The Effects of Hydrocarbon Contamination on the Soil Microbial Consortia from the Quivira National Wildlife Refuge

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ABSTRACT

Three soil samples were collected. Two samples from the Quivira Wildlife Refuge in Kansas. One of the samples was contaminated with oil and the other was from a salt marsh. The third sample was of fresh water from McPherson Kansas. The samples then went through a metagenomic shotgun sequencing which gave an in depth analysis of the microbiomes within the soil samples. The sequencing broke down the different make-ups of the bacteria ranging from the gene production and abundance analysis to the antibiotic resistance. Each of the three samples showed to share a few similar genes but the majority was different. Overall they showed to be different from each other with the different types of bacteria. The most common type of bacteria found within the samples was proteobacteria. There was not a big correlation between each of the three samples that were taken.

Keywords: *hydrocarbon contamination; microbiomes; proteobacteria; antibiotics*

INTRODUCTION

Hydrocarbon contamination is an issue that has been happening for a long time and these spills range from how large they are and how recent they have happened. One of the first spills could have come in 1859 at an oil pump in Titusville, Pennsylvania. It is believed that the first spillage of oil from the pump happened when trying to transport the oil from the pump. Then there are instances such as the Lakeview Gusher in California that spilled more than 1,200 tons of crude oil. Each oil spill has an impact on the environment. This is the focus of the research to see how hydrocarbon contamination can affect the using in the Reserve.

Diverse communities of bacteria can be found ranging from places such as the human stomach and deep-sea mud. There are even inhospitable conditions such as acid mine runoff and geothermal hot springs. Environmental DNA sequencing has revealed the expansive biodiversity of microorganisms and clarified the relationship between host-associated microbial communities and host phenotype. Studies of differently cultured microbes show how vital they are in their own environments. They provide essential ecosystems services. Although some of these microbes cannot be culture in a lab so there is limited information on organismal and functional novelty to be disovered.

In Mohammed Bahram et. al paper it explains the complexity of the microbiomes that are present in 189 soil samples that are from regions all over the world. A total of 1,450 sites were used to collect the sample. The results found that the soil microbiome depended on the soils pH levels, and that the amount of precipitation can influence how large the microbiomes are.

In K.S. Jorgensen research they used organic matter such as bark chips to try to clean up oil contamination in a soil sample. The mineral oil degradation rate was the most rapid in the first months of the research. With this type of organic matter amendment, the general microbial activity measured by soil respiration was enhanced and no particular effect of added inocula was observed.

MATERIALS AND METHODS

The soil was collected at the Quivira National Wildlife Refuge located in Kansas. The location was selected because of salt marshes that are within the Wildlife Refuge. These salt marshes have similar attributes as beaches. The state of Kansas used to be an ocean and some of the oceans characteristics are still noticeable because of the salt-water marshes and the sand dunes that are all over the refuge. The refuge features both a large salt marsh and a sand prairie. The refuge also lies right in the transition zone of the eastern and western prairies of Kansas. Along with the beach like conditions there are oil pumps that are spread throughout the refuge.

The soil samples were collected from four different oil pumps within the refuge. The samples that were taken were contaminated with the oil that came from the pumps. A shovel was used to remove the surface layer of soil that had hardened. Then a trowel was used to then get the softer soil that was underneath the hard layer of soil and oil. Along with the soil samples from the oil pumps, uncontaminated soil was gathered from about 50 meters away to compare the two different types of soil samples. After the soil samples were collected they were then stored at McPherson College. Aliquots were put into labeled 50 mL screw-top plastic centrifuge tubes, frozen to a temperature of -80°C, and then shipped to CD Genomics for analysis.

The genomic shotgun sequencing collects the microbial genetic information that is contained within the sample from the environment. The process of the shotgun sequencing can produce complete sequences of protein- coding genes. This can give a crucial knowledge about the microbial consortia within the sample that came from the oil pumps from within the Wildlife Refuge. This information is important because it gives a detailed description of what is within the soil sample.

RESULTS

There was an abundance of different genes found within the samples. A total of about 150,000 genes were found in the oil-contaminated sample. The salt marsh sample was about three times the size of the oil sample. The fresh water sample had the least amount of genes with about 50,000. All three samples had 524 similar genes found within them. Another graph shows similar genes found in each of the samples. This gives a more in depth knowledge of what different types of genes can be found in the different samples.
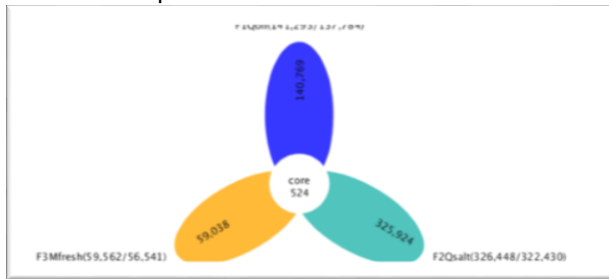

This graph shows the correlation between each of the three samples that were tested. The darker the red color the bigger the difference between the samples. Blue color shows a positive correlation. This compares each of the samples to the other to see the correlation between samples. This figure shows that there is not that big of a correlation between each of the three samples.

The Krona presentation within the research shows the hierarchy of different bacteria and their taxa. The pie chart shows the relative abundance of taxa across multiple levels of hierarchy simultaneously. The graph shows the top 10 taxa and the rest were set out as "Others". It shows the abundance of phylum and genus. The graph shows the relative taxonomy abundance of each sample in different taxonomic level. Proteobacteria makes up the majority of each of the three samples.
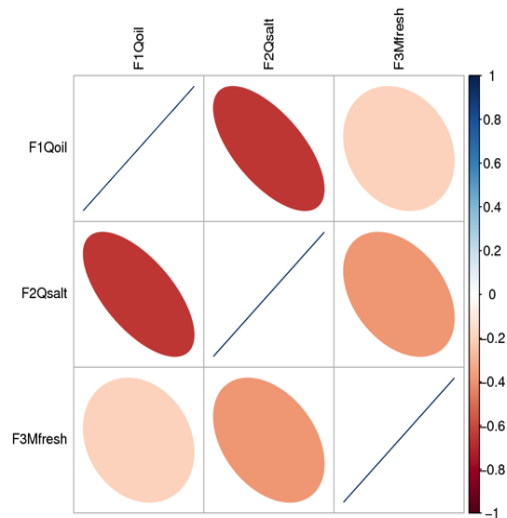
These bacteria found within each of the samples have different functions such as metabolism, process genetic information, environmental information processing, cellular processes, human diseases and organismal systems.

The soil sample that was contaminated by hydrocarbons showed to have the greatest abundance of antibiotic genes in it. It had about 140 ppm. The next closest was the fresh water sample at 50 ppm. Finally the salt sample had about 30 ppm. This shows that the oil sample has large amounts of antibiotic resistance genes found.
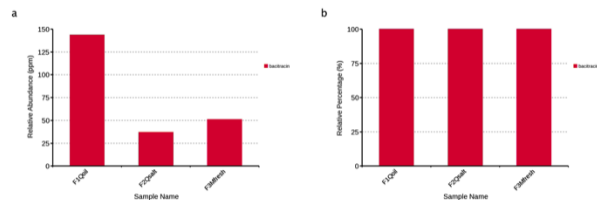
DISCUSSION

The three samples showed that they were not very correlated between each other. There were similarities but the majority different. All three samples showed 524 genes that the three samples had in common. There were at least 5 times that amount that was different between all of the soil samples. This could be expected because they were taken from separate locations. Proteobacteria was the most common type of bacteria found within the soil. In the oil sample proteobacteria made up nearly half of the samples taxa. Proteobacteria is known for being pathogens such as salmonella. It is also known to be bacteria responsible for nitrogen fixation. The sample contaminated with hydrocarbons also had the greatest abundance of antibtiotic resistant genes

found. The metagenomic shotgun sequencing showed differences between each of the three soil samples from the abundancy of different genes to its ability to resist antibiotics.

ACKNOWLEDGEMENTS

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