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Resistance Screening and Biocontrol Applications Resulting from Molecular Analysis of the Sweetpotato Pathogen Streptomyces Ipomoeae

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Agricultural Experiment Sta, Louisiana State Univ

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Goals/Objectives/Expected Outputs

The overall goal of the project is to develop applications that will be useful to sweetpotato growers and breeders. The project will focus on three specific objectives. We recently constructed avirulent mutants of the bacterium Streptomyces ipomoeae, the causative agent of Streptomyces soil rot of sweetpotato. In objective one, we will test these mutants for colonization and disease suppression capabilities. In objective two, we will develop a faster, lower-cost and more efficient method for screening sweetpotato germplasm for soil rot resistance. In objective three, we will purify a substance (an interstrain inhibitor) produced by certain strains of S. ipomoeae that inhibit the growth of other strains of this same species. We will also clone and characterize the gene(s) responsible for synthesis of this substance. Expected outputs from the project include development of a more efficient screening method for soil rot resistance and biocontrol applications that will involve using individual avirulent S. ipomoeae strains and/or interstrain inhibitors to supplement (and perhaps partially supplant) soil rot resistance in sweetpotato. Objective one is expected to be completed by year three of the project, while objectives two and three are expected to be completed by year five.

Methods

For objective one, we will test the ability of our avirulent S. ipomoeae mutants to colonize sweetpotato by tagging the bacteria with green fluorescent protein, exposing sweetpotato adventitious roots to the tagged bacteria and then observing potential plant-bacterium interactions using confocal microscopy as well as scanning electron microscopy. The ability of avirulent mutants (which still produce interstain inhibitor) to suppress disease by pathogenic S. ipomoeae strains that are susceptible to the inhibitor will be tested by exposing adventitious roots to a mixture of the two strain types and then rating any necrosis that develops using a published numerical scale. For objective two, we will evaluate sweetpotato germplasm (e.g., from established sensitive and resistant varieties) for resistance to the purified phytotoxin thaxtomin C, which we recently showed is an essential pathogenicity determinant of S. ipomoeae. This assay will be performed on agar plates in the lab. An alternative approach (also on agar plates) will be to test germplasm for resistance to S. ipomoeae strains themselves. In objective three, we will purify the so-called group II interstrain inhibitor and clone and sequence its cognate gene(s). For cloning, we will use colony blot hybridization involving a genomic library of a relevant S. ipomoeae strain and a degenerate oligonucleotide (designed using the N-terminal sequence of the purified inhibitor). Alternatively, we will identify the gene(s) by screening for inhibitor production using a heterologous Streptomyces host containing the S. ipomoeae genomic library. The project will serve as a training ground for Ph.D. graduate students interested in plant-microbe interactions. Results will be disseminated to other

sweetpotato researchers and extension personnel at annual LSU AgCenter meetings. The success of the project will be measured as follows. For objective one, a successful outcome will occur if we find that avirulent S. ipomoeae mutants show significant suppression of disease by pathogenic strains. For objective two, a successful outcome will occur if we find that purified thaxtomin C or individual S. ipomoeae strains can be used to distinguish sensitive and resistant sweetpotato varieties in vitro. For objective three, a successful outcome will occur if we isolate the group II interstrain inhibitor and clone its cognate gene(s). All findings will also be evaluated by peer review in order to publish them in high-quality journals.

23. Non-Technical Summary

To combat the devastating disease known as Streptomyces soil rot, sweetpotato varieties showing resistance to the disease have been developed through a laborious and time-consuming field testing method. However, resistance is not complete as lesions can still develop on resistant cultivars, thereby reducing their market value. Here, we will seek to create a faster, more efficient screening method for soil rot resistance (which when incorporated into a sweetpotato breeding program will enhance our ability to find resistant varieties with other desirable growth characteristics) and to develop alternative biocontrol methods for preventing the disease which may supplement (or possibly even partially supplant) the existing resistance control method. Strains of the bacterium (Streptomyces ipomoeae) that cause soil rot can inhibit the growth of one another. We will test whether mutants of this bacterium unable to cause soil rot can protect sweetpotato from disease caused by pathogenic strains. We will also screen on agar plates in the lab for soil rot-resistant sweetpotato varieties by examining which ones are resistant to a purified toxin produced by S. ipomoeae, which we recently showed is essential for the disease process. Finally, we will purify an inhibitory substance produced by some strains of the bacterium that inhibits the growth of other strains and isolate the genes responsible for production of this inhibitory substance. We expect that the fundamental and applied knowledge gained from this research will be significant enough to include in multiple publications in high-quality scientific journals. We also expect to generate an efficient screening method to identify soil rot resistance in sweetpotato varieties and a method of biocontrol, which would involve protecting the plants with avirulent mutants of the bacterium and/or purified inhibitory substances. The expected impact of these studies would be production of sweetpotatoes of higher market value, which will also possess more overall desirable growth qualities and traits.

24. Keywords

sweetpotato; Streptomyces soil rot; Streptomyces ipomoeae; thaxtomin; adventitious root; storage root

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Signature	Title	Date
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