FINAL REPORT ON

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PROJECT TITLE: HARNESSING THE STRAWBERRY MICROBIOME IS A NEW PARADIGM FOR SUSTAINABLE AGRICULTURE

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SUMMARY

Black root rot (BRR) is a complex plant disease (Coons 1924), caused by pathogenic fungi, mainly *Rhizoctonia fragariae* and *Pythium irregulare* sensu stricto, and is prevalent in North Carolina and neighboring regions (Abad et al., 1999; LaMondia, 2003). The disease can cause up to 40% yield losses, significantly threatening strawberry production (Maas 1998). In the past, soil fumigant, methyl bromide (MeBr) was used to disinfect the soil before planting, but it was phased out in 2012 due to environmental concerns. Unfortunately, no disease-resistant varieties are available to manage BRR, making it challenging for growers (Particka and Hancock, 2008). To address this issue, a study was conducted to identify beneficial bacterial strains from strawberry roots and soil that can help protect the plants from BRR and promote growth. The long-term goal is to develop an eco-friendly microbiome-based strategy that can reduce the need for synthetic fertilizers and pesticides. Through multiple years of anaerobic soil disinfestation (ASD) using organic amendments experiments (Shennan et al., 2016) and 16S amplicon sequencing data, novel bacterial strains with potential biological activities were identified. These biocontrol agents will be characterized to detect antibiotic genes, nutrition efficiency genes, and growth-promoting compounds that can enhance plant growth and protect strawberry plants from BRR. The study will provide new insights into potential solutions for managing BRR and promoting sustainable regenerative agriculture. Ultimately, these outcomes will benefit stakeholders in North Carolina and the surrounding regions.

RESEARCH OBJECTIVES

Our main objective in conducting this follow-up study was to molecularly analyze bacterial strains and evaluate their effectiveness in promoting plant growth and suppressing BRR.

JUSTIFICATION AND DESCRIPTION

MeBr was used as a soil fumigant during strawberry production before planting. However, it was discontinued due to health and environmental concerns. To improve soil health and reduce soil-borne pathogens, non-chemical cultural practices like soil amendments and cover crops have been implemented (Bernard et al., 2012; Litterick et al., 2004). Another practical approach towards sustainable agriculture is using microbiomes to enhance plant health. Recent studies suggest that plant-associated microbiomes can enhance the plant host's immune system (Vannier et al., 2019). It has been suggested that plants release compounds in the rhizosphere to selectively promote beneficial microbiomes for plant growth, health, and disease suppression (Cohen and Mazzola, 2006; Reinhold-Hurek et al., 2015; Weller et al., 2002). The rhizosphere is where plant roots meet the soil, and it plays a vital role in balancing plant physiology, nutrient availability, and plant defense against pathogen attack (Philippot et al., 2013). However, several significant gaps in our knowledge must be addressed before this biological-dependent IPM tool can be applied to strawberry production. Nonetheless, this project will pave the way for using microbiomes in sustainable crop productivity, nutrient management, and disease control in North Carolina.

MATERIALS AND METHODS

Our report summarizes the work we conducted during the 2022 season, funded by SRSFC and USDA-NIFA. Our study involved the use of ASD with various organic amendments, including molasses applied at a total rate of 5000 lbs/A, Mustard Meal applied at 2000 lbs/A (total rate), and a combination of both at half rate (2500 lbs/A, and 1000 lbs/A, respectively). A soil fumigant, Pic-Clor60 (150 lbs/A), was also injected into the bed. We had untreated controls that lacked organic amendments or fumigants. The experiment utilized a randomized complete block design with four replications. Each plot consisted of three beds 30′ long, planted with strawberries in twin rows on 12″ x 12″ spacing and offset in the twin rows. We planted the strawberry plants in mid-October, managed them over the winter, and harvested them from mid-April to mid-June based on 8 weekly harvests. At peak harvest, we collected whole plant samples to assess the plant dry weights of the crowns and leaves. Soil samples were collected before planting and at peak harvesting. We collected total yield data and analyzed it using a two-way repeated-measures analysis. In addition, we performed 16S amplicon sequencing after extracting

DNA from strawberry roots and soil. We also conducted a microbiome analysis, isolated and characterized bacterial strains in semi-selective and selective media, and detected antibiotic genes using polymerase chain reaction (PCR) techniques.

RESULTS

We used the ternary plot to identify the bacterial community at the species level to compare the relative abundance and distribution patterns between three ASD treatments and controls. The ternary plots show core bacterial species (as indicated by a high density of circles) in 2022, and the operational taxonomic units (OTUs) associated with soil and root treated with ASD using different organic amendments (Figures 1A to F). Additionally, a few OTUs associated with bacterial species were found to overlap between soil and roots. Among highly enriched bacterial species associated with soil and roots were *Bacillus azotofomans*, *Komagataeibacter saccharivora*, *Bradyrhizobium elkanii*, *Komagateibacter ccharivora*, *Arthrobacter* spp., and *Paraburkholderia nodosa*.

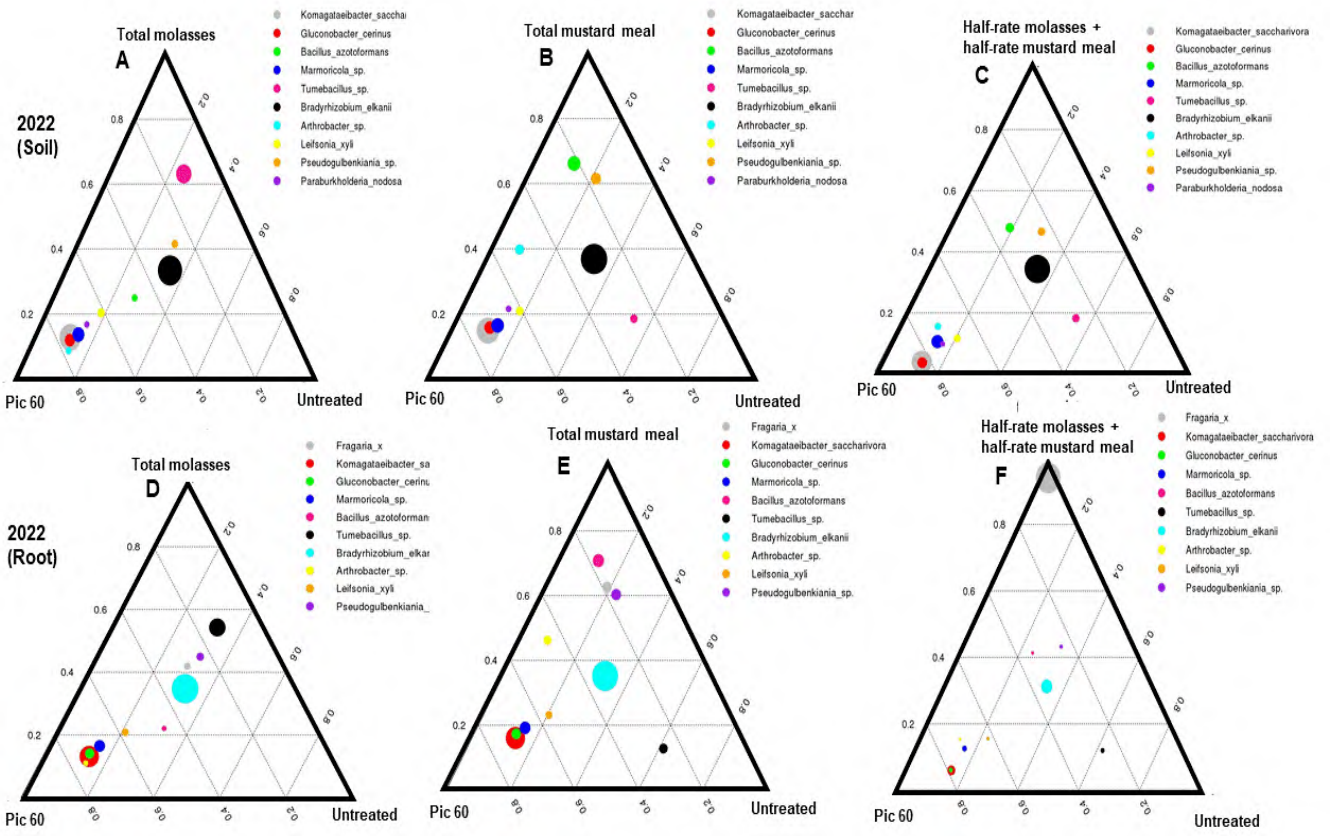

FIGURE 1. The ternary plot displays the top 10 individual species, represented by circles, and their average abundance when subjected to different organic amendments during anaerobic soil disinfestation (ASD) for both soil and roots. The treatment groups included PiClor-60 as a positive control, no fumigation or amendment as an untreated control, total rates of molasses, total rates of mustard meal, and half rates of both molasses and mustard meal. The size of the circles represents the abundance of the bacterial species.

Isolation and characterization of bacterial strains. We used 300 mg of each soil and root sample and then serially diluted them. After the dilution, we took 100 µl of the sample (10^{-4} dilution) and streaked it on three types of media: Tryptic Soy Agar (TSA), King's B medium, and Burkholderia selective media. We incubated the plates at 28°C for 24 to 48 hours. We identified and preserved approximately 150 pure bacterial colonies, which we putatively deemed as single colonies, in 30% glycerol at -80°C.

Dual culture assay. A subset (n = 35) of bacterial strains was tested for their ability to inhibit the growth of the soil-borne pathogen *Rhizoctonia fragariae* using the dual-culture method. The fungal pathogen was grown on potato dextrose agar (PDA), while the bacterial strains were cultured on TSA medium. The bacterial cultures were overlaid on PDA plates and incubated at 28°C for 7 days. Negative controls were PDA plates without bacterial cultures. The percent growth inhibition of *Rhizoctonia fragariae* due to bacterial strains over control was calculated, and three bacterial strains were found to be effective, inhibiting the growth of *Rhizoctonia fragariae* by 50 to 80% (*data not shown*).

PCR-based assays. A subset (n = 35) of bacterial strains were screened for producing nine antibiotics using PCR assays (Zhang et al., 2015). These assays aimed to detect the presence of antibiotic genes, such as 2,4-Diacetyylphloroglucinol (*PhID* gene), Phenazine (*phzFA*), Pyrrolnitrin (*prnD*), Pyoluteorin (*pltC*), Fengycin (*fenD*), Bacillomycin (*bmyB*), Bacilysin (*bacA*), Iturin A (*ituD*), and Surfactin (*srfAA*). The PCR conditions and programs used were the same throughout the study (Zhang et al., 2015).

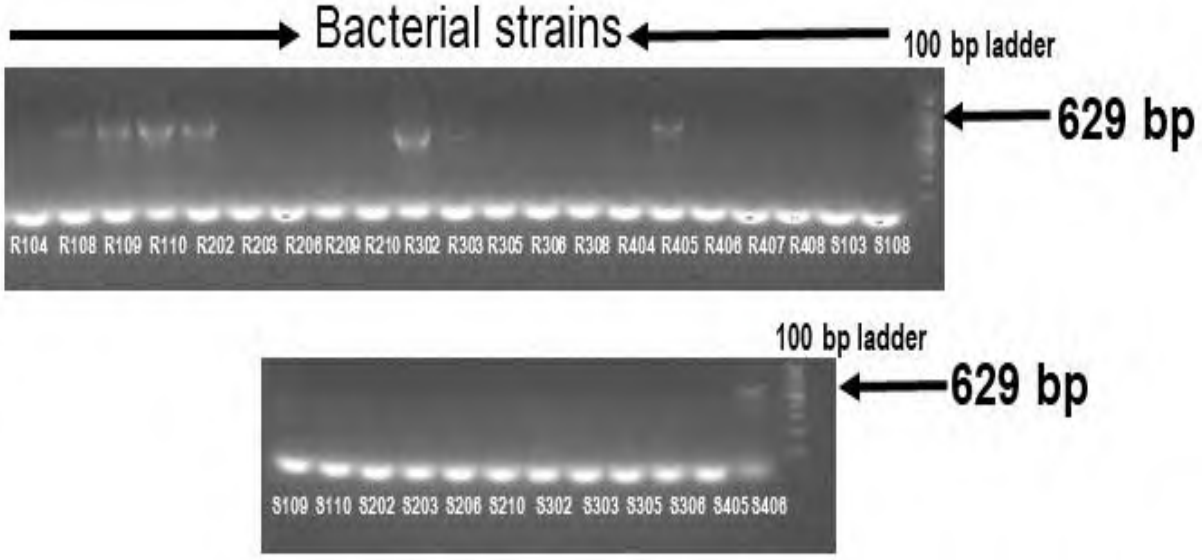

FIGURE 2. PCR detection of the gene for synthesizing 2,4-Diacetyylphloroglucinol (629 bp). The 35 bacterial strains (strains R101 and R103 are not included) were collected from roots (R) and soil (S) from strawberry treated with ASD with different organic amendments. A 100 bp ladder was used as a size marker.

PCR analysis confirmed that six strains isolated from strawberry roots contained the 2,4-Diacetyylphloroglucinol biosynthetic gene (Figure 2). In contrast, only one bacterial strain from the soil (S406) contained the *PhID* gene. The bacterial strains differed in the presence of other genes (*data not shown*).

We currently use several methods and tests to identify two to three of the most promising strains for plant growth promotion and biocontrol. These methods include 16S rDNA and gyrA gene analysis (Kim et al., 2010). We also characterize elite bacterial strains to determine their biological functions or traits. These traits include siderophore production, phosphate solubilization, indole-acetic acid (IAA) production, nitrogen fixation ability, chitinase production, and HCN production (Zhang et al., 2015).

IMPACTS

Strawberry growers face a critical issue - BRR, which results from a combination of pathogens, including *Pythium irregulare* and *Rhizoctonia fragariae*. Our study demonstrated that ASD treatments have significantly increased the yield (comparable to fumigant treatment) and fostered superior plant growth. A detailed investigation of these biocontrol agents is necessary under in vitro, greenhouse, and field conditions for plant growth and biocontrol potential to utilize this promising solution. The introduction of these biocontrol agents as bio-fertilizers and biocontrol solutions can have a positive impact on strawberry production.

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