Phylogenetic Relationships of Foliar Bacteria Within Cover Crops in an Organic Cropping System

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Abstract

Endophytes (asymptomatic microbes living inside of plant tissues) have been noted to help reduce the disease symptoms of their plant host. Understanding the relationship between endophytes and pathogens could be used to aid in pathogen control strategies in agricultural systems. **Our objective is to identify which bacterial taxa inhabit the leaves of cover crops in an organic agricultural setting.** At a working farm in Marin County, California—Star Route Farms—we sampled cover crop leaves in winter 2019, before the cash crop growing season. Leaves were surface sterilized with dilute bleach and ethanol and then leaf fragments were placed into slant tubes with malt extract agar to isolate emergent foliar bacteria. Microbial slant tube growths were quantified, and bacterial isolates were sequenced using 16S Sanger sequencing and identified via BLAST queries against the NCBI GenBank database. **The results could provide information relevant to the implementation of a new technique for improving agricultural sustainability and could also contribute towards a better ecological understanding of these complex and economically critical agricultural systems.**

Introduction

Sustainable agriculture must be implemented to ensure that repeated crop cycles will not quickly deplete soil nutrients. With the use of a more sustainable methods, producers can look towards growing crops with little to no exposure to inorganic chemicals. One technique is cover cropping, where cover crops are not only used to restore soil nutrients for the next crop cycle, but can also potentially lower the microbial burden on the cash crops. Some examples include the use of fava beans and purple vetch to fix nitrogen into the soil, and the use of daikon radish to aerate the soil. **We focus here on endophytes, which are microbes that live within plant tissues asymptomatically.** We isolated these endophytes from fava beans, *Vicia faba* (Figure 1)*;* purple vetch, *Vicia americana*; and daikon radish, *Raphanus sativus var. Longipinnatus* (Figure 2).

Figure 1:
Fava beans (*Vicia faba***),** one of Star Route Farm's cover crops used to add nitrogen to the soil

Figure 2:
Field of **daikon radish** (*Raphanus sativus var. longipinnatus*), a second cover crop used to improve soil texture

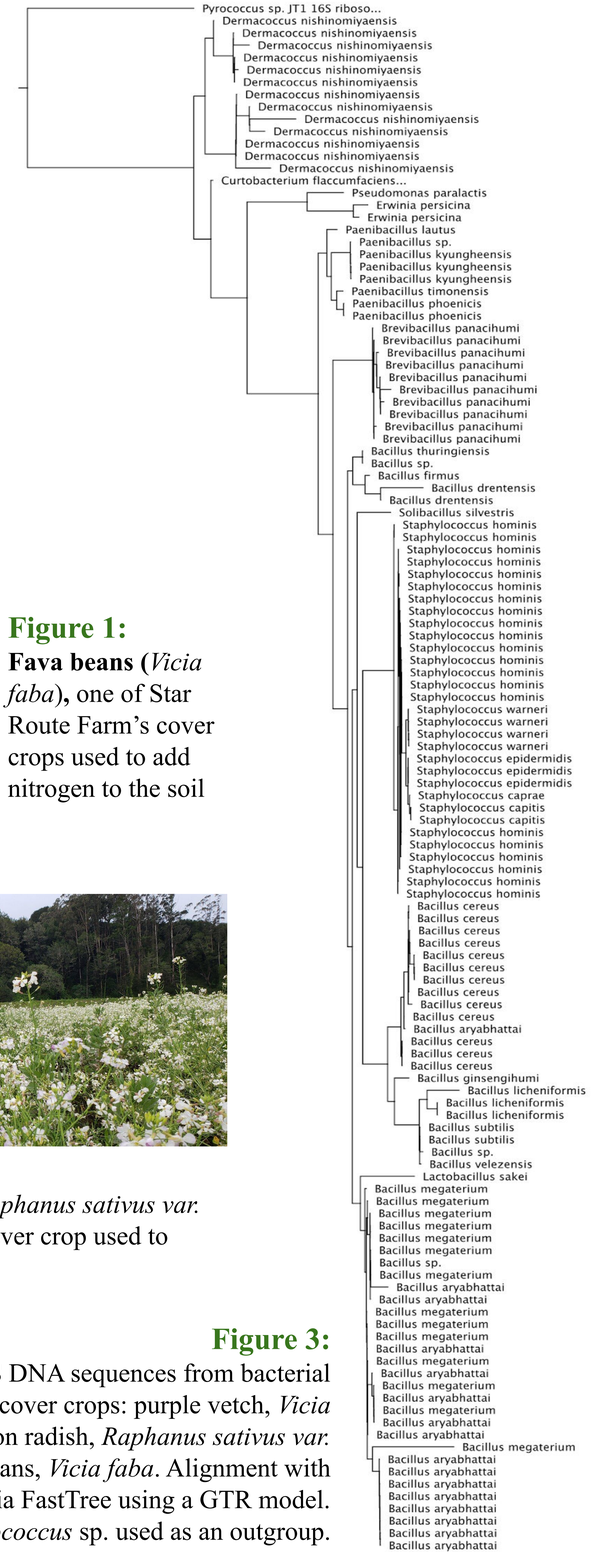

Figure 3:
Inferred phylogeny of 16S DNA sequences from bacterial isolates of the following cover crops: purple vetch, *Vicia americana*; daikon radish, *Raphanus sativus var. longipinnatus*; and fava beans, *Vicia faba*. Alignment with Clustal Omega, tree via FastTree using a GTR model. Archeon *Pyrococcus* sp. used as an outgroup.

Methods

1. **Field sampling** was done at Star Route Farms in Marin County, CA
2. **Leaf samples were surface sterilized** with ethanol and dilute bleach
3. **Cultures were isolated** from leaf fragments using Malt Extract Agar (MEA) and isolation frequencies were quantified
4. **DNA was extracted** using Sigma REDExtract-n-Amp reagents
5. **Barcode loci were characterized** via 16S PCR followed by Sanger sequencing
6. **To identify the bacterial isolates,** sequences were used to query the NCBI GenBank database using BLAST through the Geneious Prime program
7. **Cleaned, trimmed sequences were aligned** with Clustal Omega
8. FastTree was used to infer a phylogeny from the sequences, with a 16S sequence from the archeon *Pyrococcus* sp. as the outgroup

Results and Discussion

At this moment, our results are preliminary, but the project is also still ongoing. Thus far, we have successfully sequenced **126 bacterial isolates from leaves of all three cover crop species.** We found that the most commonly identified bacteria found in leaves of the cover crop species used at Star Route Farms were ***Staphylococcus hominis, Brevibacillus panacihumi, Bacillus cereus, Dermacoccus nishinomiyaensis, Bacillus aryabhattai,*** **and** ***Bacillus megaterium*** **(Figure 3)**. A literature review of these taxa showed that *Staphylococcus hominis*[1] are spherical in shape and commonly harmless to the skin of humans and animals. For *Bacillus cereus*[2], we found that these bacteria form spores and are rod-like in shape. It is commonly found within food and soil. **Another common bacteria we identified was *Bacillus aryabhattai*[3] which is a bacteria with strains that are found to promote plant growth**. In the future, we will use PHYML to infer a more robust phylogeny. We will analyze the differences in microbial communities associated with each of the different cover crop species (our current results are aggregated across all three crops).

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