

2010 Proposal to MPIC Research Committee

PROJECT TITLE: Diversity of *Streptomyces* species in Michigan and management of potato common scab using biological and non-chemical methods

Budget request: \$25,072

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Co-operating Agencies: MSU (MAES and MSUES)

ABSTRACT

Potato common scab is a major threat to the potato industry. The challenge of the disease control is that highly resistant varieties are not available, and no chemicals are effective. Moreover, the disease is caused by more than one species of *Streptomyces*. These will add more complications to the disease management. To better understand what species are predominant and how they are distributed, we will continue to survey for *Streptomyces* genetic diversity and geographic distribution in Michigan. This will include isolation and identification of *Streptomyces* spp., testing pathogenesis and Thaxtomin production, and genetic analysis of the isolates. We have done series of greenhouse experiments identifying a field with soil that suppresses potato common scab. We will continue to identify beneficial microorganisms that can be potentially used for biological control. Our emphasis will be placed on identification of specific microorganisms that are associated with suppressive soil. In the mean time, we will test several products or compounds that we selected based on literatures and our preliminary data. Some products have shown a promising result under laboratory conditions. We selected biological and non-chemical methods, which is the ultimate goal in organic and green agriculture. Greenhouse experiments will be conducted with these products on common scab control.

Diversity of *Streptomyces* species in Michigan and management of potato common scab using biological and non-chemical methods

INTRODUCTION

Potato common scab is causing significant economic losses to the Michigan potato industry. Efforts have been made to control the disease by plant breeding, cultural practices, and chemical and non-chemical methods, but there is no simple solution to this disease so far. The fact that common scab is caused by more than one species of pathogen adds more complications to the disease system. Different pathogen species means different control strategies. For example, lowering the soil pH value has been suggested for reducing the population of pathogen of *S. scabies*, but that is not effective on *S. acidiscabies*, which can survive at a low pH values (below 5.2). Therefore, knowing what pathogen is predominant in soil is critical to disease management. The focus of control strategies in Michigan has been exclusively based on *S. scabies* as the pathogen. However, our preliminary data showed that there are other species besides *S. scabies*.

The genetic diversity is supported by our published result, which showed a new strain of *Streptomyces* found in Michigan. This strain was neither *S. scabies* nor *S. acidiscabies*, but tolerant to low pH environment and cause scab symptoms on potatoes. We hypothesized that a pathogenic gene has been transferred from the known pathogen. This seems to happen frequently based on our data, which results in more pathogens being discovered. If new strains exist in Michigan, how is it populated or distributed in Michigan potato production areas? Are there more species in Michigan? How will it impact the disease management? To answer these questions, it is necessary to conduct a survey in Michigan soil to find out if there are other undocumented new pathogenic strains. Therefore, what is the genetic diversity is in the potato production field. Up to date, genetic diversity and distribution for *Streptomyces* has not been documented in Michigan.

We have identified a field near the campus of Michigan State University in East Lansing, which suppresses incidence and severity of potato common scab. Tests were done in the greenhouse and laboratory conditions. Understanding the suppressiveness phenomenon will also help in understanding the interaction between plant host and the major pathogen, and help clarify the interactions between the pathogen and other microorganisms in the soil. Some microorganisms may be antagonistic to *Streptomyces* spp. and can potentially be used as biocontrol agents, which will be especially useful as we do not currently have any effective disease control methods available. We found that this suppressive soil is correlated with biological factors, namely microorganisms that inhibited the pathogen and then reduced the potato common scab disease. Based on this finding, we will further characterize the soil by testing it with growing potatoes, looking for different groups of microorganisms, studying the function of each group, and

selecting some strong isolates as potential biological control agents. More importantly, this study will provide more evidence to support establishing a healthy soil that is not favorable to soilborne pathogens.

Management of potato common scab is our ultimate goal. Before highly resistant varieties are released, it is necessary to screen any methods that could reduce the pathogen population. We have collected some products and done some laboratory work, which have shown inhibitory effects against *S. scabies*. These will be tested in the greenhouse. Microorganisms obtained from the suppressive soil, some commercial biological control products, and organic amendments will be studied on potato common scab.

Specific projects and objectives

1. Survey for *Streptomyces* genetic diversity and geographic distribution in Michigan. This is a continuation of the project from 2009.
2. Characterize the soil that suppresses pathogenic *Streptomyces* spp..
Greenhouse experiments have been done to characterize the suppressive soil, and we will focus on molecular study, hoping to identify the group of beneficial microorganisms.
3. Management of potato common scab using biological and non-chemical methods.

EXPERIMENTAL PROCEDURES

Survey for *Streptomyces* spp. genetic diversity and geographic distribution in Michigan.

(1) Isolation of *Streptomyces* spp. from potato tubers and soil. Potato tubers with typical scab symptoms will be collected from various locations in Michigan potato production areas. The tubers will be washed to be soil free. Scab lesions on the tuber will be excised with a scalpel, surface sterilized with 0.62% of NaOCl solution for 5 min, and rinsed for four times with sterile water. The tissue will be grounded in a sterile mortar with pestle. The homogenate will be diluted 10 and 100 times. One hundred μ l of each dilution will be spread on the STR media (semi-selective for *Streptomyces*) and incubated at 28 °C for 7 to 10 days, after which colonies of characteristic *Streptomyces* will be picked and a pure culture will be obtained by transferring single colonies for two times. In some cases, the original homogenate will be boiled at 60 °C for 15 min before serial dilution and plated to reduce the background colonies. Isolations will be made from various types of lesions, ranging from superficial corky lesions to deep-pitted lesions. Yeast Malt Extract (YME) will be used for routine culture of *Streptomyces* strains. The isolated *Streptomyces* will be detected by using PCR with these primers. Soil samples will be collected from the same location where tubers are collected. The soil will be air dried at room temperature for a week to kill off some of the many bacteria

in soil that compete with those of the genus *Streptomyces*. All soil samples will be processed to estimate the density of streptomycete. Ten g of soil will be dissolved in 90 ml of sterile distilled water and shaken at 200 rpm for 2 hr at room temperature. Soil wash suspensions will be diluted and plated onto WA, STR and OMA medium. The spread plates will be incubated at 28 °C for 14 days, after which the number of culturable bacteria and Streptomycetes on each plate will be recorded and expressed as the number of colony-forming units per g dry soil. Colonies of characteristic *Streptomyces* will be picked and serially transferred until a pure culture is obtained for further analysis.

(2) Identification of *Streptomyces* isolates. The collected isolates will be identified by examining their morphology on agar plates and under microscope. Thaxtomin production will be used as an indicator of pathogenesis. All the isolates will be confirmed by polymerase chain reaction (PCR). To conduct PCR, DNA will be extracted from the pure culture by using MoBio DNA extraction kit following the manufactory's protocol. Also, DNA will be directly isolated from the tuber lesions. Potato tubers will be gently washed. One or two portions (5 mm in diameter) of scab lesions per sample will be excised. The cut tissues will be grounded in a sealed disposable maceration bags, which then will be re-suspended in 500 µl of extraction buffer (200 mM TrisHCl pH 7.5; 250 mM NaCl, 25 mM DTA, 0.5% SDS, 2% PVP), vortexed and incubated at room temperature for 1 hr with continuous shaking. It will be centrifuged at 5000 g for 5 min. After removing 450 µl of supernatant and mix with 450 µl of isoproponal it will be incubated at room temperature for 1 hr. The DNA will be palletted by centrifugation at 13,000 g for 10 min, and then air dried. The DNA will be re-suspended in 100 µl sterile distilled water. Isolates of *Streptomyces* spp. will be identified. The frequency and virulence of each species will be analyzed by location and potato cultivar.

Regions of bacterial 16S rDNA are used for phylogenetic analysis, and sequencing this region will be used in this study. Genes of *nec1*, *TomA TxtA*, and *TxtB* are associated with pathogenicity. We will use these genes as indicators of the pathogen and to help identify new isolates. Primers for these regions are available from the literature. The PCR products will be purified using the methods described in the above section, and sequenced. The sequences will be BLASTed on gene bank online.

(3) Pathogenicity test. The pathogenicity of the isolate will be examined by inoculating it onto potato tuber slices. Cultures of test strains will be grown on oat meal agar medium for 5-7 days and agar plugs will be placed onto potato tuber disks (3 cm in dia. and 0.3 cm high). The disks will be incubated in a moist chamber at 22-24°C in the dark. Seven to 10 days later, the tuber slices will be evaluated for necrotic lesions. If a lesion appears on the tuber around the inoculum plug indicates that the isolate is a pathogen.

Thaxtomin, an indicator of pathogenesis, will be examined for each isolate. Oatmeal broth (200 ml) with trace elements will be inoculated with 200 µl of a

concentrated spore suspension of *Streptomyces* strains. The cultures will then be incubated at 28 °C in an incubator shaker at 180 rpm for 7 days. Cultures then will be centrifuged to remove the cells and particulate materials. The supernatants will be extracted two times with equal volume of ethyl acetate. The combined ethyl acetate extracts will be evaporated to dry and the yellow residue will be taken up in methanol. Thaxtomin will be confirmed by color and high performance liquid chromatograph.

Green house experiments will be conducted to confirm the pathogenicity of partial strains. One hundred ml of yeast malt extract (YME) in a 500-ml Erlenmeyer flask will be inoculated with *Streptomyces* isolates from a 2-week-old YME plate. The culture will be grown with shaking for 3 days at 28 °C. Twenty milliliters of this culture and 100 ml of sterile 2× Say's solution will be added to a bag containing 600 cm³ of sterile vermiculite. Inoculated vermiculite will be incubated for 14 days at 28 °C, and mixed by shaking the bags every other day during incubation. Before using it, sub sample of the vermiculite inocula will be collected to detect the actual *Streptomyces* population by using dilution plating method. Pots will be filled with mixture of sterile soil and vermiculite inoculum containing 10⁸ CFU/g of *Streptomyces* (v:v=1:1). Seed potato Atlantic variety will be used. Potatoes will be harvested and scored for severity after 16 weeks. The scab symptom will be evaluated.

Characterize the soil that suppresses pathogenic *Streptomyces* spp.

(1) Microbial community using T-RFLP. Terminal restriction fragment length polymorphism (T-RFLP) analysis will be used for characterizing the microbial community structure, focusing on bacterial populations. Total microbial DNA will be extracted from the soils by using BIO 101 FastDNA SPIN Kit (MoBio Laboratories, Carlsbad, CA). To assess DNA yield and quality, the soil DNA will be run on 1.0% agarose gels with 1-kb ladder as a reference. To amplify target DNA in soil extracts, primers [forward FAM-63f and reverse 1387r], targeting 16S rDNA of bacteria, will be used. The final concentrations of the different components in the master mixture will be as follows: 0.5µM of each primer, 200 µM of each deoxynucleoside triphosphate, 1× reaction buffer (with MgCl₂), 1.25 U of *Taq* DNA polymerase 50 µl⁻¹. Ten µl of soil DNA (78 ng/µl) will be added to 90 µl of the PCR mixture. Duplicate PCR will be conducted as follows: 95°C for 2 min; 95°C for 1 min, 55°C for 1 min, 72°C for 5 min, 25 cycles. Size of the PCR product will be confirmed on a 1% agarose gel. The PCR products will be digested separately with *MspI* and *RsaI* restriction enzymes. The reaction mixtures will be incubated at 37°C for 3 hours followed by a deactivation step at 65°C for 20 min. Fragment size analysis will be carried out on an ABI PRISM® 3130xl Genetic Analyzer. T-RFLP profiles will be generated from the Genetic Analyzer data using GeneMapper software. The binary data will be analyzed using cluster analysis.

(2) Antibiosis assays. The *Streptomyces* and other bacterial isolates screened from the suppressive soil will be tested by a co-plate assay for inhibition to pathogenic *Streptomyces scabies* *in vitro*. Candidate *Streptomyces* isolates will be streaked on

yeast malt extract agar (YME) and incubated at 28 °C for 5 days, and the candidate bacterial isolates on TSA medium to produce single colonies. These single colonies will then be tested for antagonistic ability to *S. scabies*. One hundred µl of spore suspension of *S. scabies* will be spread uniformly onto the surface of an YME Nunc Omni tray (with 12x8 grids). Immediately afterward, spores or cells from single colonies will be spotted on the grids of the tray. The plates will be incubated at 28°C for 1-3 days. Plates will be examined for the presence of inhibition zones, and colonies that exhibited inhibition zones will be re-isolated from the original plate, cultured, and stored for further tests. These candidate isolates will be inoculated onto plants by dipping the tuber seeds before planting. Resistance induction will be examined by evaluating the common scab severity at harvesting. Isolates that show a strong inhibition will be tested in the greenhouse trial, which may further be tested in the field in the future.

Management of potato common scab using biological and non-chemical methods

A greenhouse trial will be conducted to evaluate several potential products for the control of potato common scab. Products will include biological control agents, BCA1 and BCA3 (*Bacillus* spp.) screened in Hao's laboratory, PlantShield (*Trichoderma hazianum*, SipCam Advan), Telnet (*Trichoderma asperellum* & *T. gamsii*, SipCam Advan), YieldShield (Mix of *Bacillus* spp., Bayer Crop Science), and Soilbuilder (Mix of *Bacillus* spp., Advanced Microbial Solutions); disease resistance inducer, Regalia (giant knotweed extract, Marrone Organic), and SilMatrix (potassium silicate, Monterey Agro-Biotech); soil amendment, chestnut shells and compost; and Chemical PCNB as a control. In the greenhouse, potting soil will be infested with *Streptomyces scabies* (two mixed isolates at final concentration of 10⁴ CFU/g soil) and potato tuber seeds will be planted, as described above. Prior to seeding, chestnut shells and compost will be incorporated in the soil; biological control agents and PCNB will be prepared as a solution and drenched to the soil at standard rates. Regalia and SilMatrix will be applied to plants when seedlings are showing up. There will be four replications (pots) for each treatment. The pots will be arranged as a randomly complete block design. At the end of the season, potato tubers will be harvested, and common scab will be evaluated using 0 (no disease) to 5 (the severest symptom) scales.

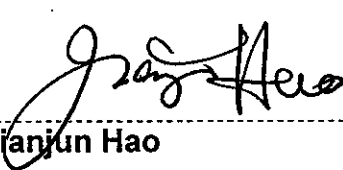
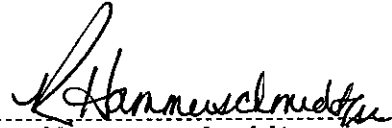
PLANS TO DISSEMINATE INFORMATION FROM STATED RESEARCH

The result will be disseminated to stakeholders in various forms, such as extension program, meetings such as 2010 Great Lakes Fruit, Vegetable & Farm Market EXPO, publication like MPIC NewsLine, websites, and peer-reviewed journals. Information will also be distributed through colleagues in the area of crop and soil science, plant pathology, and organic agriculture. Presentations will be given at research meetings of state, regional, national, and international levels.

BUDGET

The budget requested is to support a graduate student for ten months of stipend and fringe, and some materials and supplies.

Graduate student (including fringe):	\$ 24,272
Materials and supplies:	\$ 800
Total requested:	<hr/> \$ 25,072

	
Jianjun Hao	Ray Hammerschmidt
(PI)	(Department Chair)