Exploring the Microorganisms Present in the Pine Barren Soil

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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 dilution-plating method was used before incubating the plates to obtain pure cultures. Thus far, we have isolated 206 cultures from these soil samples by using serial dilutions. In addition, the soil was radiated with gamma radiation for 16 h and changes in microbial community analyzed. After radiation less than 20 cultures were isolated on the agar medium. The Biolog® Ecoplates, which contain 31 carbon sources, gave us a metabolic footprint based on characteristic reaction patterns. The microbial community analysis confirmed the changes in the microbial community. Further studies are now in progress to understand how the radiation changes the microbial community in LIPB.

Introduction

Soil of LIPB is normally acidic, sandy, and poor in nutrients. In addition, it experience wide range of temperature fluctuations between summer and winter seasons. 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.

Materials and Methods

- Soil samples were collected from Pine Barren Forest region within Brookhaven National Laboratory (Figure 1).
- Microbial inoculum were prepared by suspending the soil sample in sterile distilled water and vortexing the sample for five minutes.
- Soil was exposed to 16 h of gamma radiation using ^{137}Cs isotope as the radiation source. The rate of radiation was 1.1 Gy/min.
- Phosphate solubilizing agar, TGY: Tryptone Glucose Yeast medium, NA: Nutrient Agar, NDA: Nitrogen Deficient Agar, PDA: Potato Dextrose Agar, SOA: Sulfur Oxidizing Agar, TGC: Thioglycollate Medium, and YMA: Yeast Mannitol Agar media were inoculated.
- 1g of soil was taken from filter and placed into the eight broths. The broths were vortexed for 2 minutes each, and then 20 µl were inoculated onto the eight agar plates.
- For Biolog® Ecoplates, each well was inoculated with 150 µl of diluted inoculum.
- The plates were incubated at 30°C for 48 hours under 16/8 light/dark cycle. The incubation period was selected upon carrying out optimization studies to find out the least incubation time required for obtaining reliable data.
- Upon incubation of plates for more than 48 hours, no change in substrate utilization pattern was observed. Only the intensity of the wells increased.
- The plates were read at 590 nm using TECAN plate reader.
- Statistical analysis were carried out using STATISTICA version 8.0
- Substrate richness (S), was calculated by tabulating the number of substrates having normalized absorbance of more than 0.25
- The Shannon–Weaver index, ***H***, was calculated using the formula

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

where *pi is* the proportion of microbial activity on substrate ***i,*** in total microbial activity (***S***). The substrate evenness for the microbial composition is given by ***E = H/log S***

Figure 1. S26 N 40.51.7198 W 072.51.9739. LIPB Solar Farm Area

Results and Discussion

- As shown in Table 1, the microbial diversity in the solar farm area is less than what we found in our earlier studies. An average H value of 30 was reported last year across the LIPB. However, the diversity in the studied area is approximately 60% less. Studies need to be carried out to understand if the construction of solar farm system may cause further decrease in the microbial diversity.
- Figure 2A shows the diversity of substrate utilized by the microbial community in the non-radiated soil. All the classes of substrates were utilized with varying degree of efficiency.
- Exposure to radiation did not change the *H* values. The *S* and *E* values decreased significantly reaching zero. The numbers needs to be treated with caution as our threshold values for richness was 0.25. Color formation was observed in many wells but did not meet the threshold limits (Table 1, Figure 2B).

Sample	H	S	E
Non-radiated soil	12.8	18	10.2
Radiated soil	12.9	0	0

Table 1. Microbial Diversity Indicators

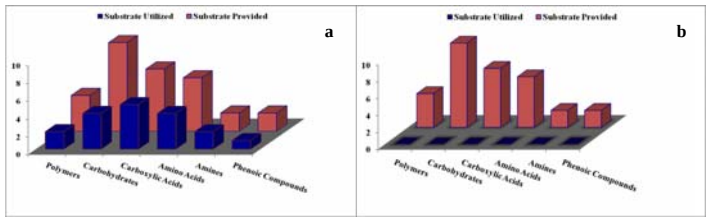

Figure 2: Substrate richness (*S*) as observed in Biolog® Ecoplates. (a) non-radiated soil, (b) radiated soil.

- To understand the type of organisms surviving radiation, different microbial media were used to grow the cultures.
- Table 2 shows that 13 cultures survived radiation.
- These cultures are now being identified and their radiation resistance is being studied.
- In non-radiated soil, around 200 cultures could be found on the same microbial media types.
- This would clearly indicate a decrease in microbial diversity. We are trying to elucidate the reasoning for the contradiction between the Biolog and culturing methods.

Media	PSA	TGY	PDA	NA	TGC	NDA	YMA	SOA
# of colonies	0	1	0	0	9	1	1	1

Table 2: Number of colonies forming organisms present in the 16 h radiated soil.
(PSA: Phosphate solubilizing agar, TGY: Tryptone Glucose Yeast medium, NA: Nutrient Agar, NDA: Nitrogen Deficient Agar, PDA: Potato Dextrose Agar, SOA: Sulfur Oxidizing Agar, TGC: Thioglycollate Medium, and YMA: Yeast Mannitol Agar)

Conclusion

- Shannon Weaver Index (*H*) indicates that microbial diversity in the soil sample collected is less when compared to the H values obtained from the other regions of LIPB (normal values around 30).
- Exposure to gamma radiation did not decrease the Shannon Weaver Index. This could be an indication of the presence of radiation resistant bacteria in the soil.
- Further studies are warranted to confirm the radiation resistance of the colonies isolated post-radiation.
- Studies are also warranted to calculate how much radiation is actually being experienced by the organisms. Soil properties may play a big role in shielding the radiation. Soils from different part of LIPB has different microbial diversity and radiation experiments on all these samples may provide wide array of radiation resistant bacteria.

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