Environmental Sampling Research

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Introduction

This research was funded by the National Science Foundation. It started at the University of Houston, then transferred to Tarleton State University to expand the geographical location of the soil samples being observed. The research is being conducted to find the distribution of organophosphate degrading bacteria found in soil samples collected by the students/researchers. The importance of finding the type of bacteria in soil helps with microbial bioremediation efforts to help eliminate environmental pollution. If we are able to isolate these organophosphate degrading bacteria and control the release of them into polluted areas, then we can begin eliminating the “footprint” that we have left on this planet. This use of biotechnology and bioremediation will help with decontamination of organophosphate compounds in an environmentally friendly manner.

Materials & Methods

Soil Collection

The collection of my soil sample took place at the coordinates 32.93979°N & 96.45585°W. This location was beside my childhood home in Rockwall, Texas. Before the house was built in 1971, this section of land was used for agricultural reasons and cow pastures. The land surrounding my house was bought out by developers and is now a developing neighborhood.
Since 1971 the house has remained in the family and that side of the house has been used as a garden, where store bought fertilizer and other chemicals were used, and as a storage area.

Figure 1. Soil Collection

Inoculation of the Soil Sample

During the first week of research in the lab after collecting a sample, we inoculated 1 gram of the soil sample that we collected with Luria-Bertani (LB) broth. LB broth is commonly used to grow *Escherichia coli* and other bacteria, because it contains a lot of the nutrients required for growth since the bacteria is not in its natural environment.

This media was grown in aerobic conditions, where anaerobic bacteria can not grow. These conditions are used to diminish the number of bacteria within our sample because there are an estimated 6,000-50,000 species of bacteria per gram of soil. Once inoculated the soil samples were left in the lab for 1 week at 30°C in aerobic conditions.

Inoculating Samples with Pesticides

The pesticides used for this step of the research were, Paraoxon ethyl (Px) and Methyl Parathion (MP). Px (Figure 2) is a very potent insecticide that can cause harm to humans and animals by absorption through the skin. MP (Figure 3) is also a harmful insecticide that can be absorbed, inhaled, or ingested and cause muscle spasms or loss of muscle function. These pesticides were diluted for the use of our research to a 1X concentration at 100 µg/mL.

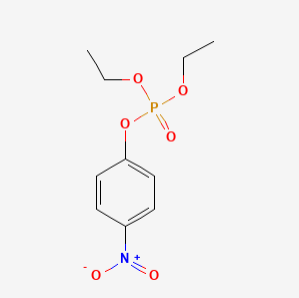

Figure 2. Molecular structure of Paraoxon ethyl (Px)

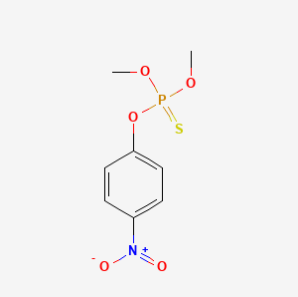

Figure 3. Molecular structure of Methyl Parathion (MP)

These pesticides were then added to 4 mL of carbon selection media (CSM) or sulfur selection media (SSM), creating 4 samples containing the different media and pesticides. We then transferred 1 mL of the inoculated LB soil sample into each of the 4 tubes containing 5 mL of the different media and pesticides, making a 1:5 dilution. These 4 tubes were then incubated at 30°C in aerobic conditions for 1 week. With our sample in this selective media there should be little to no bacteria growth, but if there is organophosphate degrading bacteria present in our sample then it should be able to break down the pesticide for nutrients in order to survive. If the bacteria breaks down the pesticides it will be indicated by a color change of our sample to yellow.

The next 4 weeks of research were conducted by taking four tubes with 5 mL of the selective media with 1X concentration of either pesticide and adding 200 µL of the previous week's culture to the corresponding current week's tube.

Revitalization

As mentioned above if the cultures turned yellow, that indicated the presence of organophosphate-degrading bacteria. Depending on how dark the samples turned yellow, determined if they were selected for further testing. This testing is referred to as the revitalization step. In this step the selected cultures were inoculated back into LB broth and then incubated overnight at 37℃.

Plating

If the selected cultures had growth after being incubated overnight, they were then plated onto glycerol minimal media with pesticide. These plated cultures were grown at 37 ℃ overnight to visualize if there were any bacterial colonies of organophosphate degrading bacteria that were able to survive after 5 weeks of research in the selective media.

Results

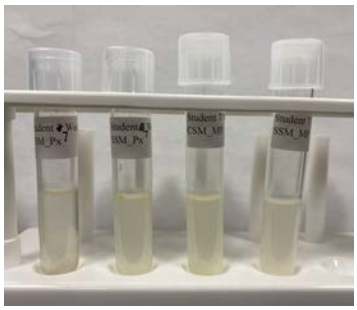

Figure 4. Week 1 Results

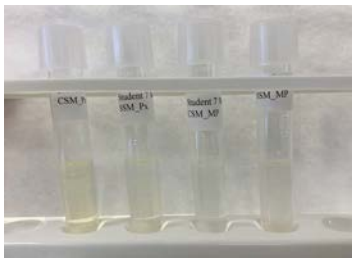

Figure 5. Week 2 Results

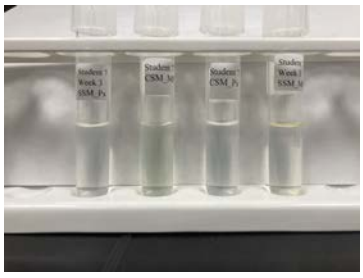

Figure 6. Week 3 Results

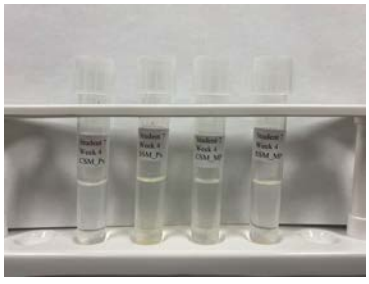

Figure 7. Week 4 Results

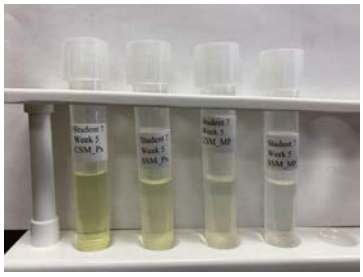

Figure 8. Week 5 Results

Discussion

The samples that I had conducted research on were not selected for further testing, I had little/no organophosphate-degrading bacteria present in my sample. As seen in Figure 4-8, there was almost no color change in my samples to indicate the presence of bacteria able to break down the pesticides MP or Px in order to survive in the selective media. Without the color change indication of my sample, I am able to conclude that the place where I collected my soil sample has not been exposed to the harmful chemicals that causes certain bacteria to adapt and find a new way of finding nutrients by breaking down the pesticides/pollutants that it has available.