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Testing for Organophosphate Degrading Bacteria Presence in Soil for Bioremediation.

INTRODUCTION

The widespread use of pesticides in agricultural practices has had a widespread effect on the Earth's ecosystem as well human health. Through the use of microorganisms that degrade these pesticides, soil can be made cleaner again and the risk to human health is greatly reduced. One of the most harmful substances within various pesticides is a class of molecules known as organophosphates, which are responsible for a host of health problems in humans. For this experiment, students traveled to areas surrounding Tarleton State University to take soil samples and test them for bioremedial bacteria.

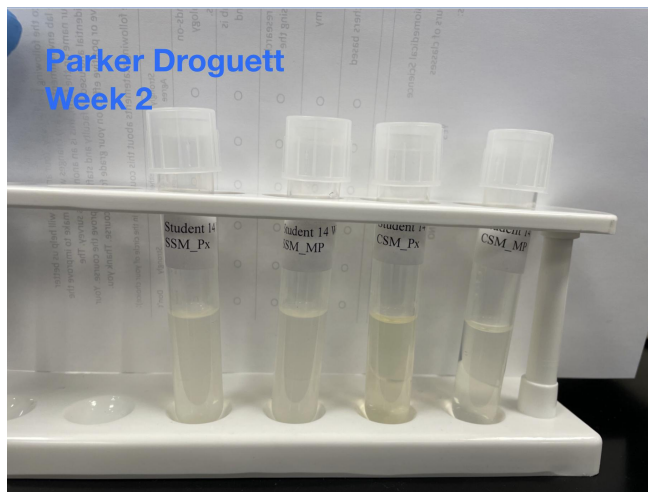
MATERIALS AND METHODS

For the experiment, a sample of soil was taken from Stephenville at GPS coordinates 32.2083601, -98.2079881. This soil would be used to create an LB Broth that would be added to the various mediums later in the experiment. The LB Broth itself provided valuable nutrients for the bacteria to grow. Approximately 1.0g of the collected soil was added to the LB Broth tube. The broth tube was incubated at 30C and 200rpm for 1 week before the experimental process proceeded.

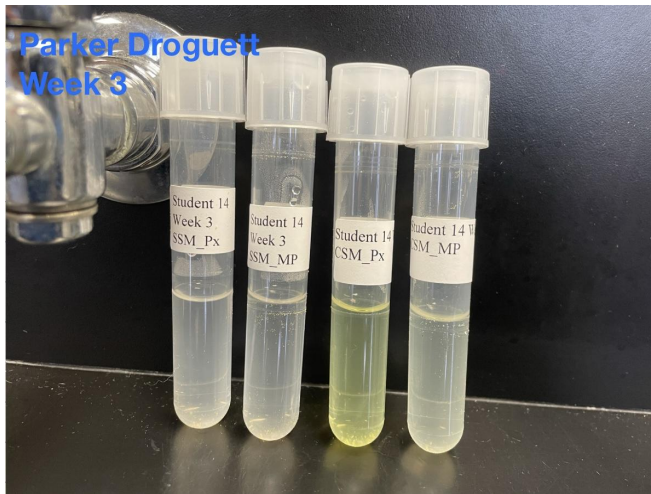
After the week of incubation, the LB Broth was ready for use. 4 test tubes filled with 4ml of media each were prepared for inoculation, those media being the pesticides Paraoxon ethyl (Px) and Methyl parathion. (MP) in a Carbon Selective Medium (CSM) and Sulfur Selective Medium (SSM). These Carbon and Sulfur mediums were meant to allow bacteria to target pesticides for degradation. CSM is void of carbon, while SSM is void of sulfur. This was intended to drive the bacteria to consume the pesticides to fill their metabolic needs and show bioremediation. 1ml of soil broth was added to each tube using a pipette. These test tubes were dubbed CSM_Px, SSM_Px, CSM_MP, and SSM_MP. The tubes were capped and incubated for a day at 37C 200rpm. We intended to incubate for a week, but poor weather prevented us from going to the lab. After that day of accelerated incubation, the test tubes were removed and photographed to be used for comparison.



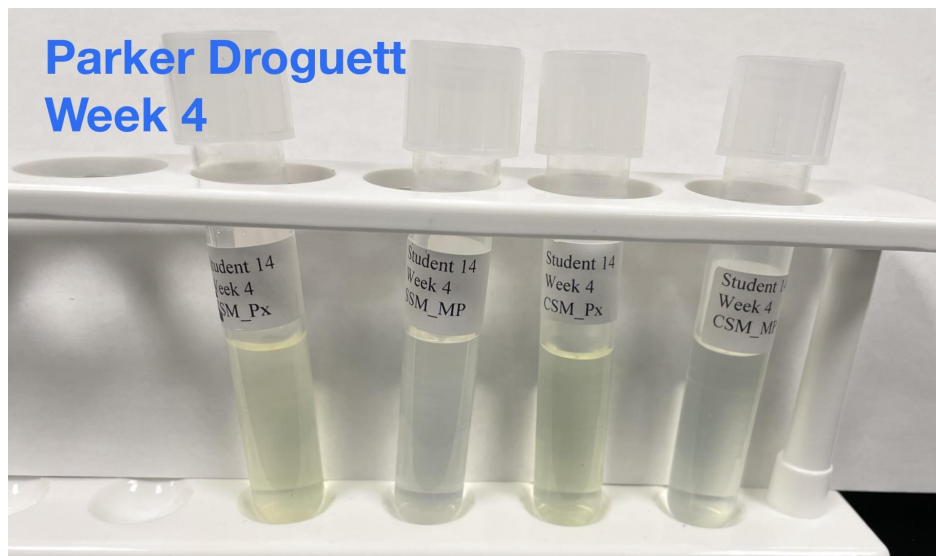
After 1 day of accelerated incubation (1 week), two of the samples had taken on distinct colors. Both the CSM_Px and the CSM_MP mediums had turned a variation of yellow. The CSM_Px sample had turned a bright yellow while the CSM_MP sample had turned a darker shade of yellow. Both sulfur-void mediums, SSM_Px and SSM_MP, were an opaque white color. As the experiment proceeded, week by week, samples were reinoculated into new 5 mL carbon and sulfur mediums. 200 μ L of the previous corresponding week's mediums were transferred into these new tubes and incubated.



After another week of inoculation, two of the samples had lost a considerable amount of color. CSM_Px, which had been a bright yellow, was now a very translucent, slightly yellow liquid. CSM_MP had gone completely clear. Both SSM_Px and SSM_MP appeared to be the same milky opaque color and consistency as before. Samples were transferred as described by the process explained earlier. For the next round of inoculation, samples were instead inoculated for 24 hours at the same 37°C, 200rpm temperature and speed as before.



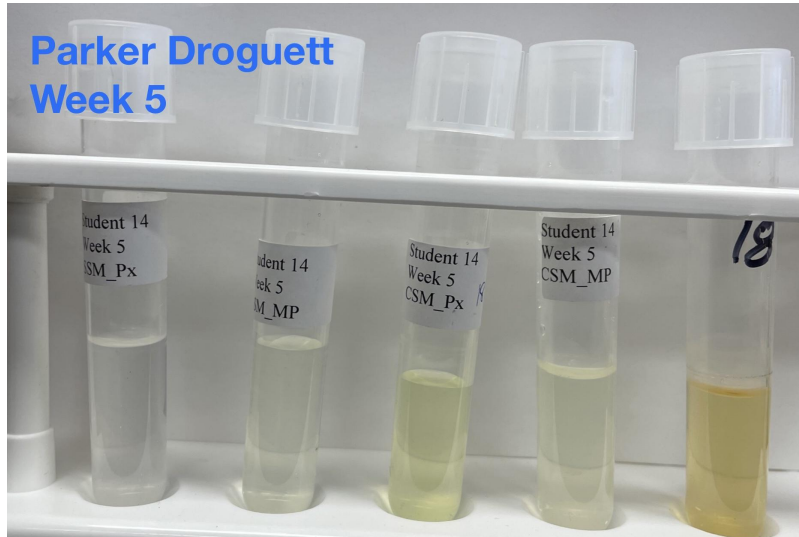
After 3 weeks of inoculation, CSM_Px was the only tube that had a considerable change, having gone from a dimmer yellow from week 2 to a much brighter yellow during week 3. Both sulfur mediums and CSM_MP have taken on the same milky opaque color as before. CSM_Mp did have a slight tinge of yellow. Our 4th-week inoculation samples were passaged for us as we went on spring break.



After 4 weeks of inoculation, CSM_Px had gone back to a muted dim yellow color as opposed to the brighter color it had been last week. The other sulfur Px sample also shares this dim yellow color. Both MP mediums however had gotten considerably clearer. Cultures were inoculated and incubated one final time before the end of the experiment.

After week 5 of inoculation, SSM_Px had turned from the light yellowish color it had in week 4 to a clear color. SSM_MP had actually turned a tinge more yellow, as well as CSM_Px. CSM_MP retained the same color throughout this week. A sample was chosen to be used for

further testing, marked by the test tube with the number 18. This concluded our side of the experiment.



RESULTS

An inoculant was chosen to be plated for further testing, designated by the test tube marked 18 in the week 5 photo. This tube was plated onto a glycerol minimal media with limited nutrients. From here 100 μ L of pesticide assay mix was added to the surface and rested for two hours at room temperature. This plating process was used to test for the presence of bioremediating bacteria that could digest organophosphates. Unfortunately, tube 18 did not show any growth, indicating there was no presence of bioremedial bacteria within the sample.

CONCLUSION

Although test tube 18 did not show any signs of organophosphate degrading bacteria, other selected samples within the experiment as a whole did. These samples help us build a strong knowledge and base of where bio remedial bacteria are found and are a crucial foundation for bioremedial research in the future.