Isolation of radiation-resistant microorganisms from Pine Barrens soil

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Abstract

Ionizing radiation-resistant organisms are valuable in studying the recovery of microbial flora after anthropological releases of radiation from nuclear waste, accidents or weaponry. These organisms could also play an important role in space exploration, as planets such as mars which lack a protective atmosphere are exposed to high levels of solar radiation. Studying these organisms would benefit efforts in both preventing possible contamination of extraterrestrial sites by exploratory vehicles and possible efforts to grow organisms outside the Earth's atmosphere.

To discover novel radiation-resistant organisms in pine barren soil, a soil sample was taken from the Pine Barrens Forest in the Brookhaven National Laboratory and irradiated with 10 kGy of gamma radiation at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. The soil was then inoculated in different types of growth media, each favoring different types of microorganisms. The cultures were periodically plated and the cultured microorganisms were isolated and characterized using standard microbiology methods. Overall, nine colonies were isolated on three different growth media. Studies are under way to identify the cultures and confirm their resistance to ionizing radiation.

Introduction

Ionizing radiation has long been understood as a cause of DNA damage in microorganisms, often resulting in mortality.[1] Given the increased likelihood of natural ecosystems being exposed to sources of concentrated high-energy radiation from human sources such as nuclear waste or weaponry, understanding ecological effects of ionizing radiation is important for preparing and responding to ecological exposure to radiation. The microorganisms in soil play a fundamental

and crucial role in the ecosystem.[2] Work has shown that ionizing radiation which destroys sensitive microbes, severely limits the biodiversity and fertility of soil.[3-5] Different microorganisms display differing sensitivities to radiation. Some organisms are highly sensitive to the DNA damage caused by ionizing radiation whereas other organism such as *Deinococcus radiodurans* display remarkable tolerance to radiation.[6,7] This resistance in many organisms is thought to be the result of, among other factors, natural DNA repair mechanisms evolved for the purpose of repairing DNA damage caused by desiccation.[8,9] Since desiccation causes some of the same single and double strand breaks that ionizing radiation can cause, the same mechanisms evolved to repair damage from desiccation are effective in repairing DNA damage from ionizing gamma radiation.[8, 10]

Radiation resistance in microbes is of interest not only for understanding the effects of exposure to high levels of ionizing radiation on natural ecosystems, and potentially using radiation-resistant organisms to aid in the recovery of the soil, isolating organisms which can withstand ionizing radiation may help in the discovery of new organisms on other planets. Planets such as Mars that lack an atmosphere are exposed to solar radiation and any life forms that are discovered or attempted to be grown on the planet surface must be able to tolerate such conditions.[11,12] Understanding radiation resistance would also help prevent exploratory vehicles from contaminating extraterrestrial sites with organisms from Earth capable of tolerating solar radiation in space.[13,14] Discovering and isolating organisms resistant to ionizing radiation allows for the opportunity to better understand the effects of ecological exposure to radiation, and aid in the discovery or growth of microbial life on other planets.

Materials and Methods

A soil sample just under the duff was taken from the Pine Barrens forest in Brookhaven National Laboratory and placed in a sterile 50 mL centrifuge tube. The soil was than mailed to the Uniformed Services University of the Health Sciences in Bethesda, Maryland and exposed to a 10 kGy dose of gamma radiation. All further work was done in a sterile laminar hood using standard microbiological techniques.

Approximately 500 mg of irradiated soil was inoculated in 5 mL of 13 autoclaved media in 15 mL centrifuge tubes (Table 1). Inoculated media were then streaked on the respective agar plates at various time intervals. Colonies were purified using multi-sector streaking method. Isolated colonies were grown in liquid media and exposed to 10 kGy of gamma radiation and replated on agar media to confirm their viability and resistance to radiation.

Results and Discussion

As seen in Table 2, nine colonies were isolated from the 10 kGy irradiated soil. Visual inspection of colony characteristics revealed there were four fungal and five bacterial colonies. The relatively high incidence of fungus is likely attributable to their ability to form resistant spores in stressful environmental conditions.[15] The survival of the bacteria is likely due to either highly effective mechanisms of DNA repair or the formation of hardy endospores.[16,17]

It can be observed that only three of thirteen media showed microbial growth. No growth was observed in selective nutrient media. This supports earlier observation made in our laboratory that organisms involved in nitrogen cycle are very sensitive to ionizing radiation.

Ionizing radiation tolerance limits for each of the isolate is currently being evaluated. Identification of the cultures will be made using rDNA sequencing methods. The role of the

ionizing radiation-resistant cultures in the Pine Barrens Forest will be elucidated by studying the physiology of the organisms.

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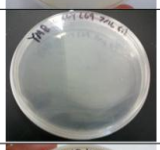
Table 1. Chemical compositions of growth media used in culturing microorganisms from gamma-irradiated soil.

Media	Ingredients (g/L)
Aleem	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	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 (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	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 (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 (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 (TGC)	29.8 thioglycollate media powder
Thiosulfate (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	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 ($FeSO_4 \cdot 7H_2O$, EDTA solution), 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 2. Colony characteristics of the cultures isolated from soil exposed to 10 kGy gamma radiation.

Name	Media	Type of organism	Size	Color	Description	Picture
LG1	TGY	Fungus	Large (10 mm)	Yellow	Filamentous growth, curvy tendrils, highly asymmetrical, raised, highly similar to LG8	
LG5	YMB	Bacterium	Large (~5 mm)	Yellow	Irregular border, jagged at edges, edges are lighter color, thicker in middle	
LG6	YMB	Bacterium	Small (~1 mm)	Yellow/ slightly blue	Rough-looking texture, non-mucoidal, slightly raised	
LG7	TGY	Bacterium	Large (>10 mm)	Light yellow	Only two colonies observed but are on streak, smooth edges	
LG8	TGY	Fungus	Very large (>15 mm)	White	Web-like, raised, highly similar to LG1	
LG9	YMB	Bacterium	Small (<3 mm)	Yellow with a clear border	Mucoidal, round	
LG10	TGC	Fungus	Large (>5 mm)	Brown/ yellow	Dense brown/ dark spot in middle surrounded by light yellow/ white nodules	
LG20	TGY	Bacterium	Small (~3-4 mm)	Yellow	Mucoidal, raised, rounded edges but not completely circular, gets very slightly darker towards center	
LG23	YMB	Fungus	Small (~2 mm)	White/ transparent	Rounded edges, non-mucoidal, not raised, 3 smaller modules in center of colony