# Isolation of Microorganisms from the Pine Barren Soil

Herman Mackey, Jr.

Murty S. Kambhampati, Ph.D

Southern University at New Orleans, New Orleans, LA

Office of Education, Faculty and Staff Teams (FaST)

Vishal Shah, Ph.D

Shreya Shah

Dowling College, Oakdale, NY

Fast and Student Teams

Timothy Green, Ph.D

Brookhaven National Laboratory

Upton, New York

August 11, 2010

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Faculty and Student Teams (FaST) Program under the direction of Dr. Timothy Green in the Environmental Protection Division at Brookhaven National Laboratory.

Participants:	
	Signature
Research Advisor	Signature
Research / Mavisor	Signature

# **Table of Contents**

	Page	
Abstract		4
Introduction		5
Materials and Methods		11
Results and Discussion		15
Conclusion		18
Acknowledgments		19
References		20
Tables and Figures		16

### Abstract

There are no studies in the literature describing the microbial flora in the Long Island Pine Barrens (LIPB), which play a central role in balancing its ecosystem. Since the soil of LIPB is normally acidic, sandy, and poor in nutrients, we hypothesized that the microbial flora in the Pine Barren soil has to be widely different from that reported in, for example, agricultural soil. In the current research, we aimed to isolate microorganisms from the soil of Pine Barrens so that we may learn how they impact the vegetation and influence this environment. After collecting soil from the Pine Barren Forest, we thoroughly cleaned the soil by removing root hairs, stones, and other debris. We then cultured the microorganisms using different media. The broth dilutionplating method was used before incubating the plates to obtain pure cultures. The Biolog® Ecoplates, which contain 31 carbon sources, gave us a metabolic footprint based on characteristic reaction patterns. Thus far, we have isolated 206 cultures from these soil samples by using serial dilutions. The microbial community analysis confirmed the growth of these isolated colonies with the help of Biolog® Ecoplates. The changes observed in the fingerprint patterns have provided us with comparable baseline data that can be used to understand the microbial community changes over time. Further studies are now in progress to understand how these microorganisms behave collectively in nature by taking the community level profile and assessing their pattern development, the rate of color change, and the diversity of the abundant species. In conclusion, later studies will be able to use these baseline observations to track the stability or changes of the LIPB's microbial community over time.

### Introduction

The Pine Barren Forest is a very unique environment that occurs throughout the northeastern United States from New Jersey to Maine, as well as the Midwest and Canada. It is commonly referred to as Pine barrens, pine plains, sand plains, or pinelands [1]. We focused mainly on the Long Island Pine Barren (LIPB) Forest. Surprisingly, there are no studies in the literature describing the microbial flora in the (LIPB), which play a central role in balancing its ecosystem. It's Long Island's largest natural area and its last remaining wilderness. The LIPB overlays and recharges a portion of a federally designated sole source for Long Island's drinking water. All of Long Island's drinking water comes from ground water wells. Almost all of the Peconic River and Carmans River, two of Long Island's four biggest rivers, as well as much of their watersheds are in the LIPB's [2]. Pine barrens are plant communities that occur on dry, acidic, infertile soils dominated by grasses, forbs, low shrubs, and small to medium sized pines. The common trees are pines and oaks while the understory is composed of grasses, sedges, and forbs. Plants like, blueberries and bearberry, and shrubs are also common habitants of the LIPB. These species have adaptations that permit them to survive or regenerate well after fires. The LIPB's support a number of rare species that have become tolerant to fire [3]. Since the soil of LIPB is normally acidic, sandy, and poor in nutrients, we hypothesized that the microbial flora in the Pine Barren soil has to be widely different from that reported in, for example, agricultural soil. In the current research, we aimed to isolate microorganisms from the soil of LIPB so that we may learn how they impact the vegetation and influence this environment. As a secondary objective, we aim to isolate ionizing radiation resistant microorganisms that may be present within the natural environment of the LIPB.

Ionizing radiation resistant bacteria has become a trending topic among the scientific community. The isolation of these bacteria could prove to have significant practical applications in improving the Earth's biosphere. Among the list of potential applications involves the ability of these microorganisms to remediate radiological wastes. From the natural ability to thrive in radiated conditions, these microorganisms could be genetically engineered to treat any environment where radiation would be the principle-limiting factor of microbial diversity. Radiation sensitive organisms wouldn't be able survive in such contaminated environments exposed to radiation and organic pollutants. It is important for us to continue our research of isolating and identifying these organisms for they have the ability to be used for more applications to improve processes, products, and the environment. Some of these applications include:

- **Bioremediation.** Treatments of environments where radiation is the principle factor limiting microbial survival and function[4]. Some of these ionizing radiation resistant bacteria have already been engineered to consume and digest solvents like heavy metals, while others have the ability to detoxify ionic mercury generated from the manufacture of nuclear weapons[5]. See Literature *Engineering radiation-resistant bacteria for environmental biotechnology*.
- **Remediation of nuclear waste material.** Radiation resistant bacteria have currently improved our current methods of consuming and digesting heavy solvents produced by nuclear waste generated from commercial uses, like electricity. They also have the ability to detoxify ionic mercury generated from nuclear weapons manufacture. Literature *Bioremediation of radioactive waste.*

• Clinical Research. Probably the most critical of the possible applications, these microorganisms have shown the ability to assemble synthetic DNA fragments into chromosomes, providing better methods of cancer therapy. We are currently trying to understand the microorganisms DNA rapid repair mechanisms. Individuals exposed to chronic or acute doses of radiation could potentially benefit from treatments that deliver purified *D. radiodurans* Mn complexes into their cells. Similarly, the toxic effects of radiation therapy in cancer patients might be ameliorated by antioxidant drugs based on such a protection paradigm[6]. Literature *Researchers Uncover Protection Mechanism* 

### of Radiation-Resistant Bacterium

## Literature

*Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies*[7]. Given the scale of the contamination associated with 60 years of global nuclear activity, and the inherent high financial and environmental costs associated with invasive physical and chemical clean-up strategies, there is an unparalleled interest in new passive *in situ* bioremediation processes for sites contaminated with nuclear waste. Many of these processes rely on successfully harnessing newly discovered natural biogeochemical cycles for key radionuclides and fission products. Recent advantages have been made in understanding the microbial colonization of radioactive environments and the biological basis of microbial transformations of radioactive waste in these settings.

*Engineering radiation – resistant bacteria for environmental biotechnology*[8]. Seventy million cubic meters of ground and three trillion liters of groundwater have been contaminated by leaking radioactive waste generated in the United States during the Cold War. A cleanup

technology is being developed based on the radiation-resistant bacterium *Deinococcus radiodurans*, which is being engineered to express bioremediating functions.

Radioresistance of Deinococcus radiodurans: Functions Necessary to Survive Ionizing Radiation Are Also Necessary to Survive Prolonged Desiccation[9]. Forty-one ionizing radiation-sensitive strains of Deinococcus radiodurans were evaluated for their ability to survive 6 weeks of desiccation. All exhibited a substantial loss of viability upon rehydration compared with wild-type D. radiodurans. Examination of chromosomal DNA from desiccated cultures revealed a time-dependent increase in DNA damage, as measured by an increase in DNA double-strand breaks. The evidence presented suggests that D. radiodurans' ionizing radiation resistance is incidental, a consequence of this organism's adaptation to a common physiological stress, dehydration.

*Isolation of Radiation-Resistant Bacteria Without Exposure to Irradiation*[10]. Resistance to desiccation was utilized in the selection of highly radiation-resistant asporogenous bacteria from non-irradiated sources. A bacterial suspension in phosphate buffer was dried in a thin film at 25° C and 33% relative humidity. Storage under those conditions for 15 days or more reduced the number of radiation-sensitive bacteria. Further selection for radiation-resistant bacteria was obtained by irradiation of bacteria on velveteen in the replication process, thereby avoiding the toxic effect of irradiated media. The similarity of radiation resistance and identifying characteristics in irradiated and non-irradiated isolates should allay some concerns that highly radiation-resistant bacteria have been permanently altered by radiation selection.

*Extensive Diversity of Ionizing Radiation Resistant Bacteria Recovered From Sonoran Desert Soil* [11]. The ionizing radiation resistant fractions of two soil bacterial communities were investigated by exposing an arid soil from the Sonoran Desert and a nonarid soil from a Louisiana forest to various doses of ionizing radiation using a <sup>60</sup>Co source. The numbers of surviving bacteria decreased as the dose of gamma radiation to which the soils were exposed increased. Bacterial isolates surviving doses of 30 kGy were recovered from the Sonoran Desert soil, while no isolates were recovered from the nonarid forest soil after exposure to doses greater than 13 kGy.

# Repair of Extensive Ionizing Radiation DNA Damage at 95 $\mathcal C$ in the Hyperthermophilic

Archaeon Pyrococcus furiosus[12]. They investigated the capacity of the hyperthermophile *Pyrococcus furiosus* for DNA repair by measuring survival at high levels of  $^{60}$ Co gamma – irradiation. The *P. furiosus* 2-Mb chromosome was fragmented into pieces ranging from 500 kb to shorter than 30 kb at a dose of 2,500 Gy and was fully restored upon incubation at 95°C. They suggest that recombination repair could be an extremely active repair mechanism in *P. furiosus* and that it might be an important determinant of survival of hyperthermophiles at high temperatures.

*Directed Evolution of Ionizing Radiation Resistance in Escherichia coli* [13]. They have generated extreme ionizing radiation resistance in a relatively sensitive bacterial species, *Escherichia coli*, by directed evolution. Four populations of *Escherichia coli* K-12 were derived independently from strain MG1655, with each specifically adapted to survive exposure to high doses of ionizing radiation. D<sub>37</sub> values for strains isolated from two of the populations approached that exhibited by *Deinococcus radiodurans*. Complete genomic sequencing was carried out on nine purified strains derived from these populations. Clear mutational patterns were observed that both pointed to key underlying mechanisms and guided further characterization of the strains. In these evolved populations, passive genomic protection is not in evidence. Instead, enhanced recombinational DNA repair makes a prominent but probably not exclusive contribution to genome reconstitution. Multiple genes, multiple alleles of some genes, multiple mechanisms, and multiple evolutionary pathways all play a role in the evolutionary acquisition of extreme radiation resistance. At least some evolutionary pathways to extreme radiation resistance are constrained by the temporally ordered appearance of specific alleles.

*Researchers Uncover Protection Mechanism of Radiation-Resistant Bacterium*[14]. Results of a recent study titled "Protection Oxidation Implicated as the Primary Determinant of Bacterial Radioresistance," will be published in the March 20 edition of PLoS Biology. The study, headed by Michael J. Daly, Ph.D., associate professor at the Uniformed Services University of the Health Sciences (USU), Department of Pathology, shows that the ability of the bacterium *Deinococcus radiodurans* to endure and survive enormous levels of ionizing radiation (X-rays and gamma-rays) relies on a powerful mechanism that protects proteins from oxidative damage during irradiation.

#### Protection Against Ionizing Radiation in Radiation-resistant Microorganisms[15].

Measured the levels of modified DNA bases following irradiation of the halophilic archaeon, Halobacterium sp. str. NRC-1. In this first study to quantify DNA base modification in a prokaryotic system after exposure to ionizing radiation (IR) a direct relationship between number of DNA lesions and IR dose was revealed. Most importantly, the data demonstrated the significant impact of modified DNA bases on cell survival. Reported for the first time in vivo evidence for the scavenging of reactive oxygen species, a product of IR, by intracellular halides in Halobacterium, resulting in increased protection of DNA and proteins from radiation damage. Results also demonstrate that Halobacterium possesses effective DNA repair systems to counter radiation-induced DNA damage. *Correlation of Bacterial Sensitivities to Ionizing Radiation and Mild Heating*[16]. DNA, isolated from bacteria, which had been heated to 52° for several minutes, sedimented in an alkaline sucrose gradient more rapidly than DNA from untreated bacteria, in a similar manner to DNA from bacteria exposed to ionizing radiation. There is a general correlation between the sensitivities to gamma radiation and to incubation at 52° of various strains of *Escherichia coli*. Heated bacteria were more sensitive to subsequent exposure to gamma radiation, indicating that recovery capacity was itself heat-sensitive. The normal function of some of the cellular systems conferring radiation resistance might therefore be the mitigation of DNA damage due to mild thermal stress at elevated and perhaps also at normal temperatures.

### Materials / Methods

A series of methodologies were used to study soil samples and microbial communities.

**Experimental site.** From the Pine Barren Forest site, soil sample was obtained to analyze the microbial community of the forest. Points were chosen by ecological and ocular methods based on differences in vegetation. The common understory vegetation was identified and marked as a point of interest. The coordinates were identified with a portable, hand-held Global Positioning System (GPS). The soil was collected at an average depth ranging from 2-40 cm from the surface. The soil was dug carefully so that we would not disturb the ecosystem. We had to make sure that the soil we put back had the same sequential order that it had when we dug it out. A total of 2 zip-lock bags of soil were collected from the sampling zone.

**Cleaning of the soil.** Once the soil was obtained, all the debris was removed. The soil was emptied in a tray and wearing gloves, all the stones, root hairs, pine needles, and debris from the tray of soil. Soil from the site in multiple bags was mixed together to obtain a single homogenized sample. Measure 30 grams of soil from each zone after thoroughly mixing both

cleaned trays of soil and place into NALGENE filters. These filters serve as microcosms. It allows us to prevent contamination of the soil from the air within the lab and other sources. In addition, it also allows us to maintain high humidity within the soil. Microcosms were incubated at 30°C, 50% humidity to simulate environmental conditions. All experimental designs must be kept under sterile conditions using the (HEPA) Laminar Flow Hood.

**Inoculating Biolog® Ecoplates.** Biolog® Ecoplates is used to characterize microbial communities by determining ability of communities and oxidize various carbon sources. (Total community substrate utilization of soil). Species diversity consists of species richness, the total number of species present, species evenness, and the distribution of species. To analyze, we took 1 gram of the soil sample from each microcosm and placed into a sterile 15 ml centrifuge tube containing 10 ml of sterile distilled water. We then vortexed each tube for a total of 5 minutes. After vortexing, 2 ml of the supernatant was placed into another 50 ml sterile centrifuge tube containing 18 ml of distilled water. The solution was then vortexed for a total of 2 minutes. Using a multi-channel pipettor, inoculate 150 micro-liters into each of the 96 wells contained on each Biolog® Ecoplate. Place in incubator 30°C at 50% humidity for 48 hours to optimize growth.

A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid <b>γ</b> Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid <b>γ</b> Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γLactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 œ- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 7 Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-AcetyI-D- Glucosamine	E3 Y- Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 Ƴ- Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 a-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 a-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 æ-Ketobutyric Acid	G4 Phenylethyl- amine
H1 œ-D-Lactose	H2 D,L-œ-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-œ-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-œ-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine
				TRATES		a .	<b>T D</b>				

FIGURE 1. Carbon Sources in EcoPlate

The substrate pattern within the ecoplate is shown in Figure 1. As one can see there are 31 substrates and water control, all in triplicates, throughout the plate.

Statistical analysis of Biolog® Ecoplates. After 48 hours of incubation, the plates were read using Tecan® Infinite M200 Plate Reader. Optical densities of bacterial communities were measured at a wavelength of 590 nm. The raw data was then transferred to Microsoft Excel. The data was interpreted using the Shannon Wiener Index, which measures the microbial community physiological profile. It is another way to account for abundance and evenness of the species present while characterizing diversity within a community. Shannon Wiener Index was calculated using the formula (figure below), where H is the Shannon's Diversity Index. *S* is the total number of species in the community (richness). Substrate richness (*S*) was calculated by tabulating the number of substrates having normalized absorbance of more than 0.25. *Pi* is the proportion of species *i*, to the relative number of species (*Pi*), which is calculated, and then multiplied by the natural logarithm of this proportion (Ln*Pi*)[17].

$$H' = -\sum_{i=1}^{S} (p_i \ln p_i)$$

The Shannon Wiener Index (H), commonly referred to, as Shannon Weaver Index is a mathematical formula derived from information theory that was developed by engineers to analyze how efficiently data could be transmitted along telephone lines[18]. The information index takes into account the evenness of the species distribution as well as the absolute number of species. The higher H value implies that within the microbial community, there is a large amount of diversity. The biodiversity of the community is dependent on two components: species richness (S) and species evenness (E)[19].

Species richness (*S*) can be defined simply as the number of species present in a sample, community, or taxonomic group. Species richness is a component of the broader concept of biodiversity. In terms of microbial community, the species richness can be influenced by ecological factors[20]. For example, species richness is often higher in areas with higher productivity, which is the amount of carbon fixed by photosynthesis per unit area per time. Disturbances, such as fires, hurricanes, and floods, can also affect species richness. In many communities, species richness is greatest at little frequency and the intensity of the disturbances[21]. This is because very frequent disturbance eliminates sensitive species, whereas very infrequent disturbance allows time for superior competitors to eliminate species that cannot compete. This decreases the chances of having a microbial community with diversity.

Species evenness (E) is also a component of biodiversity, which quantifies how equal the community is numerically[22]. The less the variation in communities between species, the higher the evenness. In ecological terms of a microbial community, for example, if there are five million

species of *Pyrococcus* and one million species of *Deinococcus*, then this microbial community would not be even. However, if there were two million of both, *Pyrococcus* and *Deinococcus*, then that would be considered an ecologically even microbial community.

## **Results and Discussion**

Sample ID	Sample Zone	Н	S	Ε
	Non radiated Control	12.8	18.0	10.2
2	Radiated control	12.9	0.0	0.0

**Table 1. Shannon Wiener Index** 

As one can see, the Table 1 refers to the calculated numbers of the Shannon Wiener Index of the two tested soil samples. Sample 1 refers to the microbial community that was not exposed to radiation whereas sample 2 refers to the microbial community that was radiated to 16 hours of ionizing radiation. *H* is the Diversity Index, *S* being the number of substrates used, and *E* stands for the species evenness. As far as the diversity, there is very little variation between the two Biolog® Samples. The difference is between the substrates used and the species evenness. The information can be misleading without further investigation. The substrates are characterized into six different categories as can be seen in Figure 2 and 3. As seen in Figure 2, most of our substrates in the categories that we had available were consumed by our microorganisms. This meant we had microbes growingly readily within the wells. The species evenness is a computed formula based on substrates used, the zero doesn't tell the full story. In both cases, we do have results, but we choose for the number to be normalized 0.25 to be considered real consumption. That leaves out the fact that some substrates were still being used, but they aren't

above our normalized numbers, so that they won't fit into our table as a real number. However, they still contribute to the species diversity.



# Figure 2. Control Baseline Microbial

Community

The figure 2 shows that between our six classifications of substrates were being used considerably when compared to the sample 2 study that involved exposure to 16 hours of ionizing radiation. This provided us with a baseline we could use to demonstrate normal diversity of the microorganisms in the LIPB.



As seen in Figure 3, we have no substrates being used by our microbial community after 16 hours of ionizing radiation exposure. Don't let the fact that the figure shows no "substrates utilized" confuse the Shannon Weiner Index. There are some substrates that are utilized, but do not fit the normalized absorbance rate of 0.25. For example, **Phenyl ethylamine**, which is an **Amine**, had an absorbance of 0.11. But because it was under 0.25, it will not be considered a real absorbance number.

To help demonstrate our diversity, we chose to plate our soil samples. Because of the misleading nature of these figures, the plating method further provided us with information that could be used to compare the microbial communities before and after radiation. As seen in Figure 4, there was still growth on our following agars: Phosphate Solubizing Agar (PSA), Potato Dextrose Agar (PDA), Thioglycollate (TGC), Trypto Glucose yeast media (TGY),

Nitrogen deficient Agar (NDA), Sulfur Oxidizing Bacteria (SOA), Nutrient Agar (NA), and yeast Mannitol Agar (YMA). These numbers indicated that there was microbial growth within the 16 hour Radiation soil sample.

Media	Sample 2
PSB	
<b>PDB</b>	
TGC	9
TGY	1
NDS	1
SOB	1
NA	
YMA	1

Figure 4. Microbial Growth on Media after 16 hrs. Radiation

## Conclusion

The numbers and figures indicate that the LIPB has tremendous microbial diversity as proven here and within literature. Therefore our hypothesis was then accepted. The Shannon Wiener Index values indicated that the microbial diversity remained consistent from sample 1 to sample 2. With the lack of substrates used in sample 2, but the same diversity, this leads us to believe that maybe the microbes were not able to grow within those given substrates. As proven with the number of species that continued to grow on the media, instead of the Biolog® substrates. Also the lack of species evenness implies that it could be that few tolerant species dominant the community after radiation. The radiation becomes the limiting factor in the competition. It shows promising results that microorganisms were still able to grow naturally and after radiation within the Biolog® and the plates stemming from the resulting number of 206 isolates that grew on media.

## Acknowledgements

We would like to thank the National Science Foundation (NSF) HRD #0928797 and DUE-0806894 for financial support and DOE for the use of their facilities and accommodations for our team at Brookhaven National Laboratory (BNL). We thank Jennifer Higbie for her help in designing the map and Robert Colichio, BNL for his help in radiating the soil sample. I would like to thank Southern University at New Orleans (SUNO) for this opportunity and the SUNO team members for the NYC experiences. Thanks to my family and friends for their support and encouragement. Thanks are to everyone who guided me through this experience, esp. Herman Richard Mackey, III.

## References

- Brown, Hutch (Summer 2000). "Wildland Burning by American Indians in Virginia". Fire Management Today (Washington, DC: U.S. Department of Agriculture, Forest Service) 60 (3): 30.
- [2] Wikipedia, The Free Encyclopedia. *Long Island Pine Barrens.* <u>http://en.wikipedia.org/wiki/Long Island Pine Barrens</u>. August 3, 2010 at 15:11.
- [3] Wikipedia, The Free Encyclopedia. *Long Island Pine Barrens.* <u>http://en.wikipedia.org/wiki/Long Island Pine Barrens</u>. August 3, 2010 at 15:11.
- [4] Jianlong, Wang. *Engineered radiation-resistant bacteria and their application in bioremediation of radioactive wastes-contaminated environment.* Journal of Radiation Research and Radiation Processing. (2005). X: 172
- [5] Demirjian, David C. *Enzymes from Extremophiles*. Current Opinion in Chemical Biology Volume 5, Issue 2, 1 April 2001, Pages 144-151
- [6] Gross L (2007) Paradox Resolved? The Strange Case of the Radiation-Resistant Bacteria. PLoS Biol 5(4): e108. Doi:10.1371/journal.pbio.0050108
- [7] Lloyd, Jonathan R. and. Renshaw, Joanna C. *Bioremediation of radioactive waste: radionuclide-microbe interactions in laboratory and field-scale studies*. Current opinion in Biotechnology. (2005). **16**: 254-260
- [8] Daly, Michael J. *Engineering radiation resistant bacteria for environmental biotechnology.* Current Opinion in Biotechnology. (2000). **11**: 280-285.
- [9] Mattimore, Valerie and Battista, John R. *Radioresistance of Deinococcus radiodurans: Functions Necessary to Survive Ionizing Radiation Are Also Necessary to Survive Prolonged Desiccation.* Journal of Bacteriology. (1996). 633-637.
- [10]Sanders, Scott W. and Maxcy, R. Burt. *Isolation of Radiation-Resistant Bacteria Without Exposure to Irradiation.* Applied and Environmental Microbiology. (1979) 436-439.
- [11] Rainey Fred A., Ray, Keren, et al. Extensive Diversity of Ionizing Radiation Resistant Bacteria Recovered from Sonoran Desert Soil. Applied and Environmental Microbiology. (2005). 71: 5225-5235.
- [12] DiRuggiero, Jocelyne, Santangelo, Nick, et al. Repair of Extensive Ionizing Radiation DNA Damage at 95 °C in the Hyperthermophilic Archaeon Pyrococcus furiosus. Journal of Bacteriology. (1997). 179: 4643-4645.
- [13] Harris, Dennis R., Pollock, Steve V., *et al. Directed Evolution of Ionizing Radiation Resistance in Escherichia coli.* Journal of Bacteriology. (2009). **191:** 5240-5252.
- [14] Daly, Michael J. Researchers Uncover Protection Mechanism of Radiation-Resistant Bacterium. ScienceDaily. (March 21, 2007).
- [15] Dizdar, Miral and Jaruga, Pawel. Protection Against Ionizing Radiation in Radiation Resistant Microorganisms.
- [16] B.A. Bridges, M.J. Ashwood-Smith. Correlation of Bacterial Sensitivities to Ionizing Radiation and Mild Heating. Journal of General Microbiology. (1969). 58: 115-124.
- [17] Begon, M., J. L. Harper, and C. R. Townsend. 1996. Ecology: Individuals, Populations, and Communities, 3rd edition. Blackwell Science Ltd., Cambridge, MA. <u>http://www.tiem.utk.edu/~mbeals/shannonDI.html</u>
- [18] Magurran, A. E. 1988. <u>Ecological Diversity and its Measurement</u>. Princeton University Press, Princeton, NJ. *Measuring Diversity*. <u>http://www.denniskalma.com/biodiversitymeasurement.html</u>

- [19] Wilsey, Brian J. "Biodiversity And Ecosystem Functioning: Importance Of Species Evenness In An Old Field". Ecology. FindArticles.com. 05 Aug, 2010. http://findarticles.com/p/articles/mi\_m2120/is\_4\_81/ai\_62332428/
- [20] McGinley, Mark and Duffy, J. Emmett. "Species richness." In: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment). [First published in the Encyclopedia of Earth February 6, 2007; Last revised February 6, 2007; Retrieved August 5, 2010]<http://www.eoearth.org/article/Species\_richness>
- [21] McGinley, Mark. <u>http://www.eoearth.org/article/Species\_richness</u>
- [22] **Species evenness.** From Wikipedia, the free encyclopedia. http://en.wikipedia.org/wiki/Species\_evenness