Increased Biopolymer Pigment Production in Bacteria and Fungi Exposed to Ionizing Radiation

By
Sara L. Wilson
Candidate for Bachelor of Science (2020)

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Sara L. Wilson *Date*
Signature of Author

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Neri Oxman *Date*
Certified by: Thesis Advisor

_____

Christine Ortiz *Date*
Certified by: Course 3 Faculty Thesis Reader

_____

Juejun Hu *Date*
Accepted by: Chair, DMSE Undergraduate Committee

Abstract

A major concern for manned space missions is ionizing radiation, which is known to pose both acute and chronic risks to many organisms. It is critical to expand strategies for radiation protection, including utilizing new materials and fabrication methods designed to support and augment health and wellbeing. The Mediated Matter Group in the Media Lab is researching the application of pigments for biocompatible radioprotection. These pigments' properties—including both UV and ionizing radiation absorption—lend themselves to interesting potential applications in biomedicine and biotechnology[1,2]. Some bacteria and fungi respond to ionizing radiation with enhanced growth and pigment production, and they have been found in a variety of extreme and high radiation environments[3]. This thesis is an exploration of the potential of pigments, like melanins and carotenoids, to protect from and react to ionizing radiation in the context of space.

Certain bacteria and fungi show a remarkable ability to persist, and even thrive, in high-radiation environments[4]. The bacteria of interest in this study are *Bacillus subtilis* and *Rhizobium etli*; the fungi of interest are *Aspergillus niger, Neurospora crassa,* and *Xanthophyllomyces dendrorhous*. These organisms form biopolymer pigments, including melanins and carotenoids, which may potentially have an important role in the radioresistance of the organisms[5]. For this reason, the Mediated Matter Group is conducting research both simulating and in space environments to understand the impact of radiation on biological systems and their adaptive strategies. In this work, we examine the growth and behavior of several species of bacteria and fungi while exposed to radiation to determine mechanisms by which they may adapt to these harsh conditions.

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1. Introduction

1.1. Types of Radiation and Where They are Found

1.1.1.1. Electromagnetic Radiation

While the range of radiation that is visibly perceptible (i.e. visible light) is narrow, we are continuously interacting with and influenced by the radiation in our environment. Radiation can be categorized in two forms: electromagnetic and particulate. Electromagnetic radiation travels in the form of waves, consisting of photons that have energy[6].

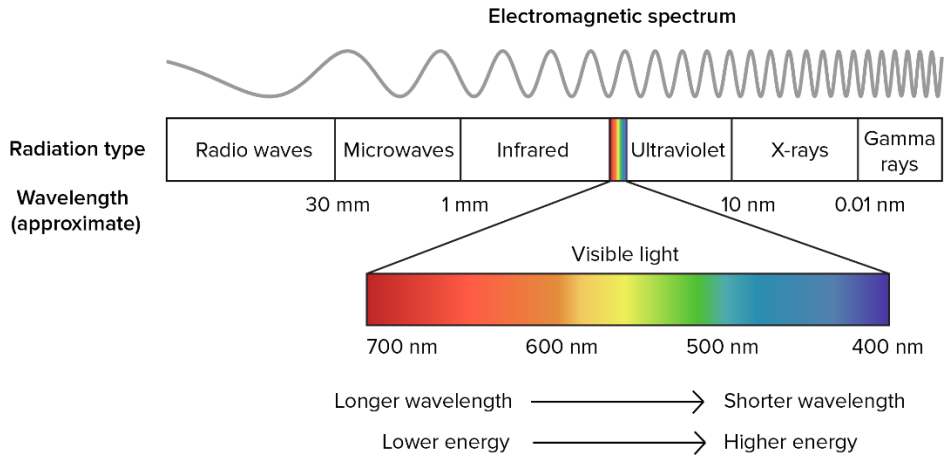

Figure 1. Electromagnetic spectrum. High energy electromagnetic radiation is ionizing[6].

X-rays and gamma rays are defined as electromagnetic radiation. These high-energy forms of radiation have the potential to ionize atoms they interact with by dislodging one or more electrons[6]. On earth, the presence of highly ionizing electromagnetic radiation is uncommon, most often found at sites of nuclear explosions, like Chernobyl, or in medical imaging equipment. In space, these forms of radiation are far more common; they are produced by neutron stars, pulsars, supernova explosions, and black holes[7].

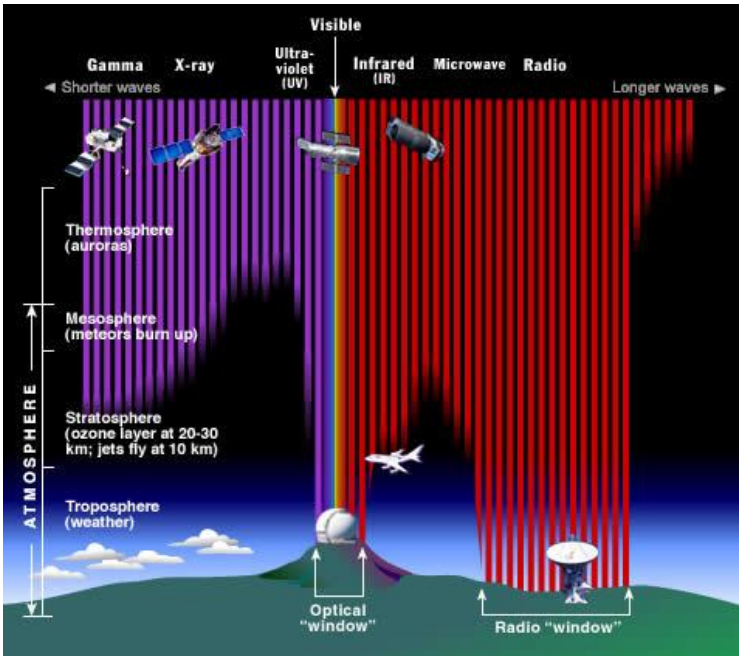

Figure 2. Earth's magnetosphere blocks most forms of high-energy electromagnetic radiation, like gamma rays, from reaching the Earth's surface. Radiation exposure increases as a function of distance from the surface of the earth[6].

1.1.1.2. Particulate Radiation

Particulate radiation, unlike electromagnetic radiation, are particles that have mass[8]. On Earth, they are formed as the result of radioactive decay or nuclear reactions. In space, they are components of galactic cosmic rays produced by solar flares[9].

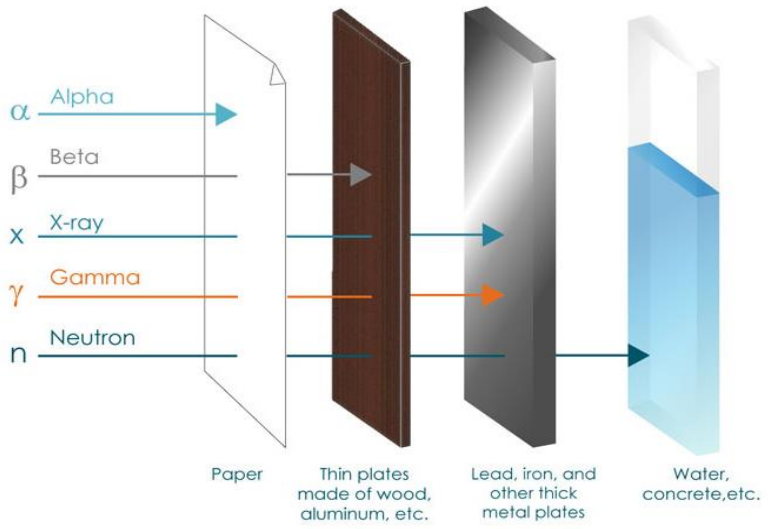

Figure 3. Comparison of penetration levels of types of electromagnetic radiation and particulate radiation. X-rays and gamma rays and electromagnetic radiation. Alpha particles, beta particles, and neutrons are particulate radiation[10].

High-energy nuclei (HZE ions), a form of particulate radiation, are especially hazardous due to their high charge. HZE ions are nuclei with no surrounding electrons[11]. Despite their rarity relative to other forms of particulate radiation (1% of galactic cosmic rays are HZE ions), they are highly penetrative, and upon interaction with other atoms produce secondary gamma radiation[11]. Due to their high charge, HZE ions break molecular bonds; upon impact with cells, these ions permanently damage DNA, which leads to mutation[12].

1.1.2. Manned Space Travel: Ionizing Radiation is a Limiting Factor

As outlined in the 2018 NASA Strategic Plan, a primary concern for future manned missions is space radiation[13]. Ionizing radiation, like HZE ions, is known to damage cells and tissue in many organisms, including humans[14]. Solar particle events and galactic cosmic rays, which are shielded by Earth's magnetosphere, are of great concern to travel beyond the Earth's orbit.

Radiation doses are categorized as either absorbed or effective. Absorbed dose, measured in Grays (G), is the amount of radiation that the object of study encounters[15]. Effective dose, measured in sievert (Sv), is the value is absorbed radiation adjusted to consider the type of radiation and the effect on the particular object and its components (i.e. a person and his or her organs)[15].

Table 1. Effective dose of radiation experienced by astronauts as compared to the general public[16].

Depth of Radiation Penetration and Exposure Limits for Astronauts and the General Public (in Sv)				
	Exposure Interval	Blood Forming Organs (5 cm depth)	Eyes (0.3 cm depth)	Skin (0.01 cm depth)
Astronauts	30 Days	0.25	1.0	1.5
	Annual	0.50	2.0	3.0
	Career	1-4	4.0	6.0
General Public	Annual	0.001	0.015	0.05

The amount of radiation received by humans within spacecrafts is affected by individual susceptibility, spatial location of the spacecraft with regard to Earth and its poles, and the solar cycle[17]. Studies of radiation within the ISS based on the Russian Radiation Control System (RCS) conducted from 2005-2009 indicate levels as high as 0.4 mGy/day (approximately 0.4 mSv/day), due to a combination of galactic cosmic rays, solar particle events, and the Earth's radiation belt[18]. This is in contrast to the estimated average natural background radiation dosage per person, which is 2.4 mSv per year (.0066 mSv/day) on Earth. Thus, long term missions or repeated missions are of concern due to increases in overall exposure which could negatively impact astronaut health[19].

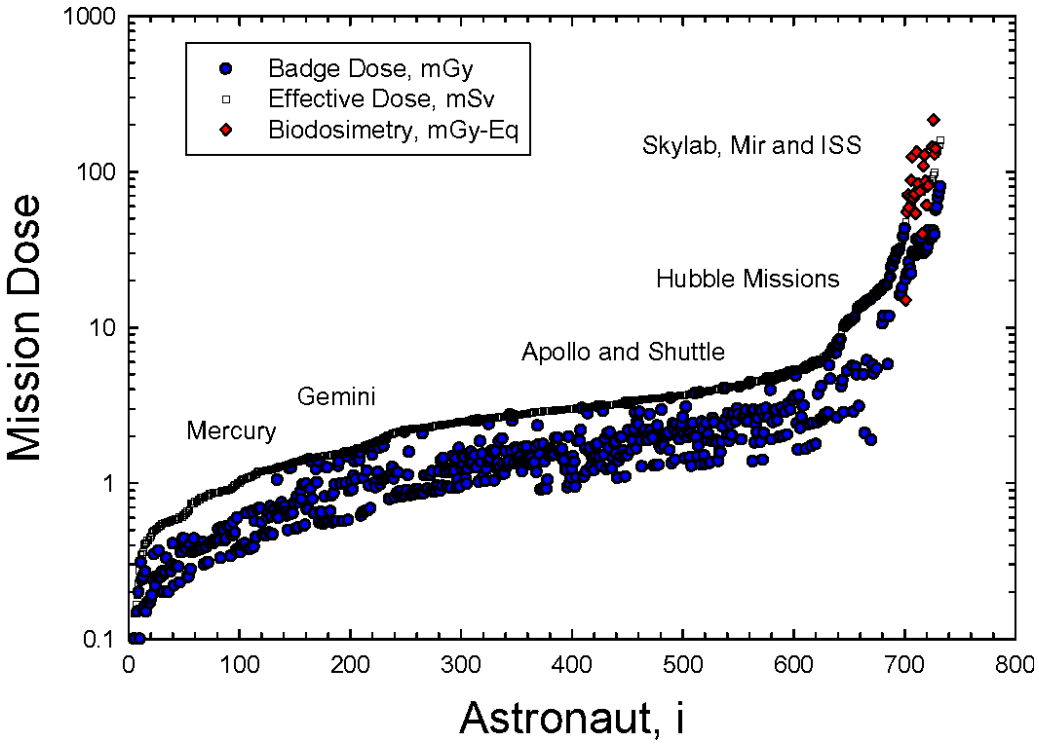

Figure 4. Radiation doses endured by astronauts on various space missions[20].

The most common radiation shield used on the ISS is high-density polyethylene (HDPE)[21]. Due to both its structure and composition, HDPE is an effective radiation absorber. Bulk HDPE is composed of linear PE chains. Due to the lack of branching in these polymers, the bulk is highly compact, minimizing the gaps between chains through which radiation could penetrate. PE, additionally, is composed of only carbon and hydrogen. Hydrogen is capable of absorbing and dispersing radiation[22]. Its electrons are tightly bound to the nucleus; it does not have numerous outer shells of electrons and is therefore not easily ionized. However, applications of HDPE are limited by the material's weight and rigidity. At this time, it is important to expand and validate strategies for radiation protection in the context of manned missions, including creating new materials and fabrication methods that can support health.

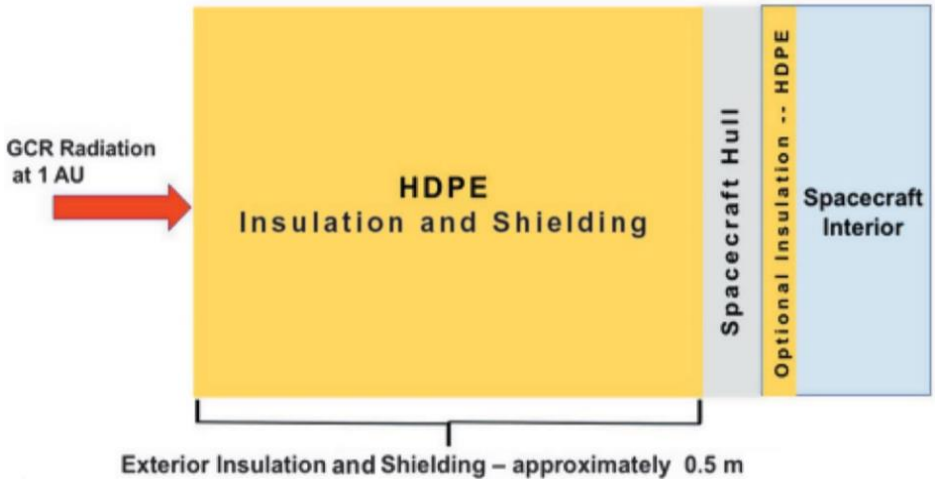

Figure 5. HDPE shield configuration on the ISS[22].

As scientific research and technologies increasingly turn towards space as the next area of application, identification of protective and adaptive compounds found on Earth is becoming more critical.

1.2. Biological Pigments

1.2.1. Case Study: Chernobyl

Nearly 34 years ago, the Chernobyl nuclear power plant disaster flung radiation into the atmosphere and across Ukraine, exposing hundreds of thousands of people to cancer-inducing levels of radiation[23]. The creation of an uninhabitable 30-kilometer exclusion radius around the plant, known ominously as "The Zone," permanently displaced 50,000 people[24]. To date, there is an incalculable number of deaths—believed to be anywhere up to 200,000—attributed to long-term radiation poisoning from the explosion[25]. Yet, amidst the seemingly deadly wasteland where radiation still permeates the air and clings to surfaces, a new ecosystem not only survives, but thrives in toxic levels of radiation. Researchers conducting studies of Chernobyl noted that within the radius, particularly at the nucleus where radiation contamination is strongest, highly melanized organisms, like mushrooms, are running rampant[26].

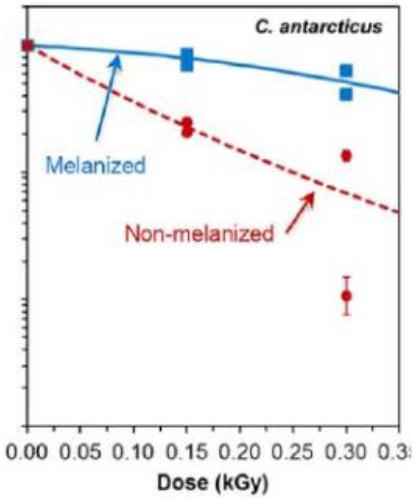

Figure 6. Survival fraction vs Irradiation dose for melanized and non-melanized fungi, *Cryomyces antarcticus*[4].

1.2.2. Biological Pigments Across Kingdoms of Life

These radioresistant mushrooms—and other fungi and bacteria also found in Chernobyl—possess neither newly evolved genes nor radiation-induced superpowers. Their origin story begins millions of years ago. Fossils of feathers, hairs, and eyes produced the earliest known traces of one of Earth's most abundant and common biomolecules: melanin[27]. Melanin, the molecule that provides pigment to our hair, eyes, and skin, is not unique to humans. In fact, melanin can be found in organisms across every kingdom of life—from bacteria to fungi to plants[27].

If you look to the pigments of nature, occurrences of color ranging from light brown to black are most likely the result of melanin[28]. Bread left too long in the fridge will sprout colonies of melanin-rich molds. Cuttlefish ink, which serves as a defense mechanism to deter predators, is primarily melanin. Even iridescent peacock feathers are full of melanin; it is the structure of the feather's barbs that then diffract visible light to produce vibrant blues and greens. Melanin, however, not only produces shades of brown. The aforementioned examples can all be categorized as eumelanin, the range from brown to black[28]. However, red pigments, such as those found in red hair or our lips, are the result of higher concentrations of pheomelanin[29].

Pheomelanin is closely related to another biological pigment: carotenoids. Carotenoids are responsible for the yellow and orange hues of carrots and oranges, as well as many other vegetables, bacteria, and plants[30]. The molecular structure of carotenoids contains key similarities to that of melanin. For this reason, carotenoids have also been found to contribute to radio-resistance in organisms that contain high concentrations of the molecule[2].

1.2.3. Melanins

Light interacts with objects through three mechanisms: reflection, absorption, and transmission. Color is the result of reflected light, and transparency is the result of transmitted light.

A molecule absorbs radiation by exciting electrons to a higher energy state. Most radiation-absorbers are characterized by delocalized pi electrons, which result from either double or triple bonds or a conjugated p-orbital[31]. The absorption wavelength is related to the size of the conjugated networks; larger networks can absorb higher energy radiation[32]. Melanin is an ideal radiation-absorber because of the widespread network of pi bonds.

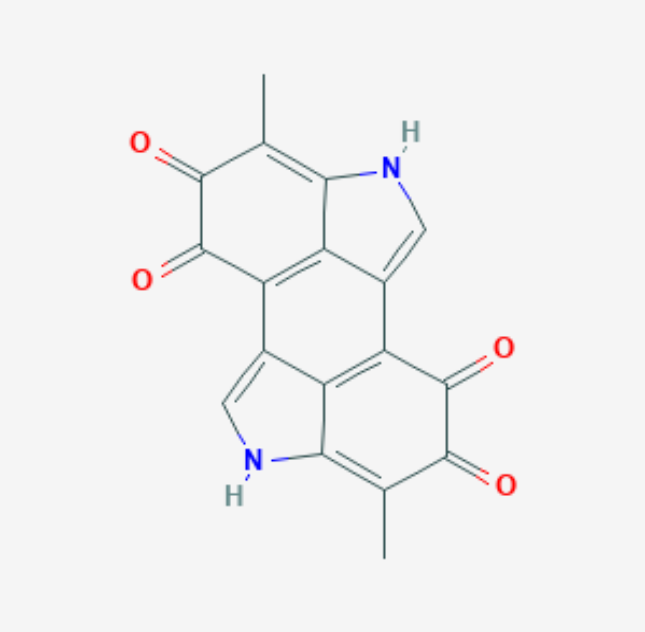

Figure 7. Melanin's structure is a conjugated network with multiple pathways (double bonds and lone pair electrons on Oxygen and Nitrogen) for delocalized electrons[33].

1.2.4. Carotenoids

Unlike melanins, carotenoids are a long hydrocarbon chain (rather than a network structure). There is one path along which delocalized electrons can travel, so mobility comparatively limited[5,34].

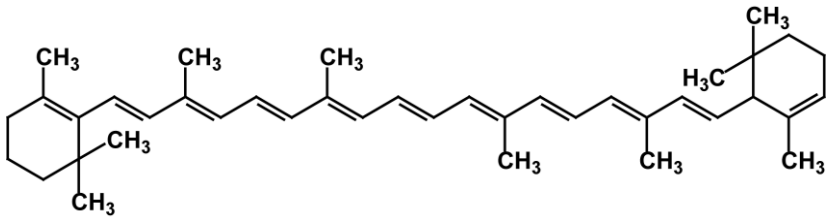

Figure 8. Alpha-carotene, a carotenoid, is characterized by a long hydrocarbon chain with alternating single and double bonds. This structure is a linear conjugated network[35].

1.3. Radio-resistant Bacteria and Fungi

1.3.1. Bacteria and Fungi on the ISS

Certain bacteria and fungi are known for their ability to persist in high-radiation environments, categorized as Ionizing Radiation Resistant Microorganisms (IRRMs)[36]. For example, discovery of fungi inhabiting the interior of the ISS led to interest in organisms such as *Aspergillus* fungi[5]. Surveys of microbes inhabiting the interior of the ISS suggest there is a variety of organisms that are able to persist in the chronic microgravity and ionizing radiation of space.

Several of the species found aboard the ISS are known to naturally produce pigments such as melanins or carotenoids as secondary metabolites, which has been demonstrated to protect from not only ionizing radiation, but UV radiation and oxidative stress[3]. These pigments hold promise

as a biocompatible shielding strategy that may be utilized as manned exploration continues into deep space.

It is for these reasons that further research into melanins and carotenoids would be useful, as certain highly pigmented organisms have been shown to survive in high radiation environments. This area of research is still comparatively new but is already of interest to the European Space Agency (ESA) in Advanced Concepts Team[37]. In particular, the ESA has noted that understanding how to efficiently incorporate melanin into existing materials and evaluate their performance in radiation shielding is a key research direction. One ESA related mission, EXPOSE-R2, included the BIOMEX experiments which focused on melanin and related pigments as protective shields (though these were external to the ISS)[38].

1.3.2. Bacteria and Fungi for Radiation Protection in Space

1.3.2.1. Research Outline

Here we study the growth of naturally pigmented microorganisms, which create melanins and carotenoids, after exposure to different types of radiation relevant to space contexts. These include on-the-ground radiation environments (from a sealed source exposure) and a 30-day exposure period aboard the ISS.

For on-earth radiation studies, we define parameters and physical setup to expose biological samples to experimental analogues of radiation profiles in low earth to deep space. After this exposure, we assess changes in growth, morphology, and material characteristics as compared to non-irradiated control samples. This study also serves as preliminary ground testing for our experiment that was conducted in the interior of the International Space Station in March 2020.

For in-space studies, we have developed an autonomous module to house and monitor a panel of biological samples for a 30-day period aboard the ISS. In this way, we evaluate a new

class of biocompatible shielding materials and lay out a path forward that combines biological materials with novel fabrication and patterning strategies for space-related applications.

These studies validate methods for creating analog ‘space’ environments through comparison to samples from the ISS capsule experiment.

1.3.2.2. Hypotheses

We hypothesize that these pigments, representative of the melanin and carotenoid biochemical families, may be involved in shielding and protection in extreme environments such as that of space. We aim to study the dynamics of these cultures, both separately and in combination and utilize extracted pigments as materials for shielding control samples that do not naturally produce pigment or survive in extreme environments.

The main questions we aim to explore through this launch centers on the actual functionality of pigments as protection from ionizing radiation. It is known that many pigmented organisms are found in environments in which radiation is present, including on the ISS, but it is not known if the pigment itself is active without the natural living system. We hypothesize that radiation resistant organisms cultured with non-radiation resistant organisms will grant some amount of protection. Through this experiment, we aim to gather data to study the role of pigments in survival in space.

1.3.2.3. Motivation

Through this research, we seek to contribute to a new and extensive class of radioprotective materials that can be used across contexts and radiation types. We will survey possible methods of application for living materials, pigment-based materials, and composites with radiation-resistant materials such as HDPE for wearable and architectural applications. We examine materials based on properties specifically relevant to long-term manned space missions, such as

stability, radio-resistance, and biocompatibility. In this way, we hope to advance the objectives of NASA and create novel technologies useful for protecting human health on Earth, in deep space, and beyond.

We also aim to expand the palette of known IRRMs, especially those that create pigments including melanins and carotenoids and assess the feasibility of these systems as living or biological materials for radiation protection and protection from other environmental factors, such as chronic microgravity and elevated CO_2 in built space. Combined with new fabrication methods, bio-inspired micro-architectures, and existing state-of-the-art radiation shielding materials, biological shielding strategies could be translated into novel approaches for protection on both the wearable and architectural scales.

Overall, we will gain a survey-level picture of the effect of ionizing radiation, one of the major concerns in the extraterrestrial environment, on several species of pigment producing microorganisms. These studies will provide greater insight into the role of pigments as protection against stresses associated with chronic ionizing radiation as well as microgravity. This foundational project also may serve to validate setups, biological, and material choices for further experimentation in other radiation and space-like environments.

2. Methods: On-Earth Study

2.1. Bacteria Selection

In this study, bacteria and fungi with high concentrations of either melanins or carotenoids were selected.

2.1.1. *N. crassa*

Neurospora crassa (ATCC 10815) is an ascomycete fungus. It produces carotenoids, including neoxanthin and is most notable for its vivid orange spores[39].

2.1.2. *A. niger*

Aspergillus niger (ATCC 16888) is a haploid filamentous fungus. It is the most abundant mold found in the environment, and it creates large amounts of both DHN-melanin and pyomelanin[40].

2.1.3. *X. dendrorhous*

Xanthophyllomyces dendrorhous (anamorph *Phaffia rhodozyma*) (ATCC 24230) is a basidiomycetous yeast that produces the one of the most common carotenoids, astaxanthin[41].

2.1.4. *B. subtilis*

Bacillus subtilis (ATCC 6051) is a spore-forming bacteria. *B. subtilis* has the ability to produce and secrete antibiotics, and melanin is present in the spores[42].

2.1.5. *R. etli*

Rhizobium etli (ATCC 51251) is a bacteria found in soil. *R. etli* expresses the tyrosinase gene (MelA), which is implicated in melanin production[43].

2.2. Bacteria and Fungi Culture Criteria

Table 2. Bacteria and fungi growth protocol.

Organism	Kingdom	Pigment	Incubation Temp (°C)	Atmosphere	Suggested Media
A. niger	Fungus	Melanin	24-26	Aerobic	Malt extract medium, Potato dextrose medium[40]
B. subtilis	Bacteria	Melanin	30	Aerobic	Nutrient broth (beef extract + peptone)[42]

N. crassa	Fungus	Carotenoid	24	Aerobic	Neurospora culture medium (yeast extract + peptone + maltose)[39]
R. etli	Bacteria	Melanin	26	Aerobic	Rhizobium X medium (yeast extract + mannitol)[43]
X. dendrorhous	Fungus	Carotenoid	18-20	Aerobic	YM medium[41]

To standardize media across the six selected organisms, the required nutrient sources for each bacteria and fungi were determined. The ideal incubation temperature is within the range of 18 °C to 30 °C (the majority of which are between 24-26 °C, with the exception of *X. dendrorhous*, which is from 18-20 °C, and *B. subtilis*, at 30 °C). Yeast extract, maltose, peptone, potato dextrose (or a combination) were the primary nutrients required for the growth medium. Among the organisms, common nutrient requirements were a sugar source (like dextrose, glucose, and maltose), and a Nitrogen source (like peptone). The growth medium selected was a 50% YM-50% Potato Dextrose (PD) agar with 1% additional peptone (by volume) and the incubation temperature selected was room temperature (approximately 24 °C).

2.3. Sample Preparation: Medium Preparation, Culture Technique, Imaging

Thirty-six petri dishes were prepared (six per organism; each condition was performed in triplicate). YM powder and PDA powder were purchased, prepared separately as broths, and the broths were mixed in a 1:1 volume ratio. For example, if 1000 mL of mixed solution was required, 500 mL was prepared as YM broth and 500 mL as PDA broth (according to instructions provided by the supplier). The solutions were mixed, and 1% peptone was added to the final solution. 2% Bacto Agar (BD Biosciences) (by volume) was added to the mixed broth to prepare the solid

medium. This solution was repeatedly microwaved in a glass beaker and stirred until the agar completely dissolved, producing a transparent solution.

To prepare the plates, all work surfaces were cleaned with 70% ethanol to avoid contamination of plates. Plates were prepared on 100mm diameter petri dishes. Using a 25mL pasteur pipette attached to a motorized pipettor, 20 mL of the 50% YM-50% PDA medium was transferred immediately after it was prepared (while still warm, as agar solidifies below 45 °C). Twenty minutes were allowed after plate preparation for solidification and cooling of the solid medium.

Solid cultures were inoculated from stocks of liquid cultures of each organism. Each Petri dish was seeded with 10 microliters of the specified liquid culture by piercing the surface of the agar in the center of the plate. One the agar absorbs the liquid culture, the petri dishes were inverted (to avoid condensation drip onto the agar). The edges of the petri dish were wrapped with Parafilm and stored at room temperature.

Imagining of bacteria and fungi growth of the control samples was conducted via timelapse in a lightbox. Images were taken every five minutes, and lighting was timed such that the samples were only illuminated while a picture was taken.

2.4. Radiation Experimental Procedure

For radiation testing, the samples were seeded and grown overnight in low light conditions before exposure to radiation at 26 °C. The samples were exposed to ionizing radiation over time with a sealed source Cobalt-60 at the MIT Nuclear Reactor. Co-60 produces gamma radiation[44].

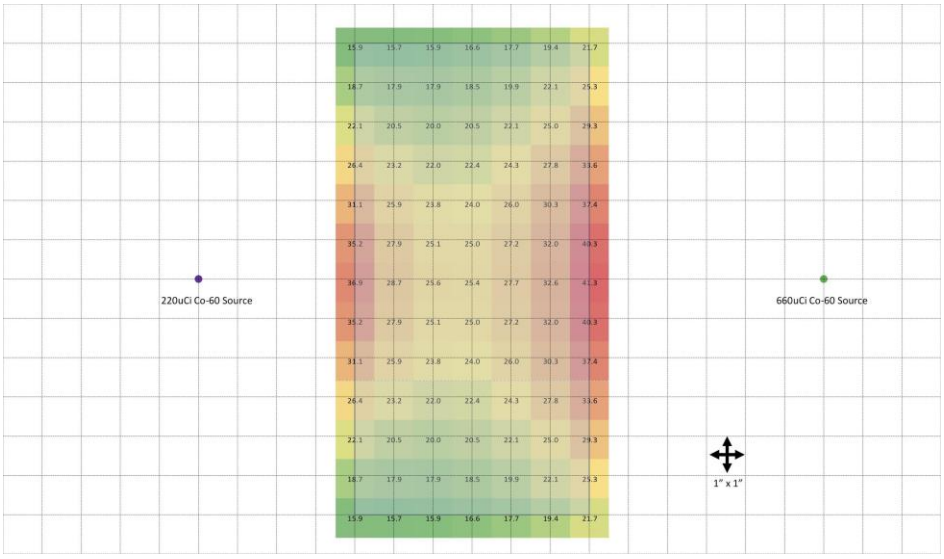

Figure 9. Radiation exposure map with Co-60 sealed source from MIT Nuclear Reactor (Credit: Ed Lamere).

Plates were labeled, wrapped individually in Parafilm, and placed within a single layer of non-air permeable containment; each plate was prepared in triplicate. They were stacked and placed in an ionizing radiation environment created through the placement of Co-60 rod sources. The experiment was conducted in indirect fluorescent light, room temperature and humidity for up to 7 days at the MIT Nuclear Reactor. Three conditions were performed (Note: 1 Gray = 100 R).

Table 3. Co-60 Irradiation Conditions.

Condition #	Co-60 Source Type	Radiation/Time	Total Dose	Total Time
1	Co-60 rods	16.5mR/hr	about 3000 mR	168hr
2	Co-60 cylinder irradiator	37Gy/min	1kGy	0.5hr
3	Co-60 cylinder irradiator	37Gy/min	100kGy	50hr

Radiation levels were chosen based upon the safety thresholds for single-dose radiation exposure; 3000 mR is the maximum safe single-dosage for an adult human[45]. After exposure, exposed plates were imaged and placed within 4 °C conditions for short term storage.

3. Methods: In-Space Study

3.1. Experimental Set-up

With the assistance of NanoRacks LLC, an autonomous capsule with liquid and solid cultures was sent to the ISS by SpaceX CRS-20 via the Dragon cargo ship atop of a Falcon 9 rocket. Beginning on March 6, 2020, the cargo ship took a 3-day journey to the ISS. The cargo was loaded onto the ISS, and the solid cultures were imaged over a 30-day period. During this time, they were exposed to chronic ionizing radiation experienced by the ISS internally.

This study examines the growth, behavior, and survival of bacteria and fungi which naturally create biochemical pigments. We have designed a capsule that can contain six 50mm petri dishes and 24 vials, as well as an autonomous time lapse imaging system. Each of the petri dishes will contain a culture or co-culture of living organisms out of the following selection. These organisms were selected based on ease of culturing and natural creation of a pigment (with the exception of *B. subtilis*, which serves as a negative control). *A. niger* creates melanins whereas *N. crassa* creates carotenoids.

These petri dishes were imaged multiple times a day using two micro-cameras controlled via two Raspberry Pi Zeros, yielding timelapse footage for analysis of growth and morphological changes. Upon return, cells will be imaged, cultured, and re-imaged to evaluate changes in

individual or colony morphology that are present across generations. We will compare results to ground controls and simulated radiation environments.

3.2. Capsule Design

Table 4. Selected live cultures for petri dishes in the capsule.

Organism	Kingdom	Pigment
Bacillus subtilis	Bacteria	Melanin
Neurospora crassa	Fungi	Carotenoid
Aspergillus niger	Fungi	Melanin

Figure 10. Diagram of capsule contents. Live cultures will grow on petri dishes; passive cultures will be housed in vials (Credit: Nic Lee and Sunanda Sharma).

Figure 11. Assemble capsule with contents. (Credit: Nic Lee).

4. Results: On-Earth Study

4.1. Analysis Protocol

Following the seven-day irradiation period, the control (which grew for seven-days without radiation) and experimental samples were imaged in a lightbox. Images were imported as JPGs into *Fiji* software. The images were processed by enhancing the pixel saturation to 10%. The shape tool was used to enclose and measure the area of the growth on the 50mm petri dishes.

4.2. Imaging of Cultures

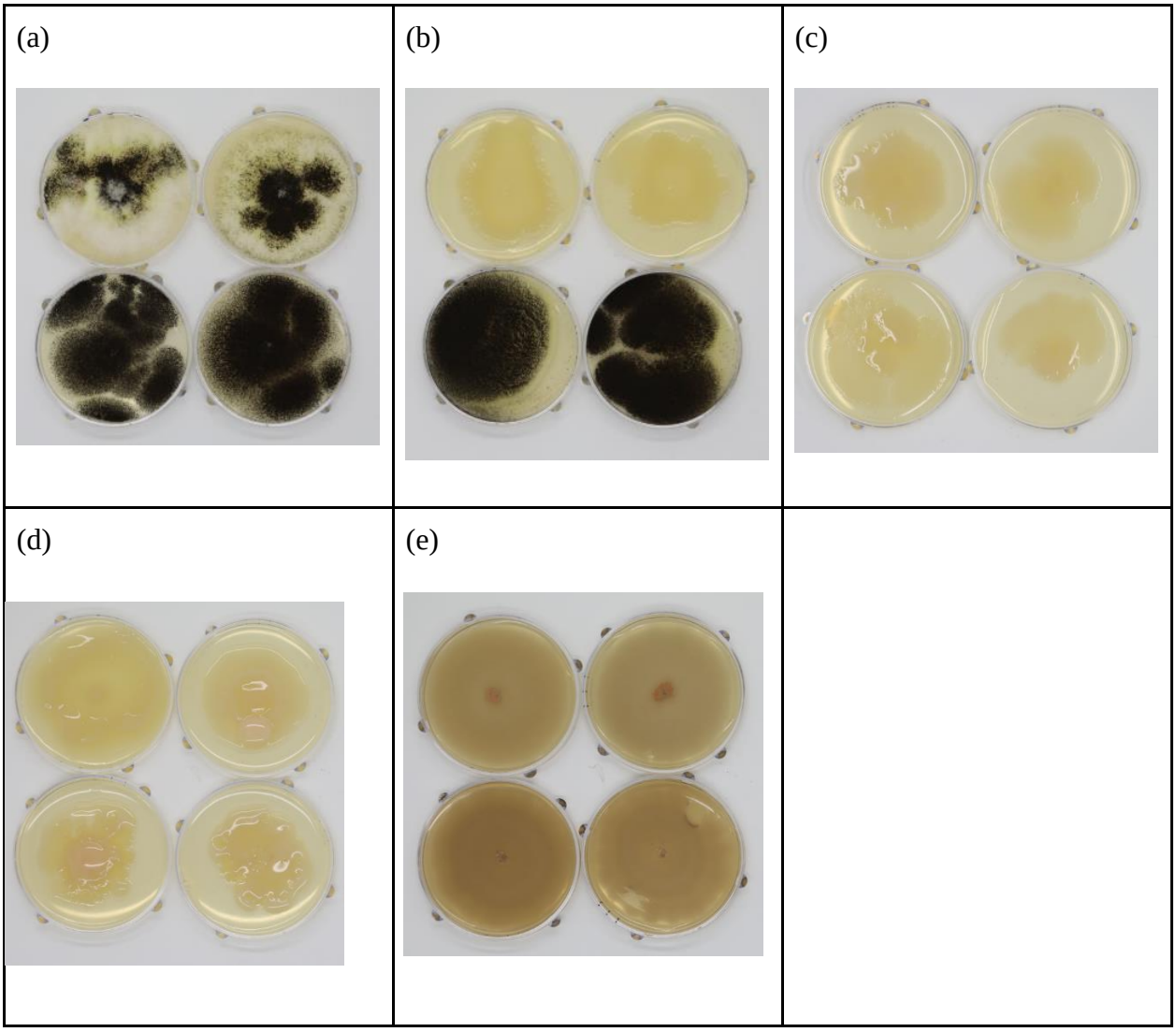

Figure 12. (a) *A. niger*, (b) *B. subtilis*, (c) *N. crassa*, (d) *R. etli*, (e) *X. dendrorhous*. In each image, the upper two petri dishes are controls; the bottom two petri dishes are experimental (seven-day irradiation from Co-60 source).

4.3. Comparison of Control and Experimental

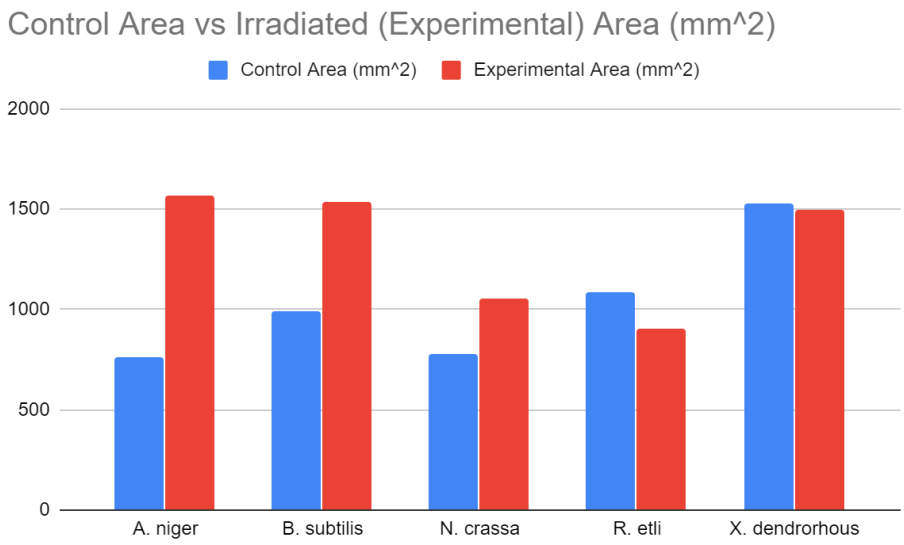

Figure 13. Average surface area of culture.

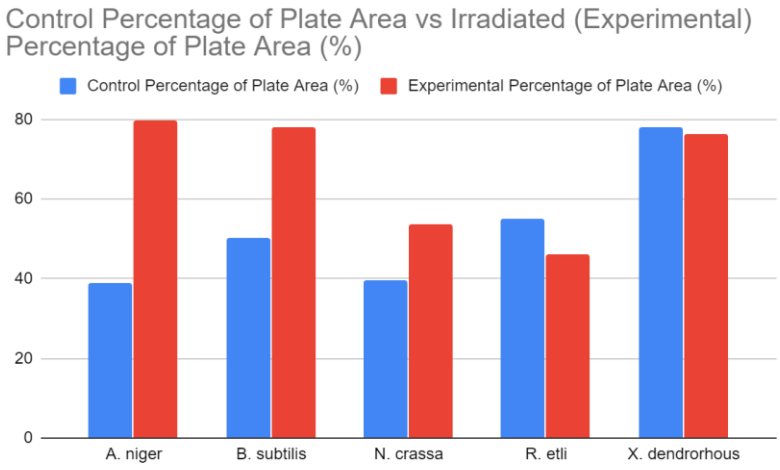

Figure 14. The percentage of surface area of the 50mm petri dish covered by colony growth.

Table 5. Percent difference in colony size between control and irradiated samples.

Organism	Percent Change
A. niger	104.8141982
B. subtilis	55.16889376
N. crassa	36.24291546
R. etli	-16.57776978
X. dendrorhous	-2.038984539

5. Results: In-Space Study

5.1. Imaging of Cultures

Table 6. Live cultures and co-cultures on petri dishes in the capsule.

Petri Dish #	Organism(s)	Kingdom(s)	Pigment(s)
1	*Bacillus subtilis*	Bacteria	None (Negative Control)
2	*Neurospora crassa*	Fungi	Carotenoid
3	*Aspergillus niger*	Fungi	Melanin
4	*Bacillus subtilis* + *Neurospora crassa*	Bacteria + Fungi	Carotenoid (+ Control)
5	*Aspergillus niger* + *Bacillus subtilis*	Fungi + Bacteria	Melanin (+ Control)
6	*Aspergillus niger* + *Bacillus subtilis* + *Neurospora crassa*	Fungi + Bacteria + Fungi	Melanin + Carotenoid (+ Control)

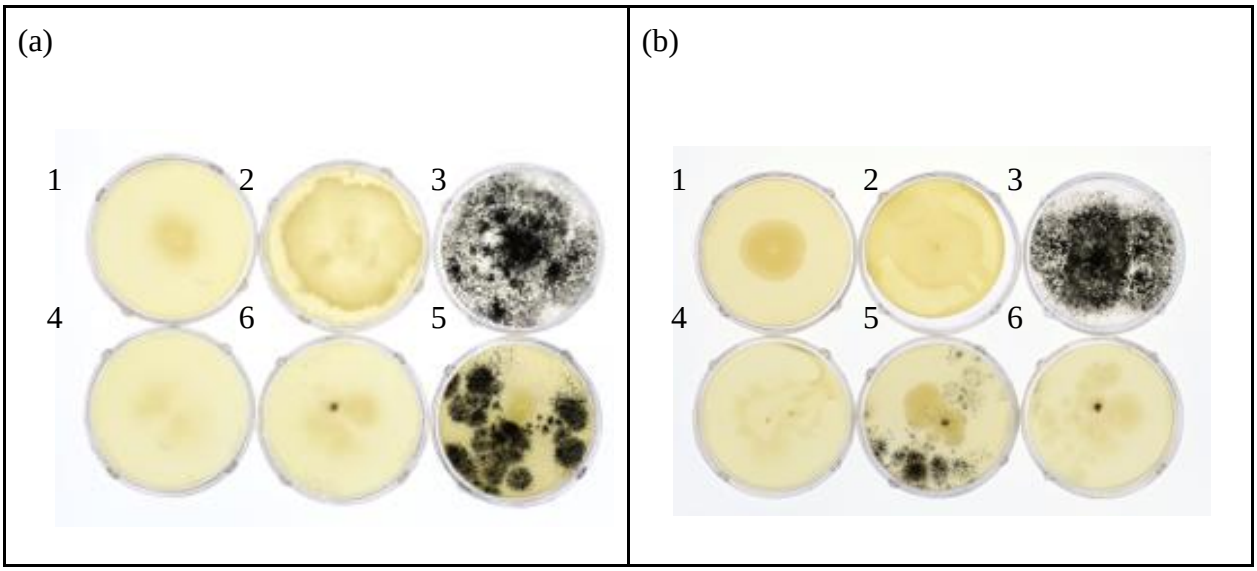

Figure 15. (a) Control (grown on Earth for duration of the ISS mission), (b) Experimental (from capsule sent to ISS.

5.2. Comparison of Control and Experimental

In all cultures and co-cultures (except dish 6, which included all three organisms) the concentration of pigment surrounding the center of the colony, where organism density is highest, perceptibly increased. The results of dish 6 aligned with on-ground studies prior to this experiment, which showed that the three cultures grow minimally when co-cultured. The visually more saturated hue is indicative of increased pigment production in the experimental samples.

6. Discussion

6.1. Application

Extracted melanins and carotenoids could be implemented in HDPE to enhance the radiation shielding while addressing the detriments of HDPE, such as weight and rigidity. Multiple

composite configurations should be considered to optimize binding between the extracted pigment and HDPE.

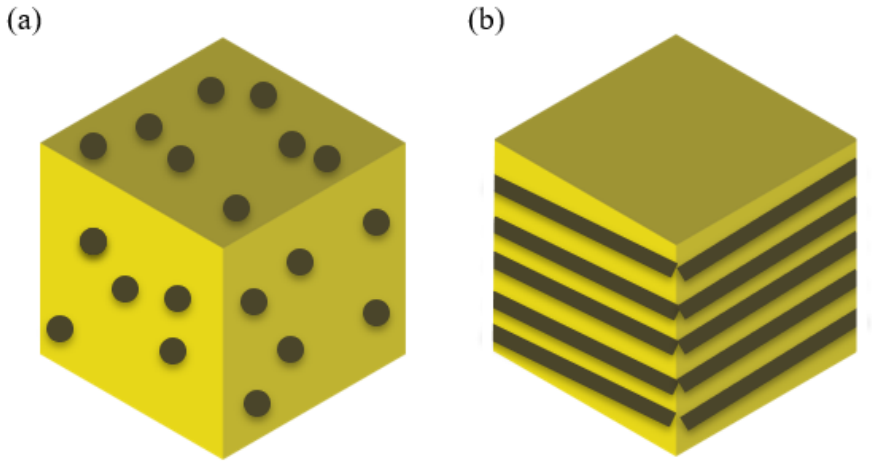

Figure 16. (a) Particulate composite with pigment clusters (brown) interspersed within the HDPE shield (yellow). (b) Laminated composite with alternating layers of HDPE and pigment.

HDPE is known to shield against neutron radiation, the most penetrative form of particulate radiation[21]. The findings of this study conclude that biological pigments, particularly melanin, shields bacteria and fungi from gamma radiation, high-energy electromagnetic radiation. The combination of HDPE and melanin in a composite shield would protect against both forms of radiation. Additionally, melanin would protect against the secondary gamma radiation formed by HZE ions.

If applied to or woven into textiles, these pigments could also augment radiation shielding in fabrics.

6.2. Next Steps

Future proposed experiments related to this work will accomplish two goals: to characterize the pigments *ex vivo* and to analyze organism evolution. This is necessary to

understand the applicability of exogenous pigments for radiation protection in two pigment families (melanins and carotenoids). It will also assist in identifying methods of directed evolution for survival in high-radiation environments through repeated exposure to increasing radiation.

Table 7. Proposed future analysis of samples.

Suggested Analysis	Specimen	Purpose
HPLC	Carotenoid-containing	Quantify concentration of pigment and identify changes to pigment structure[46]
GC/MS	Melanin-containing	Quantify concentration of pigment and identify changes to pigment structure[47]
SEM	All samples	Identify changes in surface topology of colony and morphology changes in cell[48]
RNA Sequencing	Bacteria	Identify transcriptomic changes[49]
Dead/Live Assay	Bacteria	Quantify percent of viable organisms[50]
Proteomics	Fungi	Identify changes in protein production and expression[51]

7. Conclusion

This study confirms that the fungi *A. niger* and *N. crassa* can be identified as Ionizing Radiation Resistant Microorganisms (IRRMs). These cultures thrived in on-Earth radiation studies, in which a Co-60 sealed source transmitted gamma radiation to the fungi.

This study also supports that both melanins and carotenoids can be effective ionizing radiation shields, as *A. niger* produces melanin and *N. crassa* produces carotenoids. However, species with melanins were more prolific post-irradiation than those with carotenoids.

For in-space studies conducted on the ISS, the fungi increased production of their respective pigments and also shielded the negative control, *B. subtilis*, in co-cultures. The functionality of the pigment as a radiation shield *ex vivo* is supported by the viability of this co-culture.

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