Research towards biological control methods of Southern blight disease of local crops

Background and purpose of the project

Figure 1. Challenge assay exposing *S. rolfsii* (top) to *Streptomyces* sp. GM18-153 (bottom) on PMA. Inhibition is shown by the inability of the fungus to grow close to the *Streptomyces* isolate.

Southern blight is an aggressive disease that affects a wide variety of vegetable crops and can be particularly devastating to the production of tomatoes and potatoes in California[1]. The disease is caused by the soilborne fungus *Sclerotium rolfsii* which often lies dormant as sclerotia, tough spherical structures formed by clumping of the fungal mass. Sclerotia preferentially germinate under warm and moist soil conditions, hence midseason for tomato and potato production in Kern County. The fungus infects the plants near the soil line and colonizes the plant tissue aggressively. Plants wilt and collapse fast[1,2]. Chemical control is possible when used at the right time in the field, yet this is technically very challenging. When applied via the overhead irrigation system, fungicides cannot reach the base of the plants due to the mature plant canopy, and when applied through the buried drip irrigation fungicides will not reach the soil level unless a large amount of water is used which tends to cause root rot[2]. Early season application is not recommended as the fungus is not active yet and fungicides typically lose activity over time. Hence, a long-lasting novel fungicide or a fungicide-producing microbe that can establish itself in the field would be a great way to control the disease.

We isolated a *Streptomyces* species, which we named GM18-153, that strongly inhibits the growth of *S. rolfsii* on agar medium (Fig.1). *Streptomyces* are common soil bacteria and well-known producers of metabolites with important agricultural and medical applications[3,4,5]. Several species have been put forward as potential biocontrol agents to combat harmful pathogens in the field[6,7].

The proposed research will explore the potential benefit of GM18-153 for the local agricultural community in two ways as outlined below.

- ***Streptomyces* sp. GM18-153 as a biocontrol agent of Southern blight**

The activity of GM18-153 as a biocontrol agent protecting growing crops from the fungus will be evaluated using tomato plants grown in pots set up in the growth chamber. Sterilized soil mixed with different amounts of bacteria will be used to grow the plants which will be challenged by placing plugs with the fungus in the corner of the pots. Plants will be scored at various time intervals to evaluate the level in which the bacteria can lower the incidence and severity of the disease.

- **Towards characterization of the fungicide produced by *Streptomyces* sp. GM18-153**

Data obtained so far show that the bacterium makes a very potent fungicide that is stable under extreme conditions. While we would like to keep the of the bacterial species that GM18-153 belongs to confidential, we can say that this particular species is not well researched. It is therefore possible that bacterium produces a novel compound. Steps will be undertaken towards the identity of the fungicide produced by GM18-153. Total RNA will be extracted from bacteria grown on a medium known to induce fungicide production (Fig. 1) and a rich medium that suppresses fungicide production. Sequence analysis will be done in collaboration with Dr. LeBlanc at the USDA-ARS facility in Salinas, CA. This will give us the activated genes potentially involved in fungicide production. These genes will be deleted in our lab through an established protocol for site-directed mutagenesis in *Streptomyce*[8,9]. Mutants will be tested for their inability to inhibit fungal growth in antibiosis assays as seen in Figure 1. Having a mutant will greatly benefit the continuation of this project to identify the fungicide by comparing the metabolite profile of the wild type and the mutant bacterium through liquid chromatography-mass spectrometry (LC-MS)

and isolate fractions corresponding to missing peaks in the mutant profile. NMR spectroscopy analysis will generate data that will reveal the chemical structure of the fungicide. This method has worked well for us before in collaboration with Dr. Truman at the John Innes Centre in Norwich, UK[10].

Anticipated significance and relation of the project to the local agricultural community

Southern Blight caused by the heat-loving fungus *S. rolfsii*i is of great importance for our local agricultural industry. It is responsible for significant economic losses for our growers. Moreover, the fungus is very persistent in field soil and can be easily spread through harvest equipment. As mentioned above, no reliable management practice is available. This overall goal of this project is to evaluate the biocontrol properties of the bacterium GM18-153 in two ways: the use of the live bacteria as field inoculum, and the identification of a potentially new but very powerful and stable natural fungicide. Data obtained by this grant will be used to apply to funding from local commodity boards such as the California Tomato Research Institute to be able to complete the fungicide identification and to set up field trials. Both are needed in order to propose the bacterium and/or the fungicide as novel management tools for Southern Blight for our local growers.

Proposed timeline with milestones for the project for the 5-month award period

	Milestone	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**
	RNA extraction and quality check	X				
	Sequence analysis		X			
	Mutagenesis		X	X	X	
	Evaluation of mutants				X	X
	Optimization of pathogenicity assay	X	X	X		
	Biocontrol assay			X	X	X

Funding obtained from this grant will be used to buy supplies for the project. A detailed budget list is available upon request.

References

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