Evolving Techniques in Discovering Bacterial DNA in Soil Samples

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INTRODUCTION

Dr. Krolikowski led her class on the Soil Microbiome Project to determine whether variations in the soil microbiome were correlated with plant growth and health. To this end, soil samples were taken from various points at Urban Tilth's North Richmond Farm and brought into the classroom at Contra Costa College. A series of experiments were undertaken to identify Bacterial DNA in the soil.

The current class and subsequent classes will perform DNA tagging and sequencing to identify the species of bacteria that reside within the soil. This will allow them to determine any correlation between the composition of the soil microbiome and plant growth. The current class has completed amplification of bacterial DNA in farm soil, this current presentation will summarize the work that has been done so far.

METHODS

Discovering evidence of bacterial DNA in soil samples from different sections of the Urban Tilth North Richmond farm was processed through a series of DNA extraction, PCR, and gel electrophoresis.

- 1. A bacterial positive control was created by infusing soil with known bacteria. DNA was extracted from the bacterial soil sample that will later be used in DNA tagging.
- 2. Technicians was given soil samples from different sections of the Urban Tilth farm to extract DNA from.
  - A supernatant containing DNA from each soil sample was isolated using the provided DNA extraction kit and following a specified S.O.P
  - Nanodrop analysis was used to determine the DNA concentration of each sample
- 3. Fragments of DNA from each sample were amplified through PCR using 16s primers.
- 4. PCR products were conducted through gel electrophoresis on an agarose gel. The gel was analyzed to detect any DNA bands in the correct lanes of the gel.
- 5. A failed attempt to detect DNA bands resulted in technicians to repeat the extraction procedure or PCR procedure to achieve better results.

Experimental Results

Table 2

PCR Results Spring 2018 Semester

	Bacteria Control PCR Early Semester		Farm Soil 1 PCR Middle Semester		Farm Soil 2 PCR Late Semester	
	Results	Percent of Total	Results	Percent of Total	Results	Percent of Total
Total Tests	10		29		27	
Positive Results	2	20.00%	8	27.59%	9	33.33%
Negative Results	3	30.00%	5	17.24%	10	37.04%
Positive Control Errors	3	30.00%	10	34.48%	3	11.11%
Negative Control Errors	2	20.00%	6	20.69%	5	18.51%
Total Usable Results	5	50.00%	13	44.83%	19	70.37%

- The information in Table 1, 2, and 3 were aggregated from student laboratory notebooks and PCR soil logs. We tabulated the total tests performed, the positive results, the negative results, the number of negative and positive control errors, and the total usable results, as shown in Table 2. The results of the three experiments are compared in Graph 1, demonstrating class technical skill development, in both the increasing total usable results and the decreasing control errors, over time. The total usable results will be used by the class for the final experiment of the semester, the DNA Tagging of samples from the Soil Microbiome Project.

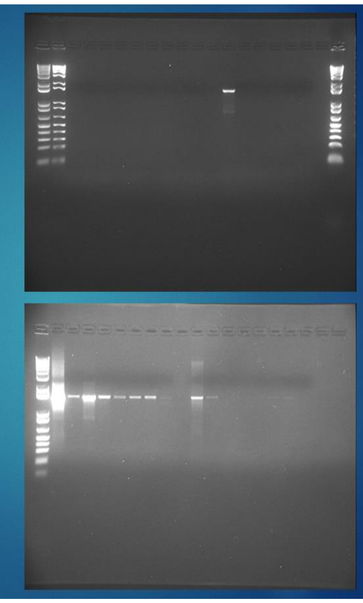
PCR Log

Farm Soil Experiment 2 PCR Results
Spring 2018

Sample Unique ID	PCR Band	PCR Pos Ctrl	PCR Neg ctrl
CCCUT04B	+	+	-
CCCUT05B	-	-	-
CCCUT06B	-	+	-
CCCUT07B	+	+	+
CCCUT07C	+	+	-
CCCUT07D	-	-	-
CCCUT09B	-	+	-
CCCUT13B	+	+	-
CCCUT17B	+	+	-
CCCUT18B	-	+	-
CCCUT19B	+	+	-
CCCUT20B	+	+	-
CCCUT21B	-	+	-
CCCUT23B	+	+	-
CCCUT23C	+	+	+
CCCUT25B	+	+	+
CCCUT25C	-	+	-
CCCUT25D	-	-	-
CCCUT27B	+	+	-
CCCUT29B	+	+	+
CCCUT32B	+	+	+
CCCUT32C	-	+	-
CCCUT33B	-	+	-
CCCUT38B	+	+	-
CCCUT290	-	+	-
CCCUT294	-	+	-
CCCUT318	-	+	-

Legend
Positive Results
Negative Results
Positive Control Errors
Negative Control Errors

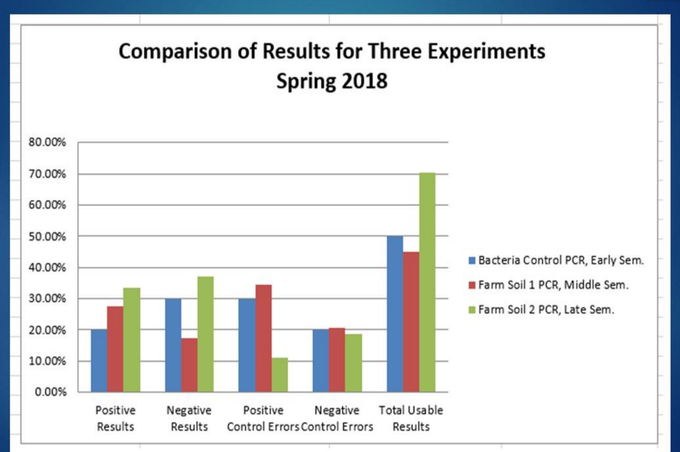
Results	Total	%
Total Tests	27	
Positive Results	9	33.3
Negative Results	10	37
Positive Control Errors	3	11.1
Negative Control Errors	5	18.5
Total Usabel Results	19	70.3



RESULTS

- Three experiments were performed, Spring 2018, to check for the presence of bacterial DNA in soil: Bacteria Positive Control, Farm Soil #1, and Farm Soil #2. Each experiment involved the extraction of DNA from soil, amplification of the DNA using PCR and bacterial DNA primers specific to the 16s ribosomal subunit, and visualization on agarose gel.
- In the beginning of the semester, the Bacteria Positive Control Experiment was performed to serve as practice for the class to learn how to extract, amplify, and visualize bacterial DNA where the presence of bacteria in the soil was assured. It was also used to test quality control steps including Nanodrop readings, PCR positive controls, and PCR negative controls.
- Mid-semester, actual soil samples from the Soil Microbiome Project were used in the Farm Soil #1 Experiment, were the presence of soil bacteria was unknown. Additional benefits from this experiment included refining SOP's, developing procedures for data collection, and planning redundant testing on the soil samples.
- Toward the end of the semester, the Farm Soil #2 Experiment was performed using improved procedures determined in the previous experiments. The results of the Farm Soil #2 Experiment are currently being used to determine which samples are ready for DNA tagging and future Sequencing.

Comparison of Experimental Results



Conclusion

The PCR procedure targeting the 16S gene produced positive results showing the presence of the 16S gene in the extracted DNA. Since the 16S gene is found only in bacteria, these results demonstrate the presence of bacteria DNA within the soil samples.

Many technicians that did not produce usable results in their first attempt at PCR and gel electrophoresis because bands failed to appear in the positive control lane, or bands appeared in the negative control lane. In these cases, the procedure was repeated in hopes of fixing errors made in the lab procedures. Technicians performing a second attempt produced a higher proportion of usable results. DNA tagging will be performed next on the samples which were shown to contain bacterial DNA.

Our data will be passed on to the subsequent class which will likely perform the task of sequencing the DNA samples, identifying bacterial species within the samples, and drawing conclusions as to how the soil microbiome affects the growth of plants at Urban Tilth.