CHARACTERIZE ROOT MICROBIAL COMMUNITIES WITH ANTI-FUNGAL ACTIVITIES IN SOYBEAN, 53-2021

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RATIONALE/JUSTIFICATION FOR RESEARCH

Plant roots associate with a microbial community that is distinct from the microbes present in surrounding soil. The microbes colonize the rhizosphere (immediately surrounding the plant's roots) and the superficial root tissues (endophytic compartment). The root microbiota comprises a wide diversity of microorganisms, and it can benefit plant health or have detrimental effects; shifting this balance towards beneficial plant-microbiota interactions is of high agronomic interest. Microbes may provide the plant with nutrients that accelerate plant growth and development, suppress soil pathogens, or help plants withstand environmental stress. The advantages of exploiting microbiota are evident and include consumers' and farmers' safety and sustainable practices that preserve the environment and protect biodiversity.

Taproot decline (TRD), caused by *Xylaria* sp., is recognized as a critical soil-borne soybean disease. Previous MSPB studies by Dr. Maria Tomaso-Peterson demonstrated TRD is widespread throughout MS soybean production, and affected soybeans were identified in 73 counties. Through the support of MSPB, we are developing a better understanding of TRD and the causal agent *Xylaria* sp. This pathogen overwinters as stroma on crop residue across successive growing seasons and colonizes the roots of corn, cotton, rice, sorghum, and wheat in greenhouse studies. We demonstrated variable degrees of virulence among *Xylaria* sp. isolates, which may be due to microbes' influence within the soybean plants' rhizosphere. Field observations show dead soybean seedlings resulting from TRD at the V4–V5 growth stage; however, most symptomatic soybeans observed in production fields are in growth stages greater than R3.

Soil microbiome investigations have been strongly promoted over the past ten years. Microbiome studies have provided insights into the composition of disease-suppressing soils. For example, soil suppressing wilt disease caused by Ralstonia solanacearum contains a higher abundance of *Proteobacteria* and *Acidobacteria* than soils with disease symptoms. Species with anti-fungal activity include non-pathogenic strains of *Fusarium oxysporum* and bacteria in the genera *Pseudomonas* and *Bacillus*. Thus, the evaluation of root microbiomes for crop growth promotion and disease resistance presents unique opportunities. We aim to understand the diversity of rhizobiome microorganisms that cause specific phenotypes in soybeans, test rhizobiome isolates for beneficial activities, and utilize this knowledge to establish methods for improved growth and biological control of soil pathogens.

Primary goals of the project:

1: Identify rhizobiome microorganisms with inhibitory activity on *Xylaria* growth

2: Characterize the rhizobiome communities of the healthy and diseased soybean to quantify the impact of *Xylaria* infection on the composition and structure of soybean rhizobiome.

3: Evaluate the biocontrol activity of rhizobiome microorganisms against soybean taproot decline in the field.

Activities and significant results:

1. **Deconvolution and phylogenetic characterization of isolates from our soybean root endophyte library**: 150 isolates were selected for this analysis. The purified DNA from each isolate was extracted, quantified, and verified for purity before sending it for sequence analysis and taxonomic identification (performed through CosmosID). For the 150 isolates selected, we prioritized those isolates with *in vitro* and/or *in vivo* anti-fungal activity.
2. **We continued the testing of the bacterial endophytes from our library using the *in vitro* methods we developed during the first years of the project (**see flowchart **in Figure 1).** For the *in vitro* studies we applied the co-cultivation method to identify endophytes with significant inhibitory activity on the growth of *Xylaria* in laboratory cultures and analyzed the data using statistical methods. In total, we tested 92 library isolates; 39 isolates were found to have moderate anti-Xylaria activity and 14 found to have very strong anti-*Xylaria* activity when compared to controls. (**Figure 2**)
3. **Testing of bacterial endophytes for anti-Xylaria/TRD activity using soybean plants grown in growth chambers in sterile Jiffy pellets.** We tested 17 isolates for their potential disease-suppressive activity in soybean. Isolates with complete taproot decline suppressive activity are: Is-40, Is-71, Is-62, Is-10, Is-113, Is-7, Is-89, Is-9, Is-78, Is-65, Is-84, Is-148. Other four (Is-15, Is-72, Is21 and Is-4) showed partial, but still statistically significant, protection against TRD compared to controls. (**Figure 3**)
4. **Testing of bacterial endophytes for anti-Xylaria/TRD activity using soybean plants grown in soil collected from agricultural fields.** We performed experiments to test weather our selected microbial isolates maintain their beneficial activity against Xylaria infection when plants are grown on MS natural soil. Pretreatments of plant with bacterial isolates Is-9, Is-10, Is-20, Is-62, Is-66, and Is-71 rendered the plants resistant to *X. necrophora* infection, as evidenced by the similar dry mass weights of pathogen-inoculated and -uninoculated plants. (**Figure 4**)
5. **Generating and testing synthetic bacterial communities (syncoms).** These experiments aimed to identify combinations of bacterial isolates with more robust inhibitory activity against *Xylaria necrophora*. We found that several syncoms showed a significantly lower inhibition activity than the single isolates, suggesting antagonistic interactions among the bacterial strains. Most syncoms showed similar levels of inhibition compared to the single isolates.
6. **Comparative metagenomic analyses of healthy and TRD symptomatic soybean (Figure 5).** In addition, we developed a statistical framework for analyzing the effect of *X. necrophora* and bacterial isolates on plant phenotypes in laboratory experiments. We developed models to discriminate antifungal effects from plant growth effects and to build predictive models of

antifungal and growth effects from in vitro measurements. Some of the results obtained from the comparative analyses have been published in the peer-reviewed journal *Microorganisms*.

Key research outcomes:

1. In planta testing of beneficial isolates using *sterile soil (Jiffy peats)*: Isolates with complete taproot decline suppressive activity are: Is-40, Is-71, Is-62, Is-10, Is-113, Is-7, Is-89, Is-9, Is-78, Is-65, Is-84, Is-148. Other four (Is-15, Is-72, Is21 and Is-4) showed partial, but still statistically significant protection against TRD compared to controls.
2. In planta testing of beneficial isolates using *soil collected from agricultural fields*: Pretreatments with bacterial isolates Is-9, Is-10, Is-20, Is-62, Is-66, and Is-71 rendered the plants resistant to *X. necrophora* infection
3. Several syncoms showed a significantly lower inhibition activity than the single isolates, suggesting antagonistic interactions among the bacterial strains. Most syncoms showed similar levels of inhibition compared to the single isolates. Notably, the four-member syncom composed of Is-9,20, 62, 72 showed a slightly higher inhibition (54%) than the strongest single-isolate performer Is-72 (49%). This result suggests a potential for synergism in the Syn 9/20/62/72.
4. Completed comparative bioinformatic analyses to characterize the rhizosphere and endophytic bacterial and fungal communities in Mississippi soybean healthy plants and plants infected by *Xylaria necrophora*

Opportunities for training and professional development provided by the project:

1. Training of undergraduate students in microbiology, molecular, computational biology and plant biology techniques:

   Tyrikus Hayes - helped with soybean growth, plant maintenance and tissue processing at the end of each experiment. Tyrikus was recently accepted into medical school (MS) and will start in August 2022

   Joshua Mitchell- helped with organizing work on the project, optimizing assays for soybean growth, plant maintenance and tissue processing at the end of each experiment. Josh has been accepted and will start Graduate School at MSU (Dept. of Biochemistry) under the supervision of Dr. S. Popescu

   Slade Smith- helped with soybean plant maintenance and tissue processing at the end of each experiment

   Sean McGrath- helped with soybean growth, plant maintenance and tissue processing at the end of each experiment

   Pradeep B K - implemented data analysis methods, helped with microbial community analysis.

2. Training of graduate students in metagenomics and lab techniques for biocontrol:

   Jasmine Uyen Weser, Master student; Jasmine has successfully presented her results in the first committee meeting (organized in October 2021). Dr. Dan Peterson, Director of IGGB at MSU and Dr. Popescu George, Assistant professor at IGGB are the members of her committee, chaired by the Project Leader, Dr. Sorina Popescu, Associate Professor.

   Philip Berg, Ph.D. student, designed metagenomics and microbial community data analysis methods, performed soybean data analysis, supervised undergraduate students working on data analysis.

3. Training of one postdoc, Dr. Xin Ye in microbiology, molecular and plant biology techniques

Products and dissemination of results to communities of interest:

1. Poster presentations at the 82nd Meeting of Southern Section of the American Society of Plant Biologists (SS-ASPB) that took place on April 16-18, 2021, by two MSState Undergraduates working in the PI's lab:

   Joshua Mitchell: '*Common Soil Bacteria as Possible Antifungal Biological Control Agents Against Xylaria sp. and Taproot Decline'*

   Aja Black: '*Bacterial Influence on Xylaria sp. Growth'*

2. Oral presentations at the 82nd Meeting of Southern Section of the American Society of Plant Biologists (April 16-18, 2021) by Jasmine Uyen Wesser, a Master's Student in the PI's lab.

   Jasmine Uyen Wesser*: 'Characterization of Soybean Root Endophytes with Protective Activity Against the Soil-Borne Fungal Pathogen Xylaria sp.'*

3. Online posting on MS Soybean Promotion Board website: Joshua Mitchell's video presentation presented at the SS-ASPB meeting In April ,2021.

4. The abstracts of the posters and presentations from the SS-ASPB meeting were published in the Conference Program booklet.

5. Oral presentations for online posting on MS Soybean Promotion Board website by Jasmine Uyen Wesser, a Master's Student in the PI's lab and Joshua Mitchell, an undergraduate working in PI's lab.
6. Oral presentation at the 2021 Mississippi State University Three Minute Thesis (3MT) Competition (November 17th, 2021) by Uyen "Jasmine" Wesser, a Master's Student in the PI's lab. Title: '*Characterization of Soybean Root Endophytes with Protective Activity Against the Soil-Borne Fungal Pathogen Xylaria sp.*'
7. Poster presentation at the 4th Annual Department Student Research Symposium (November 19th, 2021) by Uyen "Jasmine" Wesser, a Master's Student in the PI's lab. Title: '*Characterization of Soybean Root Endophytes with Protective Activity Against the Soil-Borne Fungal Pathogen Xylaria sp.*'
8. Journal paper published: Jasmine Uyen Wesser, S. Popescu, M. Tomaso-Peterson, T. Wilkerson, A. Bronzato-Badial. '**Sorina C Popescu**, Maria Tomaso-Peterson, Teresa Wilkerson, Aline, Bronzato-Badial, Uyen Wesser, George V Popescu. 2022. *Metagenomic analyses of the soybean root mycobiome and microbiome reveal signatures of the healthy and diseased plants affected by taproot decline*. Microorganisms Journal, Plant-Microbe Interactions Section.
9. The pilot study data on metagenomic analyses of the soybean root mycobiome and microbiome of the healthy and diseased plants affected by taproot decline was deposited in the NCBI database. The Illumina MiSeq sequence raw reads are now available in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA821528.
10. 2. The metagenome WGS and ITS data sequenced at CosmosID was deposited in the ENA under project PRJEB44705, assembled with the Elixir pipeline, and analyzed using MGNIFY metagenomics analysis tools.
11. 3. Oral presentation at the 83rd *Meeting of the Southern Section of the American Society of Plant Biologists* (University of Alabama, Birmingham) March 26-28, 2022) by Jasmine Uyen Wesser, a master's student in the PI's lab: *'Characterization of Soybean Root Endophytes with Protective Activity Against the Soil-Borne Fungal Pathogen Xylaria sp.'*

Impact of the project

Scholarly Impact. Publications, oral and poster presentations at regional and national conferences, training of undergraduates, Ph.D., and Master Students, and posting research results and conclusions on the MSPB website (www.mssoy.org)

Economic impacts. Taproot decline (TRD) is widespread throughout MS and Southern US and cannot be controlled through classical methods. We aim to discover and deploy methods for the biological control of *X. necrophora* for enhancing soybean production by reducing TRD incidence. Our results provide evidence and proof-of-principle for the development of synthetic bacterial communities for targeted use against *Xylaria* sp.

Our overarching aim is to produce probiotic products for use in Mississippi agricultural fields to suppress disease.

Figure 1: Flow chart of the experimental design for the isolation and testing bacterial endophytes for potential anti-*X. necrophora* activity

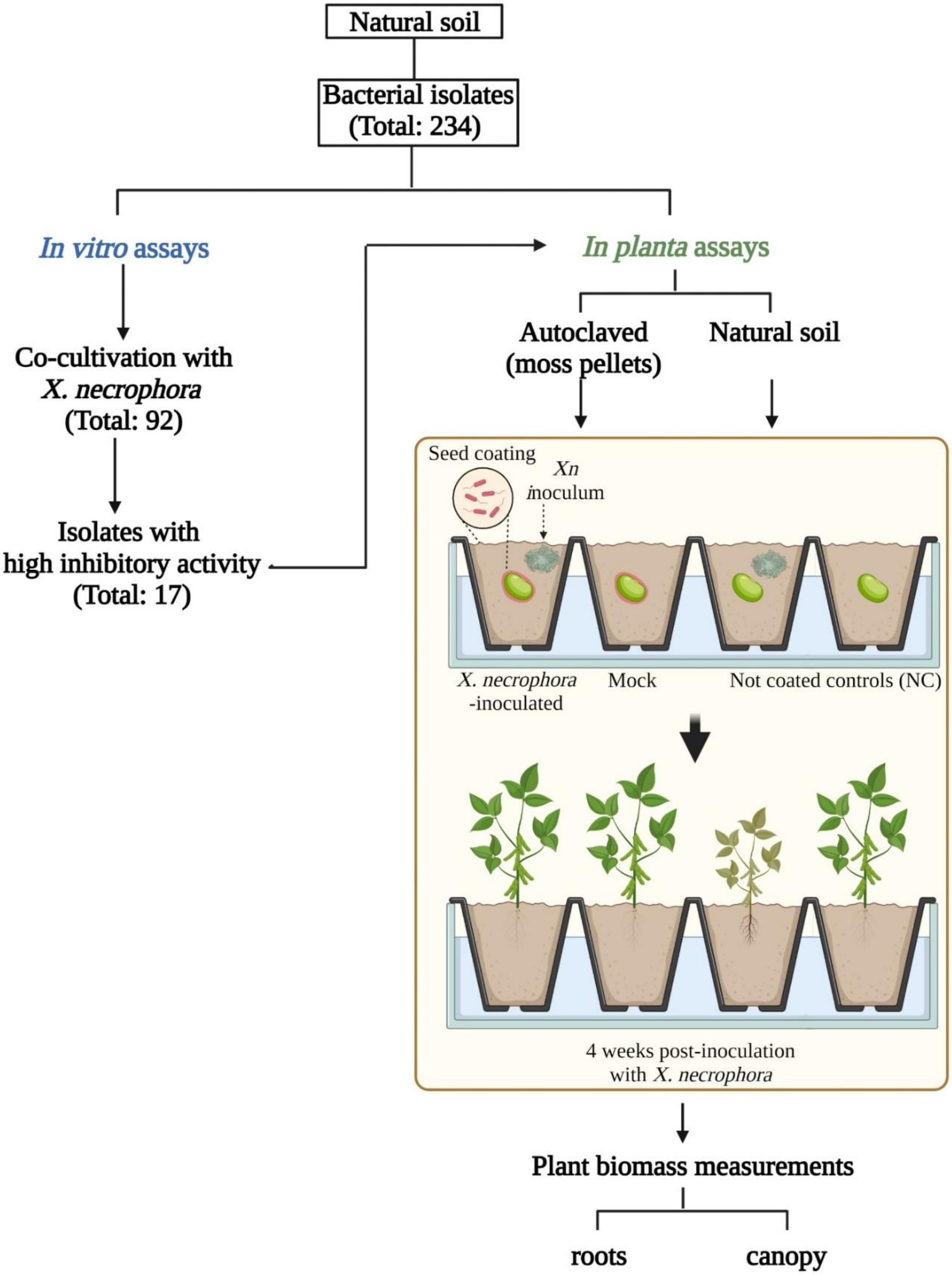

Figure 2: 92 bacterial isolates were tested in co-cultivation assays with Xn to identify isolates with anti-xylaria properties

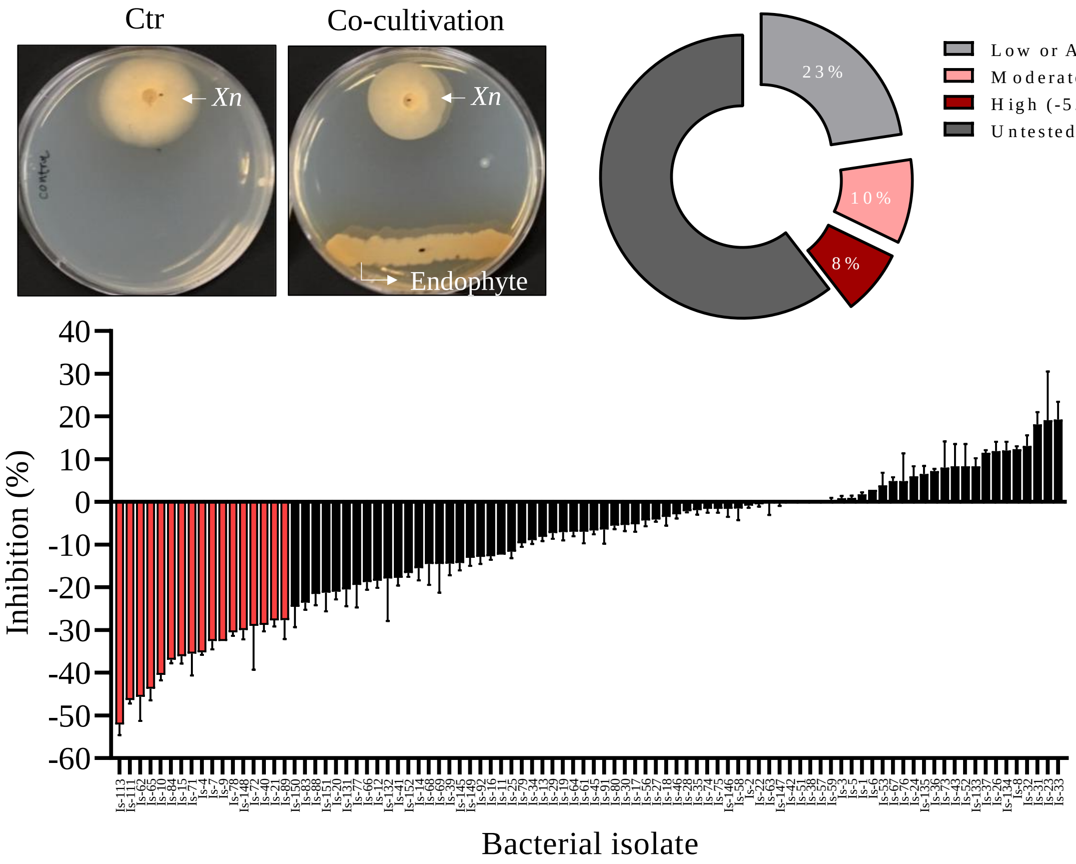

Figure 3: In planta assays using sterile moss pellets (jiffy peats) to identify bacterial endophytes with protective activity against *X. necrophora*. The endophyte strains listed in D and E were found to have significant anti-TRD effects.

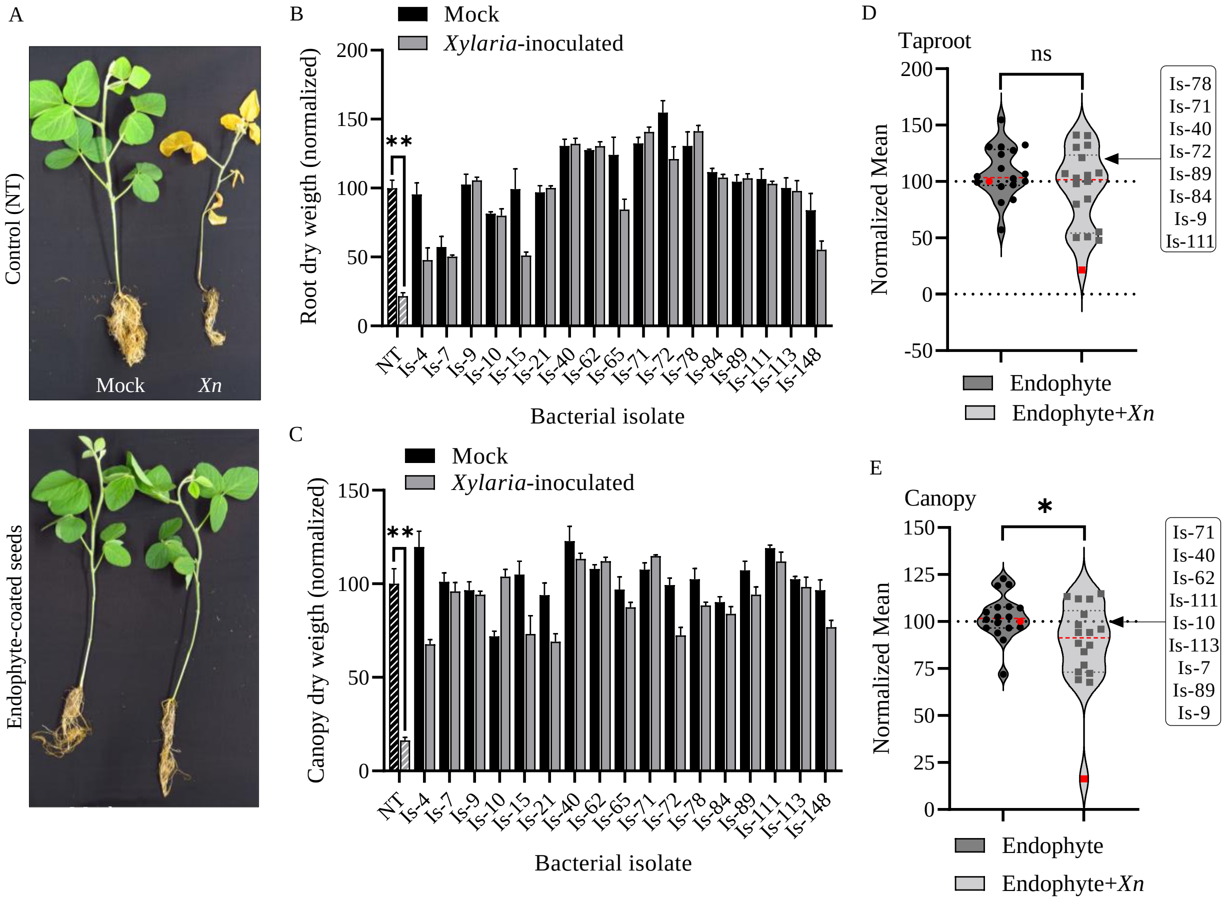

Figure 4: In planta assays to identify beneficial anti-Xylaria bacterial isolates using MS Delta soil. The endophyte strains listed in C and D were found to have significant anti-TRD effects.

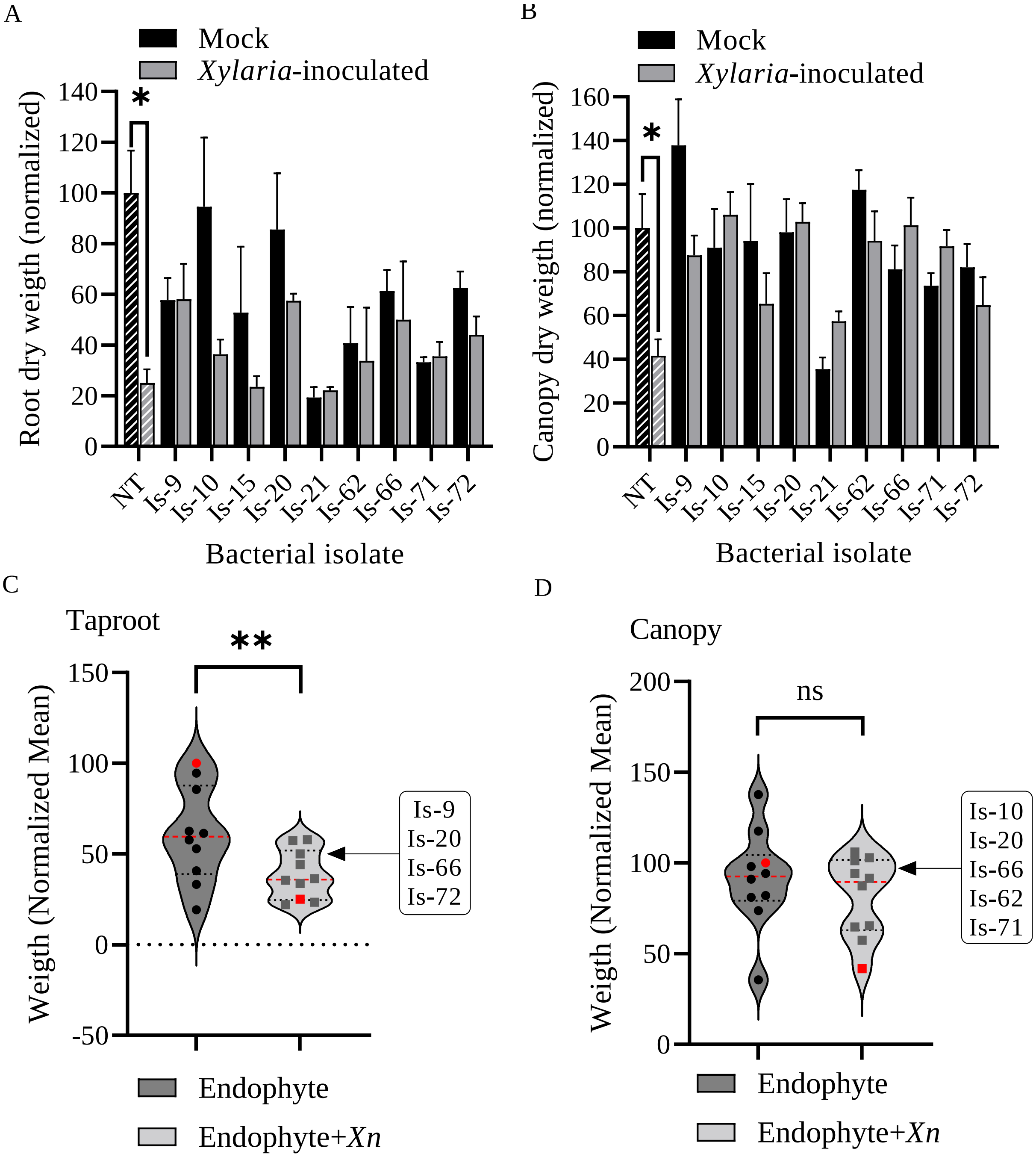

Figure 5: Flow chart of the experimental design to test the influence of *X. necrophora* on the composition of root endophytes.