Isolation of microorganisms from the Long Island Pine Barrens Forests with resistance to high doses of gamma radiation

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Abstract

Few organisms resistant to gamma radiation (<10-12 nm) have been documented in the literature. Deinococcus radiodurans is the most radiation resistant microbe found yet (growth and survival at 30 kGy of gamma irradiation), utilizing high fidelity DNA repair mechanisms that allow it to also resist desiccation. Our goal was to investigate if the Pine Barrens Forests of Long Island would contain novel gamma radiation resistant microorganisms due to its unique soil properties. Soil samples from the Pine Barrens Forest on Brookhaven National Laboratory property were obtained and irradiated at 20 kGy and 50 kGy. Thirteen types of nutrient media were inoculated with the irradiated soil and plated periodically on respective agar media. Fourteen unique microorganisms were isolated. Implications of discovering living microbes from this heavily irradiated soil would be significant for future forays into space (where ionizing radiation levels are high), and especially for nuclear energy research/safety implications.

Introduction

Gamma radiation refers to high frequency, low wavelength (<10-12 nm) radiation emitted from radioactive isotopes. It is a type of “ionizing” radiation because it has the potential to liberate electrons upon contact with atoms. This characteristic makes it especially damaging to living organisms, which have been shown to be extremely vulnerable to such radiation. 4.5 gray (Gy) of gamma radiation is considered a fatal dose for humans (expected to cause death to 50% of an exposed population within 30 days of exposure).1

Certain microbes have been discovered that can survive radiation doses higher than 5 Gy. Most famously, Deinococcus radiodurans from desert soil has survived up to 30,000 Gy of gamma irradiation.2,3 Organisms that persist have been found with strong DNA repair mechanisms to quickly and effectively recombine thousands of base-pair breaks into a coherent genome, due in part to a particularly active RecA protein. They concurrently show resistance to desiccation.4 Other radiation resistant microbes have emerged from various sources, including nuclear waste sites, sewage, and spoiled food.2 To find more of these organisms, diverse soil types containing variable microbe assemblages should be analyzed.

Soil from the Pine Barrens Forests on eastern Long Island in New York could potentially contain novel organisms with resistance to gamma radiation. Pine Barrens soil, named as such for its lack of fertility, exists in just a few localities across the US Northeast. It differs from most other soils in three facets that support possible housing of gamma radiation resistant microbes:1) It is sandy and does not hold moisture well,2 and previously discovered resistant microbes have shown resistance to desiccation in dryer environments.2 It contains larger than normal amounts of aluminum and iron that microbes may be internalizing.3 It has been proposed that increased amounts of manganese (similar in reduction potential to aluminum) accumulated in D. radiodurans help confer its resistance to gamma irradiation.3,4 It has an acidic pH,3 providing a harsher living environment that resistant organisms may be accustomed to.

The purposes of attempting to isolate such microbes are multifold. It has been suggested that radiation resistant microorganisms could be used for nuclear waste disposal and bioreactors.4 Nuclear reactions used for energy and other purposes are known to release gamma radiation as artificial byproducts.5 Finding out which microbial systems can survive this stress has important implications for nuclear safety and associated research. In addition, solar UV radiation, of sufficient intensity to be considered ionizing, bombards the surface of planets lacking an ozone layer such as Mars.5 An organism surviving gamma irradiation would be well equipped to also withstand this radiation. In this study, traditional microbiology methods were used in an attempt to isolate living microorganisms from Pine Barrens soil subjected to gamma irradiation at 20 kGy and 50 kGy. The latter is a level of radiation not yet thoroughly explored in the literature for survival of microbes.

Methods

• Soil sampled from Pine Barrens Forest at Brookhaven National Laboratory, Upton, NY [Figure 1a]
• Irradiated at 20 kGy and 50 kGy of gamma radiation
• Inoculated 5 mL of each of the 13 types of media [Table 1] with ~500 mg of soil in 15 mL centrifuge tubes [Figure 1b]
• Traditional microbiology methods utilized to isolate any life found in inoculations
• Multiple rounds of streaking onto agar media plates
• Isolation/purification of unique colonies

Results & Discussion

• 14 unique colonies isolated [Table 2] [Figure 2] • 10 from 20 kGy irradiated soil (7 bacteria, 3 fungi) • 4 from 50 kGy irradiated soil (2 bacteria, 2 fungi)
• NB, TGC, TGY, YMB most common media grown on (at least two unique colonies) [Figure 3]
• 9 additional unique colonies were isolated from the same soil irradiated at 10 kGy in a related experiment
• Next steps: Re-irradiation at respective radiation levels to confirm survival Replication to allow identification to microorganisms Analysis of microbe characteristics

References


Acknowledgements

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Table 1. Chemical compositions of each type of media used for liquid media inoculations as well as agar media streaking and isolation.

<table>
<thead>
<tr>
<th>Name</th>
<th>Media</th>
<th>Type of organism</th>
<th>Description</th>
<th>Pictures in Figure 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LG2 20 TGY</td>
<td>Bacterium</td>
<td>Small (~1–2 mm), yellow, mucoid, smooth border; circular</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>LG3 20 TGY</td>
<td>Bacterium</td>
<td>cauliflower border, raised black dot in center, rough-looking texture, non-mucoid</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>LG4 20 TGY</td>
<td>Bacterium</td>
<td>Large (9–10 mm), pale yellow; mucoid, surrounded</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>LG11 20 TGY</td>
<td>Bacterium</td>
<td>Large (~10 mm), light yellow; raised and spread; smooth surface above agar</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>LG13 20 NB</td>
<td>Bacterium</td>
<td>Small (~2 mm), yellow/green; rough, round, mucoid; non-mucoid; light yellow</td>
<td>e</td>
<td></td>
</tr>
<tr>
<td>LG14 50 YMB</td>
<td>Bacterium</td>
<td>Large (~20 mm), yellow/green; smooth, circular</td>
<td>f</td>
<td></td>
</tr>
<tr>
<td>LG15 50 YMB</td>
<td>Fungus</td>
<td>Top: dark yellow concentric zone, 1St ring dark gray, 2nd ring light yellow and thin, 3rd ring gray/dark yellow; bottom: huge gray/concentric ring, 1St ring light gray, 2nd ring lighter gray than for ring</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>LG16 50 YMB</td>
<td>Bacterium</td>
<td>Top and bottom smooth, round, mucoid, slightly raised</td>
<td>h</td>
<td></td>
</tr>
<tr>
<td>LG17 20 NB</td>
<td>Fungus</td>
<td>Irregular colony, made of many smaller circles in an almost hemis-</td>
<td>i</td>
<td></td>
</tr>
<tr>
<td>LG18 20 NCL</td>
<td>Bacterium</td>
<td>Small (~1–2 mm), rough, with mucoid border, very slightly raised, non-mucoid, round edges</td>
<td>j</td>
<td></td>
</tr>
<tr>
<td>LG19 50 YMB</td>
<td>Fungus</td>
<td>Extremely raised, somewhat mucoid, roundly edged, appears to have a small light yellow area in middle surrounded by ring of dark yellow</td>
<td>k</td>
<td></td>
</tr>
<tr>
<td>LG21 20 Kirtotel Fungus</td>
<td>Fungus</td>
<td>Top: very much black dot; bottom: widely spreading roots; “fuzzy” appearance (candy-cane-like)</td>
<td>l</td>
<td></td>
</tr>
<tr>
<td>LG22 50 Watson Fusarium</td>
<td>Fungus</td>
<td>Not raised, non-mucoid, circularly; very fast, can only really see what appears to be the fastest radiating out from a central point</td>
<td>m</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Name, level of radiation, media grown on, type of organism, size, color, description, and picture where available of each from the 14 microbe types isolated from potentially survived gamma irradiation at 20 or 50 kGy.

Figure 2. Photos of each type of microbial species as streaked on media plates, a-j (refer to Table 2).

Figure 3. Number of unique microbial species procured using each media type from soil irradiated at a) 20 kGy (blue) or b) 50 kGy (red).