The Influence Tobacco Mosaic Virus has on Rhizospheric Microbial Biodiversity

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Introduction

Research Question: How does the presence of TMV affect the microbial diversity of the rhizosphere?

Hypothesis: Microbial diversity in the rhizosphere will decrease as a result of the presence of the antagonistic Tobacco mosaic virus.

GAP IN KNOWLEDGE

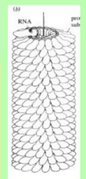
-The analysis of the rhizospheric microbiota of plants that have TMV is currently a gap in knowledge as well as how the virus changes the microbiota over time. With this project. we will gain a better understanding of how the virus spreads and interacts in plants throughout Long Island

THE RHIZOSPHERE

[5]

The dynamically influenced soil/root region holds important microbiota to the tobacco plant [3].

This microbiota is specific to each plant, it holds different eukaryotic species and is highly important to the plants growth, respiration, and nutrient exchange [3].



[1]

TOBACCO MOSAIC VIRUS

TMV is made up of a piece of nucleic acid with a surrounding protein coat, it can enter a plant through mechanical transmission [2]

Inside the plant cell: the protein coat falls away and the nucleic acid causes the plant cell to produce more of the virus protein and nucleic acid [1].

This causes the prevention of chloroplast development, resulting in infected and stunted plants with leaves showing a mosaic pattern [1]

Aims

<u>Aim #1:</u> To complete a rhizospheric microbial metagenomic analysis in the root systems within plants with and without Tobacco Mosaic Virus (TMV).

<u>Aim # 2</u>: Compare how microbes change over time in the rhizosphere of the plants with and without TMV.

Methodology

Results

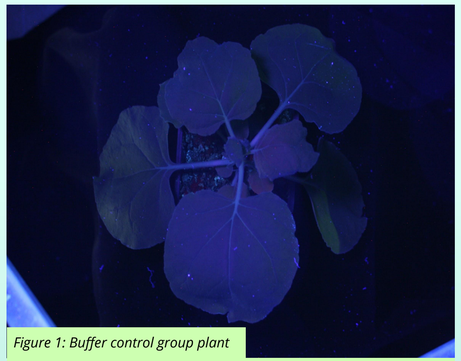


Figure 1: Buffer control group plant

Three tobacco plants were chemically inoculated with an abrasive buffer. As seen in the figure this mock-inoculation shows no presence of viral colonies. The rhizosphere samples were taken 2 weeks after, this coming from the soil and roots of the three plants.

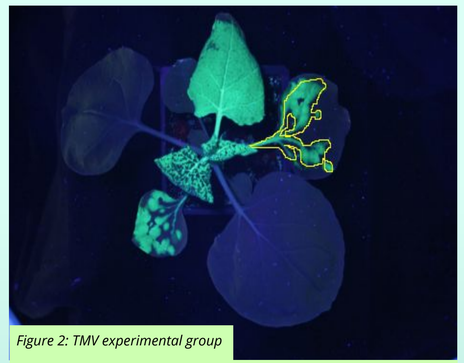


Figure 2: TMV experimental group

Three tobacco plants were inoculated with the tobacco mosaic virus through an abrasive powder. As seen in the figure the fluorescence shows presence of viral colonies causing a mosaic pattern. The rhizosphere samples of the experimental group plants were taken 2 weeks later, coming from the soil and roots.

Key
T: TMV experimental group
C: Control group
L: Leaf (1&2)
R: Root
S: Soil
ST: Stem

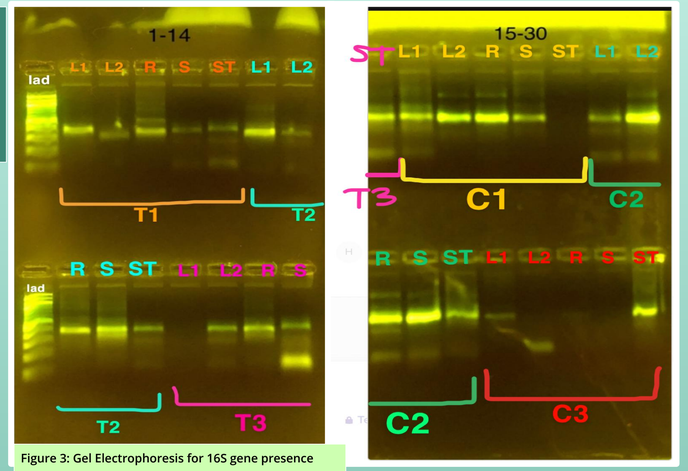


Figure 3: Gel Electrophoresis for 16S gene presence

Gel electrophoresis was utilized to test the presence of the 16S gene in the leaf, root, soil, and stem, 23/30 samples were successful in amplification. The first set, being from the tmv inoculated plants amplified more consistently while the second set, from buffer inoculated plants amplified much weaker, especially in set C3. In each experimental set, the presence of 16S gene is more prominent in the the rhizosphere (root, soil, stem) than the leaves, which can be exemplified in T3S and T3ST showing longer stands than T3L1/L2.

Tentative Discussion

In figures 1 and 2 the comparison of buffer and inoculated tobacco plants allowed us to see the increase of viral colonies after the introduction of the tobacco mosaic virus (TMV).

16S ribosomal RNA can be used to detect the variation and relative abundance of different bacterial DNA [6]. When used in the rhizosphere with the samples R, S, and ST shown in figure 3, the gels amplified and showed the 16S gene present with darker bands. It is believed that the rhizosphere has higher levels of bacterial DNA present than the leaf samples in the samples that have amplified shown with the darker bands but this cannot be confirmed until sequenced and analyzed.

The rhizosphere is a densely populated area in which the roots must compete with the invading root systems of neighboring plant species for space, water, and mineral nutrients; allowing the rhizosphere to regulate plant and microbial communities [3]. Due to the darker bands, it is believed that in the rhizosphere of the three tmv inoculated plants the abundance of bacterial DNA will show an increase of relative abundance and variation of microbes. This is because the microbiota of the rhizosphere holds importance to the plant that can affect the overall health of the plant, with the introduction of TMV into the plant the rhizosphere caused the rhizosphere to release a constant and diverse secretion of antimicrobial root exudates in the form of phytoalexins, defense proteins [3]. This potentially expresses the plants fitness in response to the TMV and added compounds that are shown in the expression of the 16s gene.

Limitations & Future Implications

- ★ School closing causing time constraints
- ★ Waiting to continue to sequence the samples
- ★ Combining the two projects to see change over time in the phyllosphere as well, including the stems and leaves
- ★ Increasing the sample size
- ★ Explore the mechanisms of the antimicrobial root exudates of the rhizosphere
- ★ What specific antimicrobial exudates are expressed based on different introductions of viruses or environments

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