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 quality 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 the 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 percent 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 to have strong DNA repair mechanisms to quickly and effectively reconnect thousands of double-strand 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 possibly 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,[5] 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.[5] 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.[6] 3) It has an acidic pH,[5] 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 biosensors.[7] Nuclear reactions used for energy and other purposes are known to release gamma irradiation as artificial byproducts.[8] 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.[3] 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

Figure 1. Activities associated with a) sampling Pine Barrens soil and b) inoculating soil.

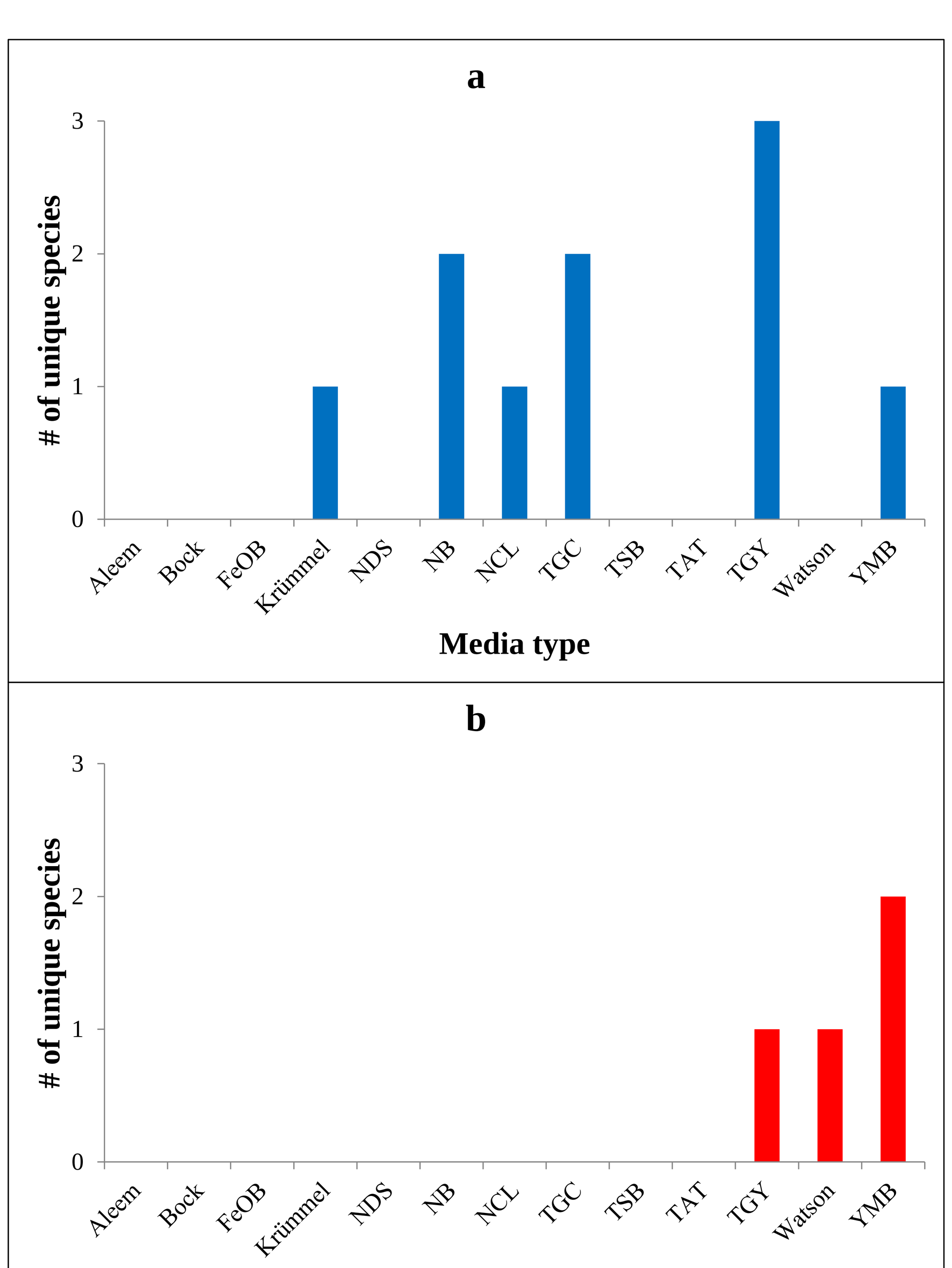

Figure 3. Number of unique microbial species procured using each media type from soil irradiated at a) 20 kGy (blue) or b) 50 kGy (red).

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
  - rDNA sequencing to identify microorganisms
  - Analysis of microbe characteristics

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Name	Ingredients (g/L)
Aleem media	0.3 KNO_2, 0.1875 $MgSO_4 \cdot 7H_2O$, 0.0125 $CaCl_2 \cdot 2H_2O$, 0.5 KH_2PO_4, 0.5 K_2HPO_4, 0.01 $FeSO_4 \cdot 7H_2O$, 1.5 $KHCO_3$, 0.1875 NaCl
Bock media	0.05 $MgSO_4 \cdot 7H_2O$, 0.15 KH_2PO_4, 0.00075 K_2HPO_4, 0.015 $FeSO_4 \cdot 7H_2O$, 0.5 NaCl, 2 $NaNO_2$, 0.003 $CaCO_3$, 0.00005 $(NH_4)_6Mo_7O_{24}$
Iron-oxidizing media (FeOB)	0.4 $MgSO_4 \cdot 7H_2O$, 0.4 $(NH_4)_2SO_4$, 0.4 K_2HPO_4, 6 $FeSO_4 \cdot 7H_2O$, 0.00365 HCl
Krümmel media	0.0493 $MgSO_4 \cdot 7H_2O$, 0.147 $CaCl_2 \cdot 2H_2O$, 0.000025 $CuSO_4 \cdot 5H_2O$, 0.0000431 $ZnSO_4 \cdot 7H_2O$, 0.584 NaCl, 0.535 NH_4Cl, 0.0544 KH_2PO_4, 0.0744 KCl, 0.0009731 $FeSO_4 \cdot 7H_2O$, 0.0000371 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.0000446 $MnSO_4 \cdot 4H_2O$, 0.0000494 H_3BO_3, 0.00005 cresol red (0.05%)
Nitrogen-deficient media (NDS)	0.5 K_2HPO_4, 0.2 $MgSO_4 \cdot 7H_2O$, 0.1 $BaCl_2$, 5 malic acid, KOH, 0.01 $MnSO_4 \cdot 1H_2O$, 2 bacteriological agar, 0.002 $NaVO_3$, 0.002 $Na_2MoO_4 \cdot 2H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$
Nutrient broth (NB)	3 beef extract, 5 peptone
Sulfur-oxidizing media (NCL)	0.2 $(NH_4)_2SO_4$, 0.5 $MgSO_4 \cdot 7H_2O$, 0.25 $CaCl_2 \cdot 2H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$
Thioglycollate media (TGC)	29.8 thioglycollate media powder
Thiosulfate media (TSB)	5 $Na_2S_2O_3$, 0.1 K_2HPO_4, 0.2 $NaHCO_2$, 0.1 NH_4Cl
Tryptone-azolectin-tween broth (TAT)	20.83 pancreatic digest of casein, 5.21 soy lecithin
Tryptone-glucose-yeast extract broth (TGY)	10 tryptone, 1 glucose, 5 yeast extract
Watson media	2 $(NH_4)_2SO_4$, 0.2 $MgSO_4 \cdot 7H_2O$, 0.02 $CaCl_2 \cdot 2H_2O$, 0.0159 K_2HPO_4, 0.001 chelated iron, 0.0001 $NaMoO_4 \cdot 2H_2O$, 0.0002 $MnCl_2 \cdot 4H_2O$, 0.000002 $CoCl_2 \cdot 6H_2O$, 0.00002 $CuSO_4 \cdot 5H_2O$, 0.0001 $ZnSO_4 \cdot 7H_2O$, 0.0005 phenol red (0.5%)
Yeast mannitol broth (YMB)	3 peptone, 5 yeast extract, 25 mannitol

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

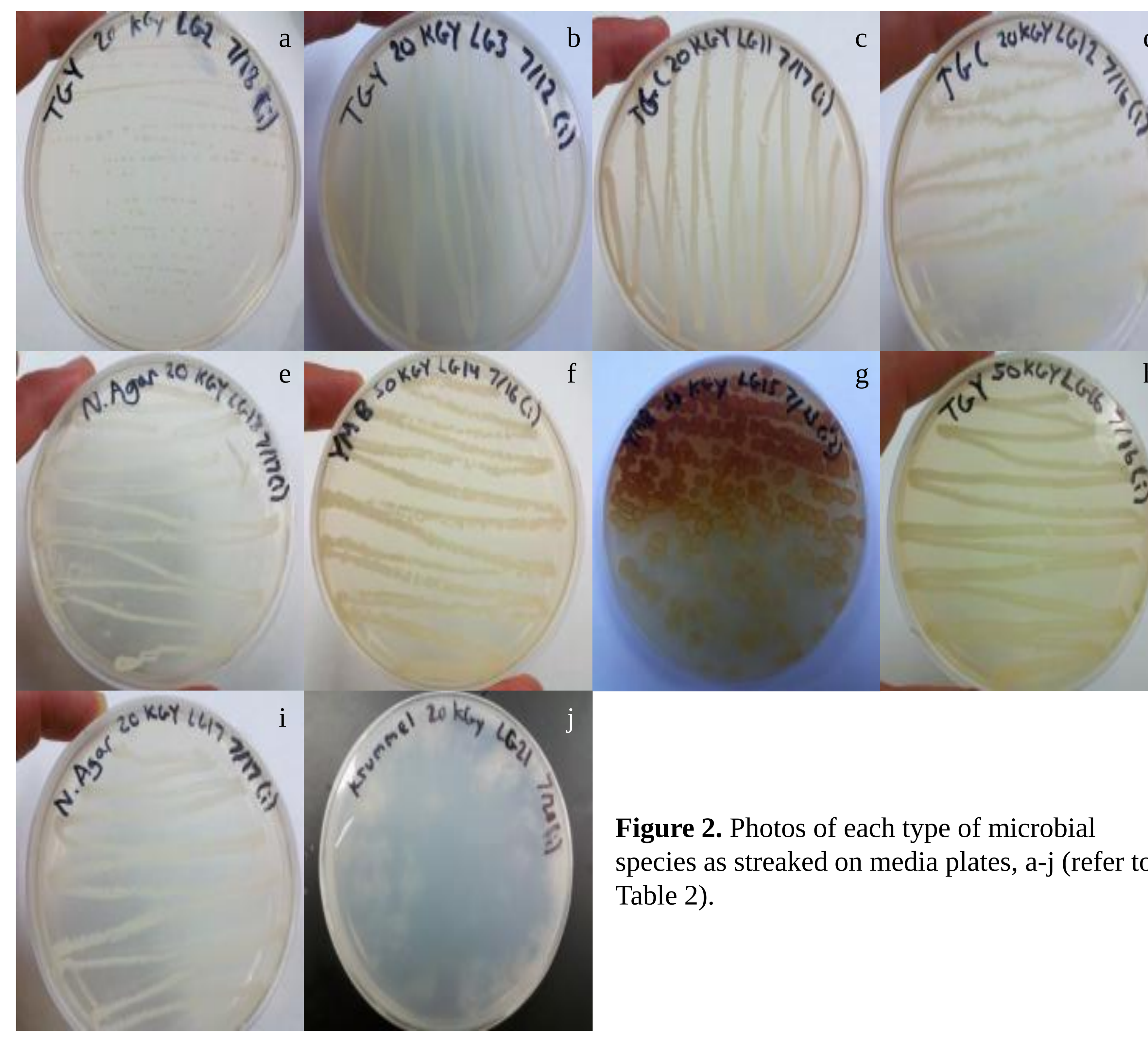

Figure 2. Photos of each type of microbial species as streaked on media plates, a-j (refer to Table 2).

Name	Radiation level (kGy)	Media	Type of organism	Description	Picture in Figure 2
LG2	20	TGY	Bacterium	Small (~1-2 mm); yellow; mucoidal, smooth border, circular	a
LG3	20	TGY	Bacterium	Large (~5-8 mm); yellow (besides center); cauliflower border, raised black dot in center, rough-looking texture, non-mucoidal	b
LG4	20	TGY	Bacterium	Small (~2 mm); yellow/blue; irregular border, jagged at edges, mucoidal at center	Not available
LG11	20	TGC	Bacterium	Large (5-10 mm); pale yellow; mucoidal, elevated	c
LG12	20	TGC	Fungus	Large (>10 mm); light yellow; web-like, a raised and textured surface above agar	d
LG13	20	NB	Bacterium	Small (<1 mm); yellow/gray; round, mucoidal, not elevated	e
LG14	50	YMB	Bacterium	Large (~10 mm); yellow; mucoidal, spherical	f
LG15	50	YMB	Fungus	Large (>20 mm); yellow/gray/green; top: dark yellow center, 1st ring darker gray/yellow, 2nd ring light yellow and thin, 3rd ring gray/dark yellow; bottom: huge gray/green center, 1st ring light green, 2nd ring lighter green than 1st ring	g
LG16	50	TGY	Bacterium	Large (>10 mm); light yellow; round, mucoidal, slightly raised	h
LG17	20	NB	Fungus	Large (~8 mm); white/yellow; irregular colony, made of many smaller circles in an almost honey comb-like composite	i
LG18	20	NCL	Bacterium	Small (~1 mm); red/pink tinge with dark magenta center; very slightly raised, non-mucoidal, rounded edges	Not available
LG19	20	YMB	Bacterium	Small (~2 mm); dark yellow/goldenrod; extremely raised, somewhat mucoidal, rounded edges, appears to have a small light yellow area in middle surrounded by ring of dark yellow	Not available
LG21	20	Krümmel	Fungus	Large (~8mm); white with black dots; top: many small black dots; bottom: widely spreading roots, "fuzzy" appearance (cotton candy-like)	j
LG22	50	Watson	Fungus	Small (~3-4 mm); transparent; not raised, non-mucoidal, relatively circular, very faint, can really only see what appears to be roots radiating out from a central point	Not available

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