Microbial Diversity of Wet and Dry Soil in Prince Edward County

Megan Curry

Biol 250

Spring 2017

**Introduction**

Microbes live everywhere, some in the hottest places in the world and some in the coldest places, but we are still learning the basis on their life structures and effects on the environment (Brockett, 2006). Sometimes natural occurrences in the environment, such as high precipitation, can destroy the microbes or make them prosper (Castro, 2010). Scientists have been studying microbial diversity in different areas and climates. The author’s search for microbial diversity started in Prince Edward County, Farmville, Virginia. A collection of environmental and microbial diverse samples were taken from Buffalo Creek, and later plated for further examination. The initial idea was to sample water migrated microbes, or microbes from wet soil. Further research showed that wetting and rewetting the same soil only increased the number of bacteria, instead of changing the amounts of microbial diversity (Barnard, 2013). With that in consideration the authors concluded that the diversity seemed larger if the samples were taken from the wetted soil right at the coast line of the creek and the dry soil on the bank of the creek. To assure that the microbial diversity was high, the samples were not rewetted. Also, the authors took samples during colder weather because microbial growth and increase of community can be prosperous during the winter (Schmidt, 2004). Researchers show that samples from under patches of snow have a higher diversity, but the resource wasn’t available at the time of sampling (Schadt, 2003).

The purpose of this study was to find microbial diversity in the samples collected in Prince Edward county. The authors took note of the needed research and questioned, what is the microbial diversity in the wet dirt and dry dirt surrounding Buffalo Creek? They hypothesized that the wet dirt surrounding Buffalo Creek will have a larger microbial diversity than the dry dirt because of the influence of microorganisms in the water itself.

**Materials and Methods**

*Site description and sampling*

Wet and dry soil samples were collected on February 2, 2017. The wet soil was directly taken from the shoreline of Buffalo Creek, located in Farmville, Virginia, and the dry soil was taken from a bankside close to the creek. Samples were taken to the Environmental Education Center in Lancer Park (Longwood University), and then weighed out to fit the criteria of .5 grams. Each amount was placed into a sterile tube and 25mL of water was added to create a direct count. 10mL was pipetted from the direct count into a new sterile tube (1:10) and vortexed for 10 seconds. 10mL from the 1:10 was placed into a third sterile tube (1:100) and vortexed for 10 seconds. Once all six tubes (wet and dry soil) were ready, each sample was placed onto an agar plate, spread out with a blue hockey stick, and stacked together to be placed into an incubator at 30 degrees Celsius for roughly four days to grow.

*Genomic DNA extraction*

One colony from each site was collected with a sterile toothpick, added to separate microcentrifuge tubes containing 300uL of microbead solution, and mixed for 15-30 seconds to suspend the bacteria cells into the solution. The solutions were micro pipetted into separate microbead tubes, and 50uL of solution MD1 was added. The tubes were transferred into a water bath for 10 minutes at 65 degrees Celsius, and then vortexed for 10 minutes at maximum speed. Next, the tubes were centrifuged at 10,000xg for 30 seconds at room temperature. The supernatants were transferred to clean 2mL collection tubes, and 100uL of solution MD2 was added to the supernatants. The tubes were then vortexed for 5 seconds, and incubated at 4 degrees Celsius for 5 minutes. The tubes were centrifuged for 1 minute at 10,000xg. Avoiding the pellet, the volume of the supernatant was micro pipetted into clean 2mL collection tubes. Nine hundred ul of solution MD3 was added to the supernatant and the tubes were vortexed for 5 minutes. Seven hundred uL was added to the spin filter and then centrifuged at 10,000xg for 30 seconds at room temperature. The flow through was discarded, the rest of the supernatant was added to the spin filter, and centrifuged the same. Three hundred uL of solution MD4 was added and the tubes were centrifuged at 10,000xg for 30 seconds. The flow through was discarded and the tubes were centrifuged at 10,000gx for 1 minute to ensure all of the ethanol was emptied out. The spin filter was placed into a new tube and 50uL of solution MD5 was added to the center of the filter. The tubes were centrifuged for the last time at 10,000xg for 30 seconds, the spin filter was discarded, leaving the DNA in the tube, and the tubes were stored at -20 degrees Celsius for a week.

*PCR amplification*

Fifteen µl of nuclease-free water, 25µl of One*Taq* 2X Master Mix, and 2.5µl of Primer Mix (10uM Forward and Reverse) were added to the PCR tubes. To mix the solution, pipettes were used to pull the solution up and down slowly. Seven and a half µl of the previous genomic DNA was added to the tubes. The PCR tubes were placed in a PCR machine to thermocycle. For initial denaturation, the temperature was set for 94 degrees Celsius at 4 minutes. For 30 cycles, it was set at 98 degrees Celsius for 10 seconds, 50 degrees Celsius for 15 seconds, and 72 degrees Celsius for 20 seconds. For final extension, it was set at 72 degrees Celsius for 5 minutes.

*Purification, restriction digest, and electrophoresis of PCR products*

For PCR (Polymerase Chain Reaction) purification, 250uL of Binding buffer was mixed with the previous PCR samples. The mixes were added to two spin filter columns and centrifuged for 1 minute at 13,000rpm (16,000xg). Flow through was discarded, 200uL of DNA Wash Buffer was added to the spin filters, and centrifuged for another minute at 13,000rpm. The previous step was repeated, and the spin filter was placed into a new 1.5mL tube. 20uL of sterile water was added to the center of the spin filter, and a nanodrop was used to measure the DNA concentration and 260/280 values.

For restriction enzyme digestion, each PCR product tubes had 5µl PCR product and 10µl MspI mix added to it and mixed with a pipette. The tubes were incubated for 45 minutes at 37 degrees Celsius.

For the electrophoresis, 5µL of 5X loading buffer was added into each PCR tube and mixed with a pipette. The electrophoresis chamber was filled and the gel was covered with 1X TAE buffer. 10µL of each sample was loaded into separate wells in the gel chamber. The power was turned on and the gel was running at 120V for 30 minutes.

*Sequencing*

The DNA was sent to EurofinsGenomics for sequencing. 8µl of the “cleaned” PCR product and 4µl of the sequencing primer was added to the DNA to prepare it for sequencing.

*Blast analysis*

The DNA sequences came back, and a BLAST (Basic Local Alignment Search Tool) analysis was conducted to identify the species and genus of the bacterium. Eight hundred good bp were copied and submitted into the BLAST database, *Pseudomonas koreenis* has a 99% match with the DNA sequence.

**Results**

*Colony description*

The purpose of this experiment was to collect samples of wet and dry soil in Prince Edward County, study the microbial diversity, and identify the bacteria amongst the samples. Both wet and dry colonies have purple, yellow, or a white color to them. Their sizes range from small, medium, and large. The wet soil’s Direct Count (DC) plate had 30-50 colonies with filamentous, lobate, undulate, and entire forms and margins. The 1:10 plate had 50-60 colonies with punctiform, circular, and filamentous forms. The 1:100 had 30-50 colonies with punctiform and filamentous forms (Fig. 1a). The dry soil’s DC plate had up to 27 colonies mixed with punctiform, irregular, circular, and filamentous forms. The 1:10 plate had 25 colonies with irregular, circular, and filamentous forms. The 1:100 plate had 41 colonies with punctiform, irregular, and circular forms (Fig. 1b).

*PCR Purification and DNA extraction*

The PCR purification for both wet and dry samples concentration and 260/280 values were extremely low. For wet soil the 260/280 value had 5.89 and the concentration was -.9. For dry soil the 260/280 value had 1.67 and the concentration had 8.6. The DNA extraction also showed weak results. The wet soil’s 260/280 was 1.119, the 260/230 was .51, and the concentration was 1.9. The dry soil’s 260/280 was 1.30, the 260/230 was .36, and the concentration was 1.6 (Fig. 2).

*Gel Electrophoresis and BLAST analysis*

The gel electrophoresis for both of our samples did not work, but Dr. Shanle reran the gel and found a working strand of MSP1 and PCR product for the wet soil. The MSP1 digest had a band at 500bp, and the PCR product had a band at 950bp. The PCR products had roughly 900 good reads out of 1092 bases. From the product, a NCBI BLAST analysis matched it with an identical strand that had 908 out of 910 matched base pairs. This match identified our bacteria as *Pseudomonas koreensis* (Table 1, Fig. 3).

**Discussion**

This experiment was conducted to compare the microbial diversity in wet and dry soil from Buffalo Creek, located at the Environmental Center in Prince Edward County. It was hypothesized that the wet soil would have a larger microbial diversity than the dry soil because of the influence of the flowing water. The hypothesis was rejected after the gel electrophoresis showed no results for the dry and wet soil, and the DNA was not prevalent. The site’s DNA could not be sequenced because of an issue in the experiment and/or the sample collected was a yeast. A bacterium shows up on the electrophoresis ladder, and a yeast shows nothing. Dr. Shanle reran the gel electrophoresis and managed to get a bacterium out of the wet soil sample, and used for further analysis.

The genus and species of the bacteria was most likely *Pseudomonas koreensis Ps 9-14.* It was also found in samples in Korean agricultural soils (Kwon, 2003). The wet soil’s bacteria related to other experiments such as the Korean experiment, but after further comparison it seemed to have more differences than similarities. Other research experiments showed that wetting and rewetting the same soil only increased the number of bacteria, instead of changing the amounts of microbial diversity (Barnard, 2013). Our hypothesis stated that the microbial diversity will be higher in wet soil, but the results showed differently. This statement also debunked our data because there was not a large microbial diversity, and the results showed hardly any bacteria. The colony’s bacteria appeared to be coccus shaped, but sampling taken from similar sources all came back to be rod-shaped, meaning that there were possible mutations in the experiment (Chang, 2016). Though the hypothesis was not supported, there were similar characteristics between our colonies and others. *Pseudomonas koreenis* is a bacterium with gram-negative cells and appears with a whitish-yellowish coloring (Kwon, 2003). Our collected samples showed many colonies with the same coloring characteristic.

Next, the main limitations to the study was the gel electrophoresis and the sample collection. If there is an error is data collection, PCR amplification, and/or DNA extraction. The PCR product did not work because a yeast had been collected. The second limitation would be the sample collection. Sometimes natural occurrences in the environment, such as high precipitation, can destroy the microbes or make them prosper (Castro, 2010). The samples experienced the same natural habitat, so this would have not been an option for our experiment.

Subsequently, the research question should change into a broader enquiry to match the results. The research question was at fault because it was too open of a question with too many issues. The results showed that there were little bacteria, the initial experiment had faults in it, and the samples contained primarily yeast. The question posed should not be “what is the microbial diversity in wet and dry soil,” it should be reworded as: what is the microbial difference and yeast association with wet and dry soil? With this styled question, the results would match the question better, and show that there are more than just bacterium living in the soil.

Thus, the next steps in clarifying the research question would be further analysis of the sampling source. Before sampling areas, they should be checked for wildlife, weather, and other contributing factors. Water from Buffalo Creek could also be tested to check what microbes have been washed into the wet soil sample because soil moisture is one of the key issues with the microbial community (Brockett, 2006). With more samples and data collection, yeast and bacteria identification can be made easier.

**Figures and** **Legends**

**Figure 1a. Percent of shaped colonies for wet soil.** This data shows the percent abundance of the colony forms for the plates. The most common were irregular and punctiform shaped colonies. All of the plates had three different colony shapes.

**Figure 1b. Number of colonies for dry soil.** The data is shown for the different colony forms and plates. Irregular and circular formed colonies were the most common among all the plates. The largest difference is the amount of circular formed colonies in the 1:100 (.11111).

**Figure 2. PCR purification and DNA extraction values**. On the left is the concentration and 260/280 values for the wet and dry soils. The numbers are lower than what is expected. To the right is the concentration, 260/280, and 260/230 values for DNA extraction. The numbers for the extraction are very small meaning there was weak DNA present.



**Figure 3.** **Identification of *Pseudomonas koreensis* from wet soil sample.** (3.l) Picture of the colony selected for further inspection. (3.ll) Alignment between *Pseudomonas koreensis* rRNA gene sequence and the sequenced DNA from the wet soil. (3..lll) Gel electrophoresis of 16S rRNA PCR product (Lane 2) and MspI digestion (Lane 3). (3.llll) High quality chromatogram results that were used for BLAST analysis.

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| Pseudomonas koreensis  | 99% |
| Pseudomonas moraviensis | 98% |
| Pseudomonas reinekei | 98% |
| Pseudomonas vancouverensis | 97% |
| Pseudomonas cremoricolorata  | 96% |

**Table 1. Percentage of matches**. The top five matches compared to the PCR product are shown on the left. On the right is the percentage of identical bases to the original strand. Pseudomonas koreensis is the closest match with only 2 misplaced base pairs.

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