Schw, as previously described, are often associated with carotenoids. Thus, it is reasonable to assume that isolates such as BF11_BFM03, BF02_Schw, and DTM06_05, that are each brightly pigmented, may contain carotenoids, or carotenoid-like pigment molecules that would aid in their survival under intense UV exposure. Isolates, like WLB02_22m8 and BF02_S3, which lack bright pigmentation may be expected to struggle under UV pressure unless they contain separate mechanisms, such as DNA repair pathways, to deal with this intense light.

Ultraviolet Resistance

As exposure to UV light increased, each isolate demonstrated various changes to their colony size and physical characteristics. As shown in Table 2, each of the five isolates were able to withstand thirty seconds of UV exposure. However, within one minute of UV exposure, a dramatic decrease in the colony size of WLB02_22m8 was observed. While the colony size of WLB02_22m8 greatly decreased at the one minute mark and persisted with increasing UV time points, small colonies survived up to five minutes of UV exposure. Each of the remaining four isolates proved to be more resistant to UV exposure as they all had at least one colony survive at the twenty minute time point. Similar to WLB02_22m8, the colonies of the other four isolates decreased in size dramatically, some also displaying morphological changes as well. As shown in Table 3, at the one minute UV exposure time point, BF11_BFM03, DTM06_05, and BF02_S3 each grew in a ring-like shape with smaller colonies growing within. This colony morphology disappeared at the five minute time point as further UV exposure decreased the overall colony

size and survival rate. Also depicted in Table 4, BF02_Schw takes on a wrinkled morphology after thirty seconds of UV exposure. As UV exposure time increases, BF02_Schw also forms an irregular shape around its edges. However, the wrinkled and irregular colony structure of BF02_Schw disappears at the 10 minute time point as the colony becomes smooth and shrinks in size.

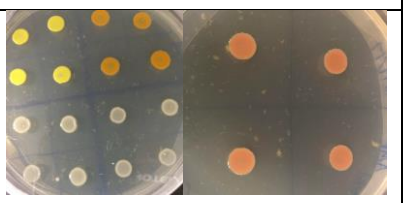
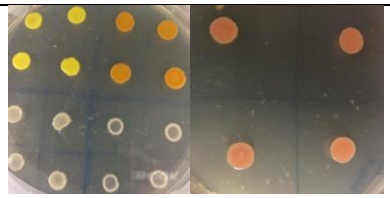
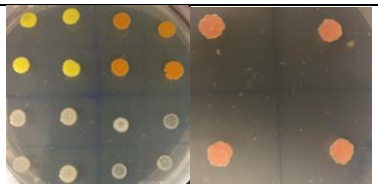
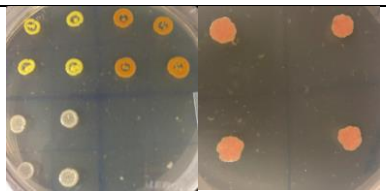
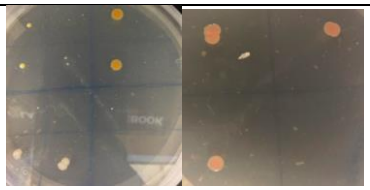
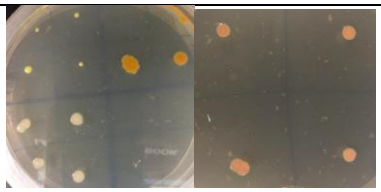
DTM06_05, yellow pigmented, and BF02_Schw, pink/orange pigmented, had the highest overall survival under UV exposure. Surprisingly, BF02_S3, which lacks colorful pigmentation, had a greater survival than BF11_BFM03 that is orange pigmented. These results may indicate that pigmentation, while being one of the possible mechanisms to combat UV damage, is not required for UV resistance in this/these isolates. Furthermore, the depth at which this isolate was collected could provide insight to how each respond to ultraviolet light. DTM06_05, BF02_S3, BF02_Schw, and BF11_BFM03 were all collected from surface environments while WLB02_22m8 was collected at 22 meters depth in the water column. Thus, because BF02_S3 was able to survive on the ice surface without pigmentation, it may have developed other unknown mechanisms to combat light exposure. This could explain its resilience in the presence of germicidal UV light. Contrasting, WLB02_22m8, another isolate lacking pigment, was collected at 22 meters depth in the water column and did not require similar adaptations to combat light exposure. This provides understanding of why WLB02_22m8 did not perform as well as BF02_S3 under ultraviolet exposure although they each lacked pigmentation.

AntiSMASH

To examine possible metabolites responsible for the pigmentation and UV resistance of the *Shewanella* sp. Isolate BF02_Schw, a previously sequenced draft genome was examined using antiSMASH. The antiSMASH web server identified four secondary metabolite gene

clusters within the BF02_Schw genomic DNA data. An Aryl Polyene gene was identified within the first gene cluster of the antiSMASH results (Draft genome contig 6). Aryl Polyenes are bacterial pigments, similar to carotenoids, which aid in protection from reactive oxygen species within the cell (Schöner et al., 2016). As bacterial cells are exposed to increasing amounts of UV stress, there is an increase in reactive oxygen species (Órdenes-Aenishanslins et al., 2016). The reactive oxygen species produced under stressful conditions damages DNA, lipids, and proteins within the cell (Órdenes-Aenishanslins et al., 2016). Thus, the presence of an Aryl Polyene gene within the BF02_Schw genome could indicate how it survived under prolonged exposure to UV light. The ability of BF02_Schw to protect its genetic material from both UV light and also oxidative stress would be a selective advantage for growth at the surface Blood Falls. Interesting, the Aryl Polyene pigments give off a yellow color. This could indicate that the Aryl Polyenes work in conjunction with other unknown pigments within BF02_Schw to produce its distinct pink/orange pigmentation (Jenkins and Starr 1985). Thus, while the Aryl Polyene gene was found in the BF02_Schw genome, other undiscovered secondary metabolite gene may also be present.

Table 2: Growth under Ultraviolet Exposure. The plate on the image on the right shows the growth of BF02_Schw (pink) under increasing UV exposure. The images on the left show the growth of DTM06_05 (yellow, top left), BF11_BFM03 (orange, top right), WLB02_22m8 (tan, bottom right), and BF02_S3 (white, bottom left). The last row titled "Total Colony Count" represents the amount of colonies that survived for each isolate at each time point combined.

Length of Ultraviolet Exposure	BF11_ BFM03	DTM 06_05	BF02_ S3	WLB02 _22m8	BF02_ Schw	Growth
No exposure	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	
10 seconds	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	
30 seconds	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	
1 minute	+ + + +	+ + + +	+ + + +	- + + +	+ + + +	
5 minutes	+ + - -	+ - + -	- - + +	+ - - -	+ + + -	
10 minutes	- - + +	+ + + +	+ + + +	- - - -	+ + + +	

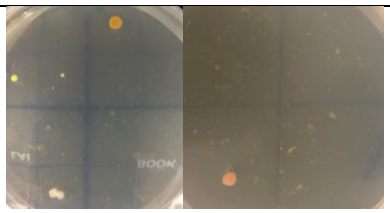
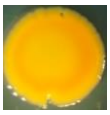
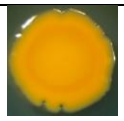
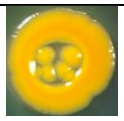
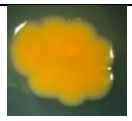
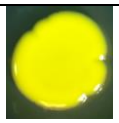
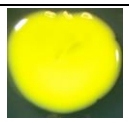
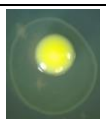
20 minutes	+ - - -	- - + +	- - - +	- - - -	- - + -	
Total Colony Count	+21 -7	+24 -4	+23 -5	+16 -12	+24 -4	

Table 3: Morphological changes of isolates. Shown below are changes in shape, size, and structure as each isolate accommodates to increased exposure to Ultraviolet light.

Isolate	10 sec	30 sec	1 min	5 min	10 min
BF11_BFM03					
DTM06_05					
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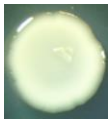
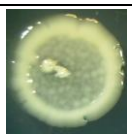
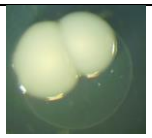
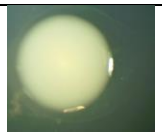
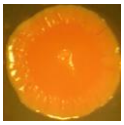
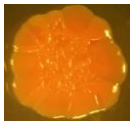
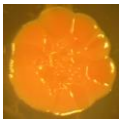
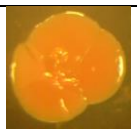
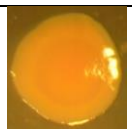
BF02_S3					
BF02_Schw					

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

Bowman, J. P., D. S. Nichols, and T. A. McMeekin. "Psychrobacter glacincola sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic sea ice." *Systematic and Applied Microbiology* 20.2 (1997): 209-215.

Campen, R. L. "Investigating the mode of action of tuberculosis drugs using hypersensitive mutants of Mycobacterium smegmatis." (2015).

Choi, Jeong-Hwa, et al. "Planococcus donghaensis sp. nov., a starch-degrading bacterium isolated from the East Sea, South Korea." *International journal of systematic and evolutionary microbiology* 57.11 (2007): 2645-2650.

Ehling-Schulz, M. & S. Scherer (1999) UV protection in cyanobacteria, *European Journal of Phycology*, 34:4, 329-338, DOI: 10.1080/09670269910001736392

Jenkins, C. L., and M. P. Starr. "Formation of halogenated aryl-polyene (Xanthomonadin) pigments by the type and other yellow-pigmented strains of Xanthomonas maltophilia." *Annales de l'Institut Pasteur/Microbiologie*. Vol. 136. No. 3. Elsevier Masson, 1985.

Karentz, D., J. E. Cleaver, and D. L. Mitchell. "Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation." *Journal of Phycology* 27.3 (1991): 326-341.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data.*Bioinformatics*, *28*(12), 1647-1649.

Kim, So-Jeong, et al. "Shewanella arctica sp. nov., an iron-reducing bacterium isolated from Arctic marine sediment." *International journal of systematic and evolutionary microbiology* 62.5 (2012): 1128-1133.

Liu, Chang, et al. "Marinobacter antarcticus sp. nov., a halotolerant bacterium isolated from Antarctic intertidal sandy sediment." *International journal of systematic and evolutionary microbiology* 62.8 (2012): 1838-1844.

Mikucki, J. A., and J. C. Priscu. "Bacterial diversity associated with Blood Falls, a subglacial outflow from the Taylor Glacier, Antarctica." *Applied and Environmental Microbiology* 73.12 (2007): 4029-4039.

Mojib, Nazia. *Study of the cold adaptive mechanisms in Antarctic bacteria and investigation of the chemotherapeutic and antimicrobial potential of their pigments*. Vol. 72. No. 08. 2011.

"Nationalgeographic-Maps - INTERKART GmbH." *National Geographic Maps - antique historical maps and atlas maps*, www.nationalgeographic-maps.com/national-geographic-atlas-wall-maps/antarctica.html#.

Órdenes-Aenishanslins, N., et al. "Pigments from UV-resistant Antarctic bacteria as photosensitizers in Dye Sensitized Solar Cells." *Journal of Photochemistry and Photobiology B: Biology*162 (2016): 707-714.

Pruesse, E., Peplies, J. and Glöckner, F.O. (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics, 28, 1823-1829

Reddy, G. S., et al. "Arthrobacter flavus sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica." *International Journal of Systematic and Evolutionary Microbiology* 50.4 (2000): 1553-1561.

Schöner, T. A., et al. "Aryl polyenes, a highly abundant class of bacterial natural products, are functionally related to antioxidative carotenoids." *Chembiochem* 17.3 (2016): 247-253.

Sudhir Kumar, G. Stecher, and K. Tamura (2015) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution (submitted).

Tig6Polished - 4 clusters - antiSMASH results, antismash.secondarymetabolites.org/upload/bacteria-7c5d6a45-008c-44cd-97e3-bba866b6c888/index.html

Appendix

16s rRNA sequences

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CTAB DNA Extraction

(Version 1.1)

Materials:

Reagent	*Concentration*	*Storage*	*Notes*
TE Buffer	1x	RT	
Lysozyme	50 mg/mL	4 °C	
Proteinase K	10 mg/mL	-20 °C	
SDS	10% (w/v)	RT	
NaCl	5M	RT	
CTAB solution	10 % (w/v) with 4 % NaCl (w/v)	4 °C	Pre-warm this solution to 65 °C to help re-dissolve
Isopropanol	90 – 100%	RT	Pre chill on ice
Ethanol	70%	RT	Pre chill on ice
PCR grade H_2O	n/a	4 °C	
Chloroform		Chem Hood	

Equipment

Water bath set to 65 °C
Water bath set to 80 °C
Heating block initially set to 37 °C.

Protocol:

1) Spin down 10 mL of liquid culture at 2700 xg for 20 min to pellet cells
   - Spin longer if necessary to fully pellet the cells
2) Re-suspend the pellet in 400 µL of 1x TE Buffer and transfer to a 1.5 or 2 mL microcentrifuge tube
   - Bring OD below 1.0 A
   - Add 2 mL TE buffer to pellet, pipet to mix, then take OD
3) Heat kill the cells at 80 ˚C for 20 min and then allow to cool to room temperature on tube rack
4) Add 0.5 mg of lysozyme and incubate at 37 ˚C for 1 hour in a heat block
   - Add 10 uL of a 50mg/mL Lysozyme stock. Vortex and spin down Lysozyme before use
5) Add 0.15 volumes of 10% SDS and 0.01 volumes of proteinase K
   - If following exactly: 61.5 µL 10% SDS; 4.1 uL proteinase K
6) Briefly vortex sample to mix, then incubate at 65 ˚C for 10 min in a water bath
   - Only vortex enough to mix, don't over vortex
7) Add 0.2 volumes of 5 M NaCl and 0.2 volumes of CTAB (pre-warmed to 65 ˚C)
   - If following exactly: 95.1 µL of 5M NaCl and 95.1 µL of CTAB mix
8) Invert until the solutions turns white then incubate at 65 ˚C for 10 min in a water bath
   - Only vortex enough to mix, don't over vortex
9) Add 1 volume of chloroform
   - If following exactly: 660 µL of Chloroform
10) Invert briefly and centrifuge at 12,000 xg for 8 min to separate the aqueous and organic phases
    - Only vortex enough to mix, don't over vortex
11) Remove the aqueous phase (top layer) and add 0.6 volumes of isopropanol to precipitate out the DNA

- Prioritize clean removal of the aqueous phase over maximizing the amount of aqueous phase removed. Look for DNA after addition of isopropanol
- If following exactly: expect around 600 µL aqueous phase; add 360 µL isopropanol

12) Centrifuge at 12,000 xg for 15 min to pellet the DNA
13) Remove all the supernatant and add 1 mL of chilled (4 °C) 70% ethanol
    - There will be a pellet of DNA at the bottom of the tube; be sure not to touch it with your pipette, but do remove as much supernatant as possible.
14) Centrifuge at 12000 xg for 5 min to pellet the DNA
15) Remove all of the ethanol possible with a pipette tip, then let the pellet air dry with the tube lid open, and covered with a clean Kim-Wipe.
    - Take care to not touch the DNA pellet with your pipette
    - Air drying will work faster if more ethanol is removed by pipette. You can spin the tube down after removing most of the ethanol to help removing the last of it.
16) Re-suspend DNA pellet in 50 µL PCR grade H_2O and store at -20 ˚C
    - Gently pipette the water up and down to re-suspend DNA.