Culture-Based Assessment of Microbial Communities in Soil Suppressive to Potato Common Scab

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Abstract


A field in East Lansing, MI, showed a decline of potato common scab compared with an adjacent potato field. To confirm that the decline was due to biological factors, the soil was assayed. In the greenhouse, putative common-scab-suppressive soil (SS) was either treated with various temperatures or mixed with autoclaved SS at various ratios. Pathogenic Streptomyces scabies was incorporated into the treated soil at 10^6 CFU/cm^3 of soil, followed by planting of either potato or radish. Disease severity was negatively correlated with the percentage of SS in the mixture and positively correlated with temperature above 60°C. The soil was screened for four groups of potential antagonists (general bacteria, streptomycetes, fluorescent pseudomonads, and bacilli) pairing in culture with S. scabies. The frequency of antagonistic bacteria in SS was higher than common-scab-conducive soil (CS) in all four groups but only pseudomonads and streptomycetes were significantly higher. The population of pathogenic Streptomyces spp. in the rhizosphere of CS was significantly higher than SS. Dilution plating of CS and SS samples showed no clear trends or differences in populations of total fungi, total bacteria, streptomycetes, fluorescent pseudomonads, and bacilli but terminal restriction fragment polymorphism analysis revealed two distinct microbial communities were present in SS and CS.

Potato common scab (PCS) is caused by Streptomyces spp. Since its first report in North America in the late 19th century, the disease has been found worldwide wherever potato is grown. Streptomyces spp. are persistent soil inhabitants that survive saprotriphically in soil for long periods in the absence of hosts. Usually, the disease affects tuber quality by resulting in superficial, raised, or pitted lesions on the periderm (19,20). In some cases, potato yield can be reduced due to severe infection. PCS threatens the $3.5 billion potato industry (NASS, 2010) due to lack of effective control methods (9).

Management of PCS is difficult. Scab-resistant cultivars are the ultimate goal but current commercial varieties have only partial resistance or tolerance (35). The only widely used chemical, pentachloronitrobenzene (5), has been withheld in the United States recently by the Environmental Protection Agency (EPA) due to its carcinogenicity and nondegradability in soil (EPA website). Increasing irrigation intensity and lowering soil pH are partially implicated in the disease suppressiveness of these fields. Knowing the specific soil profile of suppressive soil may help us to improve soil health through manipulating the microbial communities.

In recent years, the authors have observed a decline in PCS in a potato field near the campus of Michigan State University, East Lansing, and decided to evaluate the soil microbial communities implicated in the disease suppressiveness of these fields. Knowing the specific soil profile of suppressive soil may help us to improve soil health by adding various amendments. Various techniques are available to enumerate, identify, and culture microorganisms, such as selective media-based methods (32) and molecular approaches: surveying the entire microbial community present in a soil (33). The main objectives of this work were to determine whether the soil was suppressive to PCS, characterize the structure of microbial communities likely contributing to the disease suppressiveness, and culture specific beneficial microorganisms or microbial communities that can be reestablished to control common scab. A preliminary report has been published (23). A related study using pyrosequencing to characterize soil microbial communities will be reported separately (29).

Materials and Methods

Field history and soil properties. A field (N42°43.014′, W84°27.972′) near the campus of Michigan State University in East Lansing has been cultivated with potato consecutively for more than 25 years. This field, designated as the scab-suppressive (SS) field, was used as a scab nursery for potato variety evaluation. Because the disease declined gradually over several years, the varietal test was moved to a new field (N42°42.937′, W84°27.975′) in 2005, designated as the scab-conducive soil (CS) field, in the same area, separated by a distance of 200 m. This field showed higher disease pressure of PCS according to disease ratings from the past 5 years. Soil samples collected from SS and CS fields were analyzed for physical and chemical properties at the Michigan State University’s Soil and Plant Nutrient Laboratories using different methods for each element as recommended for the North-Central Region. The soil type from both SS and CS fields is loamy sand (Table 1).

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**Field rating of PCS.** The SS and CS fields were divided into 5-by-10 quadrants with 4 by 7 m²/quadrant. Potato plants (‘Snowden’) were dug and the tubers were scored for common scab disease. Fifty tubers were randomly selected from each plot for disease evaluation. Disease lesions on potato tubers were scored as previously described (8), with slight modification: 0 = no symptoms; 1 = 1 to 10, 2 = 11 to 25, and 3 = 26 to 50% surface area with superficial or raised lesions; 4 = >50% surface area with superficial or raised lesions or <25% pitted lesion area; and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease severity index was calculated as \(\Sigma\) (score × number of tubers with that score)/total number of potato tubers evaluated. Scab ratings were conducted for both SS and CS after harvest in 2007 and 2008.

**Greenhouse assay of soil-suppressing scab diseases of radish and potato.** *Plant inoculation.* Pathogen inoculum was prepared following the method described by Wanner (34) with slight modification. *Streptomyces scabies* strains 1019 from Michigan and 49173 from the American Type Culture Collection were cultured on yeast malt extract (YME; EMB Chemical Inc., Gibbstown, NJ) agar incubated in the dark at 28°C for 5 days or until spores were produced. A spawn bag (19 by 8 by 5 in.; Fungi Perfecti, LLC, Olympia, WA) was filled with 2,000 cm³ of vermiculite (medium size, premium grade; Sun Gro Horticulture Distribution Inc., Bellevue, WA) and 200 ml of water per bag, and autoclaved for 1 h twice in 24 h. Spore suspensions (10⁷ CFU/ml) determined by enumerating colonies on YME plates of *S. scabies*, were prepared by adding 10 ml of sterile distilled water to culture plates and scraping colonies using a sterile scalpel. A mixture (1:1 in volume, 10 ml of each) of *S. scabies* isolates 1019 and 49173 was added to 180 ml of 2× Say solution (8), which was then mixed into the sterile vermiculite in the spawn bags. The vermiculite inoculum bags were incubated at 28°C and mixed every other day by shaking. To determine the inoculum density, 1 cm³ of vermiculite with *S. scabies* was transferred into 9 ml of sterile distilled water. Serial dilutions were prepared and plated (100 µl) on YME agar. After 5 days of incubation in the dark at 28°C colonies of *S. scabies* were enumerated.

In a greenhouse, cut seed tubers of potato (‘Atlantic’) were placed in soil infested with *S. scabies* (see below) in 6-liter pots. Fertilizer (N:P:K ratio = 24:8:16; Scotts Miracle Gro Products, Inc., Marysville, OH) was applied once every other week after seedlings were 2.5 cm high. There were four replications (pots) for each treatment. Growth conditions in the greenhouse were 24°C with a 14-h photoperiod, supplemented by light at 200 µmol m⁻² s⁻¹. After 5 days of incubation in the dark at 28°C colonies of *S. scabies* were enumerated.

**Disease lesions on potato tubers were scored for common scab disease.** Fifty tubers were randomly selected from each plot for disease evaluation. Disease lesions on potato tubers were scored as previously described (8), with slight modification: 0 = no symptoms; 1 = 1 to 10, 2 = 11 to 25, and 3 = 26 to 50% surface area with superficial or raised lesions; 4 = >50% surface area with superficial or raised lesions or <25% pitted lesion area; and 5 = >50% surface area with superficial or raised lesions or >25% pitted area. The disease severity index was calculated as \(\Sigma\) (score × number of tubers with that score)/total number of potato tubers evaluated. Scab ratings were conducted for both SS and CS after harvest in 2007 and 2008.

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**Effect of heating temperature on disease suppression of SS.** SS was sampled with the same method described above and placed in high-temperature resistant bags (10 liters/bag; Associate Bag, LLC, WI), and treated at 40, 60, 70, 80, and 90°C in a water bath for 30 min, starting when the center of the soil bag reached the expected temperature and measured with a metal temperature probe (NovaTech International Inc., TX). An additional bag was autoclaved (121°C) for 30 min. In a second trial, SS was sampled, bagged as before, and treated at 30, 45, 60, 75, and 90°C for 30 min in a water bath, and 121°C (autoclaving) for 30 min. There were four replications (pots) for each treatment. For both trials, after the temperature treatment, the soil was mixed with *S. scabies* inoculum in vermiculite at the final concentration of 10⁶ CFU/cm³ of mixed soil. Nontreated and autoclaved soil without infestation was used as control. These two trials with different temperature sets were tested on both potato and radish.

**Characterization of soil microbial communities in disease-conducive and -suppressive soils.** *Microbial communities assayed by dilution plating method.* Soil samples were collected from CS and SS fields. Bulk soil was collected in the area between plants. Sample volumes consisted of about 2 liters, around five trowels full taken from the top 15 cm soil, at five random locations. After being transported to the laboratory, the soil was mixed thoroughly in a plastic bag. Each soil sample (10 g) was suspended in 90 ml of sterilized phosphate-buffered saline in 125-ml Erlenmeyer flasks and shaken for 20 min at 300 rpm. For rhizosphere soil sampling, potato roots were dug out and placed in a plastic bag. In the laboratory, the roots were hand shaken loosely, and soil attached to the roots surface was used.

Suspensions were serially diluted and 100 µl of each soil dilution was spread onto three replicate plates of four different semiselective agar media. The media used and the target microbial communities were as follows: rose bengal agar (EMD Chemicals) with chloramphenicol (0.1 g/liter) for total fungi, 1/10-strength tryptic soy agar (TSA; EMD Chemicals) for total bacteria, SI (31) for fluorescein pseudomonads, and *Streptomycetes*-selective (STR medium) (4) for streptomycetes. TSA was also used to culture *Bacillus* spp. from soil dilutions heated to 80°C for 30 min before plating. All plates were incubated at room temperature (22 ± 1°C) and enumerated after 2 days (total bacteria, *Bacillus*, and fluorescent pseudomonads), 3 days (fungi), or 7 days (*Streptomycetes* spp.).

**Microbial communities assayed by terminal restriction fragment length polymorphism.** Soil was collected as described above from both CS and SS fields and each mixed thoroughly. Half a gram of soil from each sample was used for total soil genomic DNA extraction, using the FastDNA Spin Kit for soil (MP Biomedicals, Solon, OH) following the manufacturer’s instruction. Polymerase chain reaction (PCR) was conducted with primer FAM-63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') labeled with 6-carboxyfluorescein, and 1387R (5'-GGG CCG WGT GTA CAA GGC-3') (21), which are universal primers amplifying the 16S rDNA gene of bacteria. Each PCR reaction had a total volume of 25 µl, containing 5 U of Taq DNA polymerase, 5× Taq polymerase PCR buffer (Promega Corp., Madison, WI), 200 mM dNTP mixture, 0.2 mM

<table>
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<th>Disease-conducive soil</th>
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<td>Soil type</td>
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**Table 1.** Physical and chemical properties of disease-conducive and -suppressive soils.
each primer, and 1 µl (2 to 25 ng/µl) of template DNA. Amplifications were performed on a thermocycler with an initial denaturation step of 5 min at 94°C; followed by 36 cycles of 40 s at 94°C, 40 s at 58°C, and 1.5 min at 72°C; and a final extension of 7 min at 72°C. After confirmation by electrophoresis on 1.2% (wt/vol) agarose gel, PCR products were purified using the PCR Purification Kit (Denville Scientific Inc., Metuchen, NJ), and digested with RsaI and MspI enzymes (New England Biolabs Inc., Ipswich, MA) following the manufacturer’s instructions and purified by ethanol precipitation. Restriction digests were separated by capillary electrophoresis using an ABI 3130 x1 DNA analyzer and data analyzed using GeneScan 3.7 software (Applied Biosystems, Carlsbad, CA) to determine digested fragment sizes.

Frequency of antagonistic bacteria against *S. scabies*. Isolates of *Bacillus* spp., fluorescent *Pseudomonas* spp., *Streptomyces* spp., and total bacteria, isolated from dilution plating (as described above), were tested for antagonism against *S. scabies* in vitro using a co-plate assay (38). Spore suspension of *S. scabies* strain 1019

![Graphs showing disease index, fresh weight, and suppressive soil percentage](image)

**Fig. 1.** Characterization of disease-suppressive (SS) soil in potato and radish in the greenhouse. SS was either A1, B1, and C1, mixed with autoclaved SS at various portions; or A2, B2, and C2, treated with various temperatures for 30 min, followed by infesting the soil with *Streptomyces scabies* at final concentration of 10⁶ CFU/cm³ of soil. Either potato (A1 and A2) or radish (B1 and B2) was seeded in the treated soils in a pot. Disease was rated and potato common index was calculated according to published procedures (8,34). Weight of fresh radish roots was measured in both trials (C1 and C2).
was prepared as described above. Antibiosis assays were done as follows: spore suspension (100 µl) of strain 1019 with the concentration of 10⁷ CFU/ml (determined by dilution plating) was spread on a YME agar plate and allowed to air dry in a laminar flow hood. Different isolates growing on the selective media (as described above) were selected to culture with *S. scabies* strain 1019. Ten isolates were tested on each plate and replicated three times. Plates were incubated at 28°C for more than 3 days and the number of antagonists were recorded. This trial was conducted over 3 years, from 2007 to 2010.

**Frequency of pathogenic Streptomyces spp.** *Streptomyces* spp. from SS and CS were obtained from isolation by dilution plating on STR media (as described above). From each soil (including bulk and rhizosphere soil), 150 isolates were randomly selected. All these isolates were purified and grown on YME agar for 7 to 10 days. Potato tubers were sliced and cut into disks 0.5 cm in height and 2.0 cm in diameter. The disks were placed on moistened filter paper in petri plates. Mycelial plugs (5 mm) of *Streptomyces* spp. isolates were placed in the center of the tuber disk (18). Noninoculated YME agar plugs were used as controls. Necrotic lesions on potato disks around the *Streptomyces* inoculum were observed and measured at room temperature after 3 to 5 days in the dark.

**Statistical analysis.** Data were analyzed using SAS software (version 9; SAS Inc., Cary, NC). Procedure GLM was used for analysis of variance and Fisher’s least significance difference. Multiple comparisons were performed for mean separation. Procedure REG was used for linear regression. Procedure Spearman rank correlation was used to analyze disease and plant growth response to temperatures. Terminal restriction fragment length polymorphism (T-RFPLP) Cluster analysis was performed using the R-statistical package (v. 2.10; Revolution Analytics, Palo Alto, CA).

**Results**

**Field rating of PCS disease severity.** Scab ratings of the SS field was 1.43 ± 0.45 and 1.55 ± 0.56 in 2007 and 2008, respectively, which were significantly lower (*P* < 0.05) than scab ratings from CS field, which ranged from 2.6 ± 0.62 to 2.6 ± 0.54 in 2007 and 2008, respectively.

**Greenhouse assays of soil suppressing scab diseases in radish and potato.** Effect of soil mixtures of suppressive and autoclaved soils on PCS disease. Disease suppressiveness was measured by assessing the disease severity in both potato and radish planted in a soil mixture of untreated natural SS and autoclaved SS. As the percentage of the SS increased, the disease severity decreased in both potato and radish (Fig. 1A1 and B1). No common scab symptoms were observed in potato planted in 100% SS (Fig. 1A1). This relationship fit a simple linear regression model with *R*² = 0.95 (*P* < 0.05; Fig. 1A1) and *R*² = 0.92 (*P* < 0.05; Fig. 1B1). Plant fresh weight was positively correlated (*R*² = 0.93, *P* < 0.05) with the percentage of SS; higher radish weights were obtained from higher percentages of SS, and vice versa (Fig. 1C1).

**Effect of soil temperature treatments on PCS disease suppression.** Temperature treatment impacted the disease suppression in both potato and radish. The disease severity showed a positive relationship with the temperature used for soil treatment. Disease suppression was significantly reduced by temperatures above 60°C (Fig. 1A2 and B2). The values of Spearman’s rho (*ρ*) were 0.996 (*P* < 0.01) for potato and 0.904 (*P* < 0.025) for radish. Plant fresh weight was negatively correlated with temperature treatments: higher radish weights were obtained from soil treated with lower temperatures, and vice versa (Fig. 1C2; *ρ* = −0.904, 0.01 < *P* < 0.025).

**Characterization of soil microbial communities from disease conducive and suppressive soils.** Microbial communities assayed by dilution plating. Analysis from eight sampling times gave mixed results (Table 2). There were no clear trends in microbial populations among CS and SS. In most trials, SS had higher population numbers of total fungi, total bacteria, streptomycetes, fluorescent pseudomonads, and bacilli in most soil samples tested. However, the majority of these differences were not statistically significant (*P* < 0.05) within the same sampling dates (Table 2).

**Microbial communities assayed by T-RFPLP.** Analysis of T-RFPLP fragments digested with Rsal and MspI resulted in two major clusters for SS and CS (Fig. 2), indicating that two distinct microbial communities inhabited these two types of soils. The variation among samples from the same field was low.

**Frequency of antagonistic bacteria against* S. scabies.** In total, 5,285 single bacterial colonies were obtained from semiselective media plates and included isolates from total bacteria, fluorescent pseudomonads, streptomycetes, and bacilli. All together, 961 (18%) isolates that included 573 and 388 isolates from SS and DS, respectively, exhibited antagonism against *S. scabies* (Fig. 3). In all four groups sampled from 2007 to 2010, the frequency of antagonistic bacteria in SS (0.27, 0.19, 0.49, and 0.24 from bacilli, total bacteria, pseudomonads, and streptomycetes, respectively) was higher than CS (0.23, 0.13, 0.30, and 0.018 from bacilli, total bacteria, pseudomonads, and streptomycetes, respectively) but only differences in pseudomonads and streptomycetes were significant (*P* < 0.05; Fig. 3).

**Frequency of pathogenic Streptomyces spp.** The frequency of pathogenic streptomycetes was higher in the rhizosphere than in the bulk soil. Rhizosphere CS had a significantly higher frequency of pathogenic streptomycetes than SS. Moreover no difference in the frequency of pathogenic *Streptomyces* spp. from bulk soil was found (Fig. 4).

**Discussion**

We have demonstrated that soil suppression of PCS corresponds with differences in microbial community structure. The disease suppression is transferable from soil to soil, and can be reduced or eliminated by high-temperature treatments. Continuous crop monoculture could be the driving force for the development of the

<table>
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<th>Date of soil sampling</th>
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<tr>
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<td>–</td>
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</tr>
<tr>
<td></td>
<td>CS</td>
<td>–</td>
<td>5.14 b</td>
</tr>
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</table>

*Microbial population was expressed as logarithmic transformation of CFU per gram of soil. Multiple comparisons were performed using Fisher’s least square difference test at significance level *α* = 0.05. Values followed by same letters have no significant differences in the same column of the table; – indicates data were not available.*
observed disease suppression, as demonstrated previously with common scab and other disease systems (17,28,36).

The disease suppression was affected by temperatures higher than 60°C. The corresponding organisms for suppressiveness could be elucidated based on the maximum temperatures they can tolerate. In this study, the suppression does not, or decreases little below 60°C, indicating that the contribution due unicellular bacteria, such as pseudomonads, may be limited. Loss of suppressiveness around 80°C supports the role of streptomycetes and that, at higher temperatures, support spore-forming organisms such as bacilli or other fungi may be important (22,36,38).

After narrowing the scope of organisms of interest responsible for the suppressiveness, further investigation was conducted based on the microbial community comparison between SS and CS. The successive planting of potato may have enhanced the soil microbial community and resulted in the accumulation of host-specific beneficial organisms, including antagonists against plant pathogens (2). In this study, the frequency of antagonistic bacteria from SS was significantly higher than CS, particularly in the case of streptomycetes. *Streptomyces* spp. produce a range of antibiotics (20) that may contribute to disease suppression. The frequency of pathogenic *Streptomyces* spp. in the SS in this study was less than that in the CS but total streptomycetes were higher in the SS. Nonpathogenic streptomycetes, such as *S. diastatochromogenes* and *S. albogriseolus*, can produce antibiotic-like compounds active against *S. scabies*, and are considered to be important biological components of disease suppression (3,17). Greater numbers of nonpathogenic *Streptomyces* spp. are associated with less severe common scab, suggesting that an interaction between host plant and *Streptomyces* microbial communities affects disease severity of

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**Fig. 2.** Dendrograms of terminal restriction length polymorphs. Soil samples are designated with number-letter-number format, where letter S means disease-suppressive soil and D means disease-conducive soil. The number before the letter indicates soil samples and the number after the letter indicates replications of DNA extraction. DNA was digested with either MspI or RsaI restriction enzyme.

**Fig. 3.** Frequency of antagonists from bulk soil (result was derived from combined data sampled on 10 April 2007, 4 November 2008, 3 July 2009, and 13 May 2010 in both disease-suppressive (SS) and -conducive (CS) soils. An asterisk (*) indicates significant differences between SS and CS at α = 0.05.

**Fig. 4.** Frequency of pathogenic *Streptomyces* strains isolated from bulk and rhizosphere soils in both disease-suppressive (SS) and -conducive (CS) soils. Bars over the values are standard deviation and an asterisk (*) indicates significant differences between SS and CS at α = 0.05. Result was derived from combined data sampled on 10 July 2007 and 13 May 10.
PCOS (3,30). Disease-causing strains of S. scabiei can be controlled by nonpathogenic \textit{Streptomyces} strains when the pathogenic and nonpathogenic isolates coexist at a certain ratio (9,27). This inhibition is especially important if nonpathogenic \textit{Streptomyces} compete for nutrition and colonization, and can produce inhibitory compounds toward pathogens (25,30).

According to the results of different soil temperature treatments, other possible organisms responsible for the suppressive mechanism are \textit{Bacillus} spp., which have been proven as pathogen antagonists in nature and can be used as biological control agents, such as \textit{Bacillus subtilis} (11) and \textit{B. amyloliquefaciens} (1). In this study, the population of bacilli in SS was larger than that in CS, without being significantly different. This is in agreement with results of pyrosequencing analysis in our companion study (29). However, if the relative abundance of bacilli did not play an important role, it is possible that the species or population composition of this group is different between the two soils.

There may be other candidate microbes that could be involved in disease suppressiveness. Results from pyrosequencing analysis showed that \textit{Lyso bacter} spp. were significantly higher in disease-suppressive soil than in the CS (29). Several isolates within this genus display potential biocontrol activities and inhibit disease-causing bacteria, fungi, and oomycetes (6,26). The role of \textit{Lyso bacter} spp. in this suppressive soil remains unclear and needs further study.

Based on our study, the disease suppressive was due to a group of microorganisms, including bacilli, florissant pseudomonads, and nonpathogenic streptomycetes. Results from culture-based and molecular studies, taken together, suggest distinct communities made up of different functional groups capable of PCOS diseases suppression. More groups of microorganism have been identified using alternative methods (29).

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**Literature Cited**


