Acta Spartae Vol. 2 No. 1 2016
Pages 9–13

Protective Qualities of UV-resistant Bacteria

Sloan Goldberg and Eric Freundt[1]

Department of Biology, University of Tampa, Tampa, FL 33606, [1]Faculty Advisor

ABSTRACT

Ultraviolet radiation causes detrimental effects on the cell by mutating its DNA. *Micrococcus luteus* and *Micrococcus radiophilus* are two bacterial species known to be able to withstand high levels of UV radiation. However, it is unknown if they have any protective effects on other cells, like *Escherichia coli*. Using UVB radiation from a light box, minimum lethal doses (MLD) were determined for *M. luteus*, *M. radiophilus*, and *E. coli*. The MLDs of the *Micrococcus* species were substantially higher than the MLD of *E. coli*. *E. coli* was mixed with the *Micrococcus* species and exposed to UV radiation to test for protective effects. It was found that *E. coli* was able to grow past its MLD in a few cases with both *M. luteus*, and *M. radiophilus*. However, it was noted that exact timing was difficult to obtain with our methods. It was found that the *E. coli* colonies that did grow in higher UV exposure were usually surrounded by a *Micrococcus* species, potentially showing protection. Exploring microbes for their UV protective qualities may provide an alternative for chemical containing sunscreens, offering a potentially safer product for both people and the environment.

1 INTRODUCTION

Non-ionizing radiation is a powerful controlling agent due to its mutagenic effects on the cell (Goodsell, 2001). UV radiation, especially UVB and UVC, causes mutation by creating thymine dimers, or cyclobutane pyrimidine dimers (Cooper, 2000). Thymine dimers are a major mutation in the DNA strand because they cause a kink in which results in an unreadable and dysfunctional DNA strand (Piersen et al., 1995). In order to overcome this, cells use a process called nucleotide excision repair to fix the damage caused by UV radiation (Grossman & Leffell, 1997). Nucleotide excision repair uses a multi-subunit enzyme called UVrABC, or exonuclease, to recognize damage-induced structural alterations in DNA, including thymine dimers (Goodsell, 2001). Once detected and removed by UVrABC, the DNA can be repaired and replication can continue (Cooper, 2000).

The ability of certain bacterial cells to resist UV light usually depends on the efficiency of its repair mechanisms. The better the cells repair mechanism, the less UV sensitivity it will display (Setlow & Carrier, 1964). Conversely, if a cells DNA repair mechanisms are unable to keep up with the damage, and the dimer continues to go uncorrected, it will be incorporated into DNA during mitosis, allowing the mutations to multiply and build up in the cell (Goodsell, 2001). The accumulation of mutations in the cell will eventually render the cell dysfunctional, killing the cell (Setlow & Setlow, 1962).

Several species of *Micrococcus* have shown high levels of resistance to UV radiation (Lavin et al., 1976). *Micrococcus radiophilus*, has shown resistance towards UV and x-ray radiation. The ability of *M. radiophilus* to defy UV radiation is accredited to its rapid and efficient excision repair (Lavin et al., 1976). In addition to this, *M. radiophilus* has proven to be more resistant than other *Micrococcus* strains, like *M. radiodurans*, suggesting that different strains of *Micrococcus* have varying levels of resistance and may use varying methods (Lewis & Kumta, 1972). Production of an enzymatic pigment could potentially be what gives *Micrococcus* species their high UV tolerance (Lewis & Kumta, 1972).

Micrococcus luteus can be found in many places such as the human skin, water, dust, and soil. It is considered a normal part of human skin flora. *M. luteus* is being studied by Promar AS, a biotechnology company, in order to find pigments to potentially use in future sunscreens (SINTEF, 2013). Despite this intriguing research, the information gathered regarding the cellular components of the ability of *M. luteus* to protect itself from UV radiation is inconclusive. The majority of studies focus on endonuclease activity and its ability to repair dimers in DNA. These studies on endonuclease activity have found that the endonuclease isolated from *M. luteus* resembled the *Escherichia coli* repair proteins, endonuclease III and MutY, however not endonuclease V (Piersen et al., 1995). This suggests that the repair mechanism for *Micrococcus* is similar to a UV-sensitive cell, like *E. coli*.

It is currently unknown if *M. luteus* has the ability to protect other cells from UV radiation. Many studies have opposing views about *M. luteus* and its ability to deal with ultraviolet radiation. While some studies concluded *M. luteus* secretes a pigment (SINTEF, 2013), other studies found that the UV resistance is due to enzymes regarding its excision repair (Carrier & Setlow, 1970). Unlike *M. luteus*, there are no studies to suggest that *M. radiophilus* uses any type of pigments as protection against the UV radiation, and instead, resistance is likely due to the excision repair system (Lavin et al., 1976). This study sought to examine mechanisms of UV protection from bacterial cells by testing the survival of *E. coli* at varying time doses of UVB radiation while alone, and mixed with *M. luteus*, and *M. radiophilus*. If protective pigments are present in *M. luteus* and *M. radiophilus*, it is hypothesized that *E. coli* would be able to survive past the time its growth was completely inhibited when exposed alone to UV radiation.

It is important to study UV resistant bacteria because UV radiation is a major threat to humans when spending time in the sun. To protect from UV damage, UV filters such as sunscreen are commonly used. However, UV filters in sunscreens have been found to be potential health hazards due to the absorption of the compounds through the skin (Kilmová et al., 2015). Additionally, multiple inorganic and organic compounds in sunscreen are damaging to coastal ecosystems such as delicate coral reefs (Downs et al., 2016). Exploring microbes for their potential UV protective qualities may provide an alternative for the current UV filters used in sunscreens, ultimately leading to a product that may be safer for both people and the environment.

2 MATERIALS AND METHODS

Bacterial Isolates

Micrococcus radiophilus, *Micrococcus luteus* and *Escherichia coli* isolates were grown in The University of Tampa Microbiology Laboratory. Pure samples of *M. radiophilus*, *M. luteus*, and *E. coli* were obtained from Carolina Biological Supply. The pure cultures were used to make a streak plate in order to obtain isolated colonies. Nutrient Agar was used as the media for all three bacterial species.

Using aseptic technique, streak plates were created for each bacterial species. *M. luteus* and *E. coli* were incubated at a temperature of 37 °C, while *M. radiophilus* was incubated at 30 °C. *E. coli* was incubated for 24–48 hours, while *M. radiophilus* and *M. luteus* were incubated 3–4 days for necessary growth.

Minimum Lethal Dose

In order to determine the Minimum Lethal Dose (MLD) for *E. coli*, *M. luteus* and *M. radiophilus* 18 plates were made using nutrient agar. One colony of *E. coli* was taken from the isolated colonies previously grown, and placed into 5 mL of nutrient broth. The nutrient broth mixture was vortexed to ensure the bacteria was evenly distributed throughout the culture. A sterile swab was placed into the broth and then spread evenly onto 6 nutrient agar plates. This process was repeated for *M. luteus*, and *M. radiophilus*, which were also plated onto 6 nutrient agar plates.

The 6 plates containing *E. coli* were exposed to UVB radiation of 302 nm at intervals of: 0 s, 10 s, 20 s, 40 s, 60 s, and 120 s. The exposed plates were then incubated at 37 °C for 48 hours. The 6 plates containing *M. luteus* were divided in half and exposed at 12 different time increments that ranged higher than for *E. coli*: 0 s, 10 s, 20 s, 40 s, 60 s, 120 s, 240 s, 480 s, 960 s and 1920 s. The exposed plates were incubated at 37 °C for 48–72 hours. This process was repeated for the remaining 6 plates containing *M. radiophilus* however, it was incubated at 30 °C for 4 days. This procedure was not replicated as we were just finding an increment of time that successfully killed each of the bacterial species.

Broth Dilution Mixtures

To test for the presence of protective qualities in the *Micrococcus* species, *E. coli* was mixed with both species, *M. luteus* and *M. radiophilus* separately. The first mixture was the combination of *E. coli* and *M. luteus*, and to combine them a broth dilution was created using 5mL of sterile nutrient broth into a sterile 10 mL tube. Using a sterile loop, one colony of *E. coli* was added to the nutrient broth. Then, using another sterile loop, two large colonies were added to the same 5 mL of nutrient broth.

The nutrient broth mixture was then vortexed to ensure the bacteria was evenly distributed throughout the tube. The mixture was transferred onto 9 sterile nutrient agar plates using a sterile swab. The sterile swab was dipped into the broth and was evenly spread onto the plate, using a new swab for each plate. The 9 plates were then divided in half, and were labeled according to the UV exposure they would receive. The first two increments of exposure were 30 s and 35 s, followed by 38 s and 42 s, and increasing to 45 s and 50 s. Each of these three plates were repeated two more times resulting in three replicates of each time increment. This exact procedure was repeated with *E. coli* and *M. radiophilus*.

UV radiation exposure

In order to expose the mixtures to UV radiation, a light box was used. The UV light box was given at least 10 minutes before each trial to warm up. The 18 plates consisting of *E. coli* and *Micrococcus* mixtures were then exposed to UV radiation at a wavelength of 302 nm. The plates were exposed accordingly to the time increment assigned to the specific plate. To expose the same plate to two different time increments, the plate was positioned in such a way that when the lowest time increment on the plate was met, that half was pulled out of the UV light. This left the remaining half of the plate exposed to the UV light until the time specified was met. This process was repeated for all plates and their replicates: 3 plates of 30 s and 35 s, 3 plates of 38 s and 42 s, and 3 plates of 45 s and 50 s (for each *Micrococcus* species). The 18 plates were then incubated for 72 hours at 37 °C.

3 RESULTS

Minimum Lethal Dose

MLD was determined by finding a time increment that killed all of the bacteria. For *E. coli*, there were no colonies present after 40 seconds of being exposed to 302 nm UV radiation (Table 1). Also notable was the amount of *E. coli* colonies present after 10 and 20 seconds of being exposed to 302 nm (Table 1). After 10 seconds, only 6 colonies remained, and at 20 seconds only 8 colonies were present (Table 1).

Species	MLD
Escherichia coli	40 s
Micrococcus luteus	120 s
Micrococcus radiophilus	240 s

Table 1. Determined MLD of E. coli, M. luteus, and M. radiophilus. The Micrococcus species have a substantially higher MLD when compared to E. coli. Signifies the sensitivity of E. coli when exposed alone to UV radiation.

Exposure Time (s)	Surviving Colonies
10	6
20	8
40	0
60	0
120	0

Table 2. Table depicting the MLD experiment done on *E. coli*. Even at low exposure to UV radiation, *E. coli* shows very few colonies. After any exposure above 20 seconds, there are no colonies that survived.

The MLD of *M. radiophilus* at 302 nm was determined to be 240 seconds and the MLD for *M. luteus* was determined to be 120 seconds (Table 2). There were sixteen colonies growing on the inside of the 120 seconds plate for *M. luteus*, but no colonies in

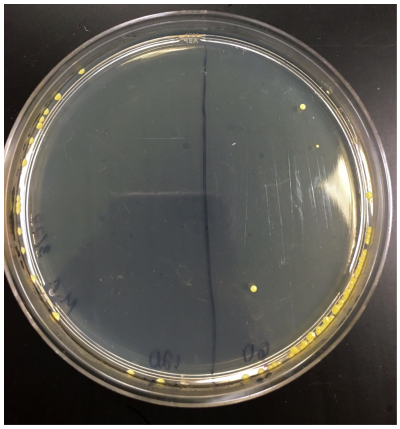

Figure 1. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

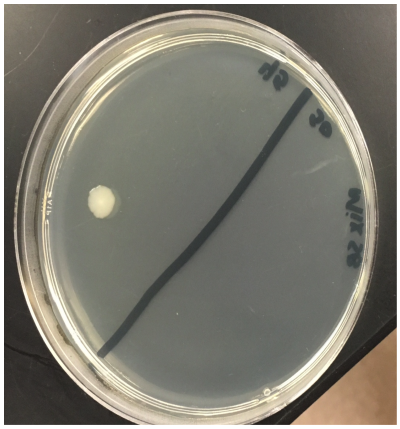

Figure 3. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

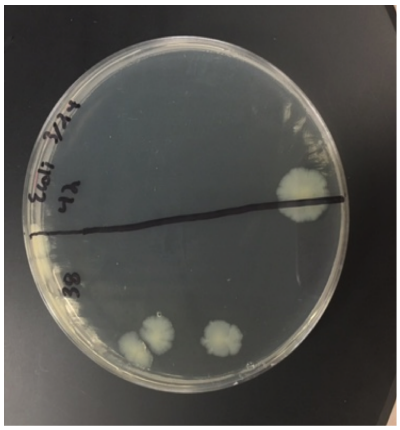

Figure 2. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

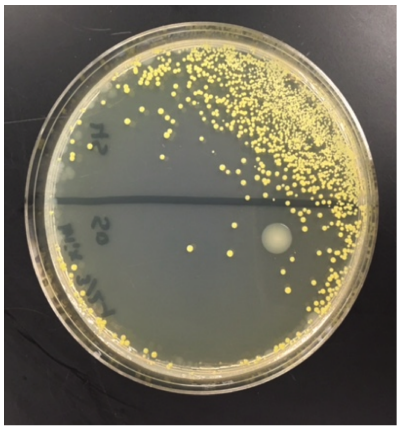

Figure 4. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

the center or reaching away from the plastic side (Figure 1). In the control plate of *M. radiophilus*, which had no exposure, many types of microorganisms were able to grow including mold and other possible contaminants (Figure 5). The control plates of *E. coli* in the mixture portion of the project were used to further specify the MLD. Growth of *E. coli* colonies varied (Table 1), at every time point at least one of the replicates was able to produce colonies. Two of the plates at 42 seconds showed growth, but one of the 42 seconds plates had a colony growing in the middle near the dividing line (Figure 2). The dividing line was separating 42 seconds, which is over the determined MLD, and 38 seconds, which is under the MLD of *E. coli*. Plates exposed to UV radiation for 30 seconds and 35 seconds were able to inhibit growth in more than one replicate (Figure 6).

Protective qualities of *M. radiophilus* and *M. luteus*. After UV exposure of a mixture of *E. coli* with each Micrococcus species, each time increment was checked for growth of *E. coli* around or above the determined MLD (40 seconds). In the *E. coli* and *M. radiophilus* mixture, it was shown that there was growth in every time increment, but there was inconsistency among the three experimental replicates (Figure 7). Growth was observed in time increments above the MLD, however there were never three replicates of the same time increment that produced the same outcome (Figure 7). For the 45-second increment of the *M. radiophilus* mixture, 33%, (Figure 3 and 7) had growth and for the 50-second increment, two of the three replicates for each had growth (66%; Figure 7).

E. coli was mixed with *M. luteus* and exposed to 302 nm of UV radiation for the same time increments; the results were similar to that of *M. radiophilus* (Figure 7). In the mixture plates with *M. luteus*, one colony of *E. coli* was able to grow after 50 seconds of UV exposure, which was 10 seconds longer than the determined MLD for *E. coli* alone (Figure 4 and Table 1). Out of the Micrococcus sp. And *E. coli* mixtures, 33% of the plates were able to grow colonies

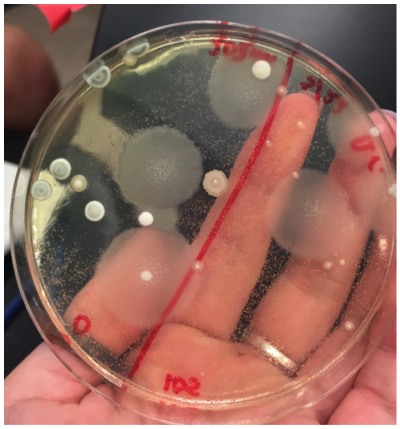

Figure 5. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

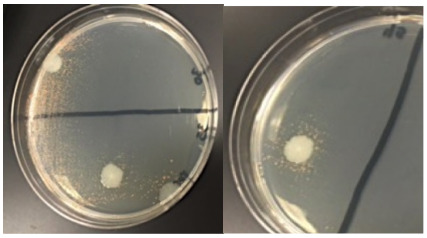

Figure 6. MLD determining plate of *M. luteus*. The left side of the plate was exposed to UV radiation for 120 seconds. Colonies are only growing on the side of the plate

in both the 45 second, and 50-second interval. However, no plates exhibited colonies growing after 42 seconds of exposure (Figure 7). Of the replicates of Micrococcus mixtures produced, 66% of the replicates that were increments under the determined MLD (30 s, 35 s, and 38 s) exhibited growth, leaving the remaining 33% void of any colonies (Figure 6).

4 DISCUSSION/CONCLUSION

Studying UV resistant microbes may potentially result in an eco-friendly filter to protect against non-ionizing radiation. *Micrococcus* is a genus of bacteria that is know to have high tolerance to Ultraviolet radiation (Lavin et al., 1976). However, it is unknown what mechanisms allow there bacteria withstand high UV exposure. Some studies have suggested a highly developed excision repair mechanism (Lewis & Kumta, 1972), while more modern studies have suggested secretion of a protective protein (SINTEF, 2013).

In an effort to test the protective qualities of different *Micrococcus* species, we exposed mixtures of *Escherichia coli* and *Micrococcus luteus*, and *E. coli* and *Micrococcus radiophilus* to multiple time increments of ultraviolet radiation. It was experimentally determined that *M. luteus* and *M. radiophilus* showed high minimum lethal doses to ultraviolet radiation when compared to *E.*

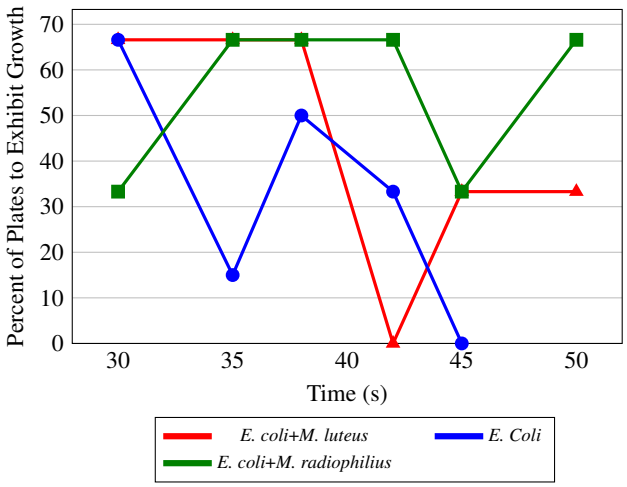

Figure 7. Chart depicting percent of growth of *E. coli* colonies for the control *E. coli* plates and the mixtures of *M. luteus* and *M. radiophilus*

coli. This suggests that the *Micrococcus* species have some way of dealing with UV-radiation. However, based on the results, we cannot be certain if this protection is due to excision repair, secretion of a protective protein, or both. More data would need to be collected and statistically analyzed in order to rule out random chance and confirm a protection method.

As seen from the literature, an experiment like this has not been done for decades and is not a topic that has been extensively explored. Many studies of this nature were conducted in the 1960s and 70s, including papers done by Lavin et al. (1976), and Setlow & Carrier (1964). The aim of these experiments was to determine how *Micrococcus* species endure exposure to UV radiation, however no conclusions could ever be made (Lavin et al., 1976). This study hopes to serve as a fresh start for an abandoned topic. The potential protective qualities of *Micrococcus* species were based on the original minimum lethal dose determined for *E. coli* (40 seconds). It was shown that even at low time increments (10 and 20 seconds), *E. coli* exhibited major sensitivity to UV radiation. This is an important observation because it indicated how sensitive *E. coli* is when exposed to UV radiation when compared to *Micrococcus* species.

For the mixture plates containing *E. coli* and *M. luteus*, colonies of *E. coli* were observed on both the 45 and 50-second exposure plates. Similar findings were observed for the mixture of *E. coli* and *M. radiophilus* plate, with colonies of *E. coli* surviving the 45 and 50 second exposure. This kind of survival was not seen in the MLD plates for *E. coli* alone, suggesting that *E. coli* could have been protected. However, it is important to note that one control plate surpassed the determined MLD for *E. coli* (40 seconds) with 33% growth appearing at 42 seconds. The exact cause of this potential experimental error is not known. Due to discrepancies within replicates, a clear relationship couldnt be determined statistically, as there is not a clear slope defining each relationship. It was noticed that there were never three replicates of the same time increment that produced the same outcome (Figure 7). If this experiment was replicated, increasing the amount of *Micrococcus* and *E. coli* could

ensure that enough bacterial cells were on the plate. More bacterial cells could potentially allow for a more consistent outcome.

An important observation was that the *E. coli* colonies that survived the higher time intervals (45 and 50 seconds) were surrounded by many colonies of *M. luteus* or *M. radiophilus* (Figure 8). This could potentially represent protection of the *E. coli* colonies due to secretion of an enzyme or pigment by the *Micrococcus* species. Another observation regarding the growth of *E. coli* and *M. luteus* colonies is that in higher time intervals (above 38 seconds and above 120 seconds, respectively) growth was only observed around the rim of the agar plate. However, these colonies were not considered as growth resulting from UV-resistance, as they could have been a result of protection from the plastic lip of the plate.

The varied results obtained could be attributed to multiple factors. A main potential error may have resulted in human reaction time. Even though a timer was used, it was difficult to remove the plates from the UV exposure at the exact time since more than one plate was being exposed at once. Another possible error could have come from the UV light box itself considering we didnt know if it had consistent exposure. Although we attempted to maintain consistency for each trial, we do not know how the strength of the UV machine in the lab changed with time.

Another potential error with this experiment is contamination. While having the plates open on the UV machine, there was exposure to air and other potential contaminants on the table. The plate with the most prominent contamination (Figure 5), where the plate contained multiple fungal colonies. Although contamination by fungi could have been prevented with the use of MacConkey agar, we would have been unable to see the ring of Micrococcus around the surviving *E. coli* species. This is concerning because *M. luteus* and *M. radiophilus* were observed to be slow and sensitive growers in the first week of testing, and it is unknown how they would have reacted to contaminants. It is also possible that a contaminant slowed or stopped the growth of the *E. coli*.

A final potential error was in the sampling of *E. coli* and *Micrococcus* species. In each broth dilution mixture, there was more *M. luteus* and *M. radiophilus* than *E. coli*. This was to ensure that there would be enough *M. luteus* and *M. radiophilus* to cover the *E. coli*. However, this may have resulted in insufficient *E. coli* being inoculated onto the plates. All things considered, our observed increase in the MLD of *E. coli* in relation to *M. luteus* and *M. radiophilus* is an intriguing result that should be explored further.

In summary, we are unable to support or challenge the existence of protective qualities in *Micrococcus* species. However, the data displays evidence that protective qualities could potentially exist. It was determined that *Micrococcus* species had a much higher MLD than *E. coli*. It was also determined that *E. coli* was able to grow in time increments higher than its MLD, suggesting protection from the *Micrococcus* species. However, further testing needs to be conducted in order to determine the extent of the qualities *Micrococcus* exhibits. For future studies, more controlled UV radiation should be used; this includes exact warm up and exposure times that need to be strictly followed. Another improvement would be to ensure there are no sampling errors of the *E. coli* and *Micrococcus* species themselves.

REFERENCES

Carrier, W. L. & Setlow, R. B. 1970. J Bacteriol, 102:178–186

Cooper, G. M. 2000. DNA Repair; in The Cell: A Molecular Approach, 2. Sunderland (MA): Sinauer Associates

Downs, C.A., Kramarsky-Winter, E., Segal, R. et al. Arch Environ Contam Toxicol 70: 265

Goodsell, D. S. 2001. The Oncologist. 6 (Cooper, 2000): 298–299

Grossman, D. & Leffell, D. 1997. Arch Dermatol, 13:1263–1270

Klimová, Z., Hojerová, J. & Beránková, M. 2015. Food Chem Toxicol 83:237–250

Lavin, M. F., Jenkins, A. & Kidson, C. 1976. J Bacteriol 126:587–592

Lewis, N.F. & Kumta, U.S. 1972. Biochem Biophys Res Commun 47:1100–1105

Makarova, K. S., Aravind, L., Wolf, Y. I., Tatusov, R. L., Minton, K. W., Koonin, E. V., Daly, M. J. 2001. Microbiol Mol Biol Rev 65:44–79

Piersen, C. E., Prince, M. A., Augustine, M. L., Dodson, M. L. & Lloyd, R. S. 1995. J Biol Chem 270(40):23475–23484

Sánchez-Quilesa, D. & Tovar-Sánchez, A. 2015. Environ Int 8:158–170

Setlow R. B. & Setlow, J. K. 1962. Proc Natl Acad Sci USA. 48(7):1250–1257

Setlow, R. B. & Carrier, W. L. 1964. Proc Natl Acad Sci USA 51:226–231

SINTEF "Super sunscreen from fjord bacteria." ScienceDaily, 6 August 2013