Developing Microbial Communities to Suppress Bacterial Diseases of Tomato Report

Personnel:
Kevin Hockett (Principle Investigator)
Hanareia Ehau-Taumaunu (Graduate Student)

Project Summary:
This project was initiated in the Summer 2019 with two primary objectives: 1) assess the effect of natural tomato microbial communities to suppress foliar diseases and 2) transfer microbial communities recovered from objective 1 to select those communities that provide the greatest disease suppression.

We are happy to report that by the conclusion of the usable season (before temperature and light conditions were no longer conducive for tomato growth in the Penn State greenhouses), we acquired encouraging results that it is indeed possible to develop a disease suppressive community following serial passage. We achieved ~60% reduction in disease severity and 10% reduction in disease incidence by the final passage compared to the peak severity and incidence when assessing natural infection alone, and a 20-30% reduction in disease severity and 10% reduction in disease incidence by the final passage compared to peak severity and incidence when assessing natural infection + supplemented pathogen.

As this project and its approaches are novel (we are not aware of any published reports using a similar strategy to develop a foliar disease suppressive community), it was expected that there would need to be an amount of trouble shooting to establish protocols and analyses appropriate for our objectives. We believe, however, that our results are promising and establish a strong foundation for continuation of this research program in the summer of 2020.

Results:
Objective 1 | *Assess the effect of natural tomato microbial communities to suppress foliar diseases.*

Although we aimed to acquire several natural communities from commercial production settings, we were unable to do so. Thus, we were unable to directly address this objective in Summer 2019. We were able, however, to acquire two natural communities (though not from commercial production settings) that we utilized in objective 2.

Objective 2 | *Transfer microbial communities recovered from objective 1 to select those communities that provide the greatest disease suppression.*

Healthy plant material was collected from two sites in State College, PA. Two foliar samples were used to initiate this objective, one from a from a community (CG) and the other from a home garden (HG), which were collected in July and August, respectively. Plants were sprayed with either community, followed by inoculation of the pathogen *P. syringae* pv. *tomato* (Figure 1). At the end of each passage the microbial communities from three plants exhibiting the

lowest disease severity were combined and transferred to a new set of ten healthy plants. The number of infected plants and the percentage of symptom coverage was recorded to measure disease incidence and severity, respectively. Because there was no way of separating selectively removing the speck pathogen from the community once it was introduced, the pathogen + buffer control (where new pathogen was introduced at every passage) cannot be directly compared to the HG and CG treatments. However, the pathogen + buffer control does act as a representative of the environmental conditions at the time, allowing us to control for environmental effects on disease severity and incidence.

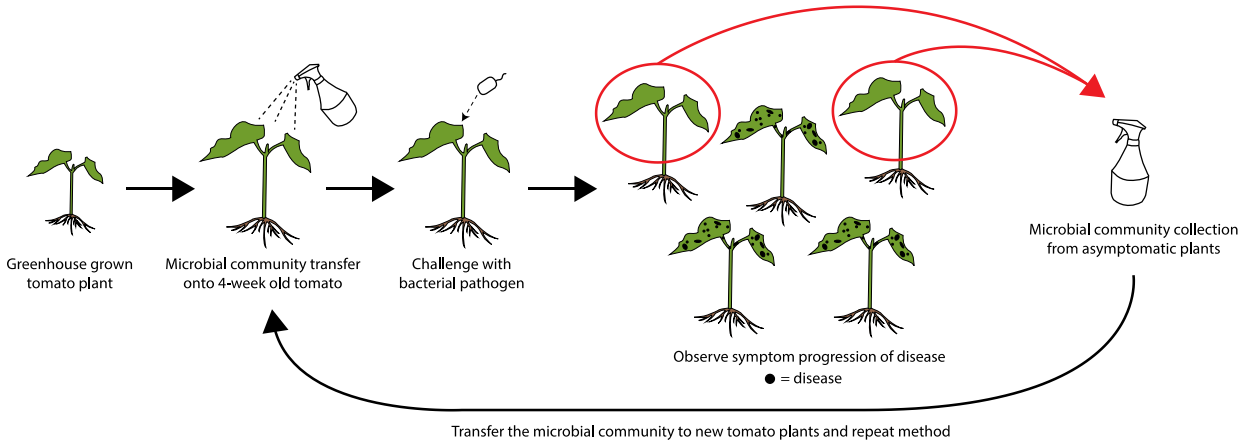

Figure 1. *Diagram depicting the transfer of foliar microbial communities. Tomato leaves from either a home garden or community garden were used as the source for the starting microbial communities. These communities were sprayed onto 4-week tomato plants grown within a greenhouse setting. Two days after community application, plants were challenged with the bacterial speck pathogen. After an additional 5-7 days, which was sufficient for disease development, the top 3 plants that exhibited the lowest disease severity were used as community sources to initiate the next passage.*

The disease incidence (table 1) and severity (table 2) for the community transferred treatments (HG and CG) were much greater than either measure for the no-community control (pathogen + buffer). This is not unexpected for two reasons. First, the amount of pathogen added in the no-community control was consistent from passage to passage, whereas we expect that the amount of pathogen from HG and CG would have likely increased from passage to passage, as a result of pathogen build up from disease. Second, we think that potentially the pathogen coming from a leaf environment is more virulent than one coming from a laboratory culture environment. All communities reached 100% disease incidence by passage 5 and maintained that level of incidence until passage 8. For most communities, only 2-3 passages were required for all replicate plants to have some level of disease symptoms present. For either HG or CG + buffer, the pathogen was introduced in the first passage, but was not reintroduced in subsequent passages. This is different that HG or CG + pathogen, where the pathogen was introduced in the first passage, and was then reintroduced at the same level as the pathogen + buffer control.

Table 1. Disease incidence (number of plants infected out of 10 plants inoculated) for each community over multiple passages. Values are absent for Passage 1 and 2.

Passage	HG_Spray + Pathogen	HG_Spray + Buffer	CG_Spray + Pathogen	CG_Spray + Buffer	Pathogen + Buffer (Control)	No pathogen (Control)
P2	No data	No data	10	0	No data	0
P3	7	0	9	10	6	0
P4	10	4	10	10	4	0
P5	10	10	10	10	1	0
P6	10	10	10	10	3	0
P7	10	10	10	10	5	0
P8	9	9	9	9	7	0

Disease severity was recorded on a continuous scale (table 2). For HG, disease severity steadily increased from passage 3 to 7. In contrast, CG disease severity reached high percentages by passages 3 or 4. The pathogen + buffer control remained under 10% disease severity for all passages.

Notably, there was a significant decrease in disease severity from passage 7 to 8 for all treatments except the control. At passage 8, the disease severity of the HG and CG spray communities with pathogen is reduced by 20-30%.

Table 2. Disease severity for each passage represented as percentages. Communities with more healthy plants will be more suppressive of disease. Values are absent for Passage 1 and 2.

Passage	HG_Spray + Pathogen	HG_Spray + Buffer	CG_Spray + Pathogen	CG_Spray + Buffer	Pathogen + Buffer (Control)	None (Control)
P2	No data	No data	15	0	No data	0
P3	5.6	0	30	15	7.2	0
P4	10	1.6	67.5	58	3.2	0
P5	27.6	22	51	47	1	0
P6	30	41.5	75.5	68.5	1.2	0
P7	42.2	65	60	57.5	7.5	0
P8	22.5	9	30	9	5.6	0

Conclusions

Our results show that following serial passage of a pathogen within a community we were able to reduce disease severity significantly by the final passage. Additionally, we found similar,

though more subtle, results with regard to disease incidence. We believe our results match with what is known regarding certain suppressive soils, where disease pressure builds over successive seasons of growing the same crop, followed by a sharp decline in disease.

To confirm our results, we will initiate another round of community passage in Summer 2020 and hope to expand our efforts to include the bacterial spot pathogen, *Xanthomonas perforans*.

Results from this project were used to secure additional funding from the Northeast Sustainable Agriculture Research and Extension (NE SARE) to continue this work. We also anticipate seeking additional, larger federal funding, which will rely on these preliminary findings.

We believe this proof-of-concept research supports the idea that community passage is a viable route to develop controls for bacterial diseases of tomato.