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Molecular and functional characteristics of streptomycete communities in relation to soil factors and potato common scab

Pingping Sun [a, b], Lindsey K. Otto-Hanson [b], Brett E. Arenz [b], Qing Ma [a, *], Linda L. Kinkel [b]

[a] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA

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ABSTRACT

Naturally-occurring disease-suppressive soils provide control of plant pathogens via the activities of indigenous microbes. While all soils contain antagonistic microbes, there is little systematic understanding of the correlates of variation in indigenous antagonist populations and on their relationships with plant diseases. We characterized the population densities, inhibitory capacities, and phylogenetic composition of soil streptomycete communities in a potato field over time in relation to edaphic factors, antagonist inoculation, and potato common scab severities. Antagonistic *Streptomyces* populations were highly variable in time and space. Similarly, metagenomic analyses of streptomycete communities showed extensive spatial and temporal variation in composition. Soil characteristics (pH, potassium, organic matter, nitrate, and phosphorous) sometimes explained up to 50% of the spatial variation in *Streptomyces* population densities, proportion of inhibitory isolates, or pathogen suppressive capacity among locations in the field. Soil pH was positively correlated with common scab severity, and negatively correlated with the proportion of pathogen-inhibitory *Streptomyces* among locations in the field. This suggests that high pH may have direct beneficial effects on pathogen populations and disease development, and/or indirect effects on pathogen populations via reductions in pathogen-inhibitory *Streptomyces* populations. Mean pathogen suppressive capacity of antagonistic *Streptomyces* was negatively correlated with common scab disease. *Streptomyces* inoculants had no discernible effect on streptomycete community composition, antagonistic capacities, or potato common scab severities, likely reflecting poor colonization of inoculants. Further understanding of the relationships between indigenous antagonist populations and plant diseases will be important to harnessing the potential of indigenous communities to contribute to sustainable disease management.

1. Introduction

Soils support a diverse microflora whose activities are central to nutrient cycling, including decomposition and carbon sequestration, and the microorganisms can have significant effects on plant productivity via their roles in mediating nutrient availability, as plant pathogens, and as beneficial symbionts. As beneficial symbionts, microbes can enhance plant nutrient acquisition [1], increase growth via production of plant growth hormones and signaling compounds, induce plant resistance to pathogen infection [2–4], and suppress plant pathogens in soil [5–12]. Disease-suppressive soils, which are notable for the capacities of their indigenous soil microbial populations to minimize soilborne diseases, have been well-studied in multiple systems (wheat, potato, melon) [13]. However, microbes with capacities to antagonize plant pathogens are ubiquitous in soils in both agricultural and native habitats. The ubiquity of antagonistic populations in soil suggests a much greater potential for pathogen and disease suppression in soil microbes than has been recognized or quantified to date. Moreover, research has shown that populations of indigenous antagonists are negatively correlated with subsequent crop disease for multiple pathogens [12,14]. However, there have been very few studies of indigenous antagonist communities in relation to plant disease in agricultural systems.

There have been efforts to actively manage indigenous pathogen antagonists, and specifically to enrich the capacity of indigenous communities to suppress plant diseases in diverse agricultural systems [15–18]. However, though green manures and composts in

* Corresponding author.
E-mail address: maqing@nwsuaf.edu.cn (Q. Ma).

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particular have met with some success in reducing plant disease, disease control is generally inconsistent. Moreover, rigorous quantification of indigenous antagonist populations in relation to disease suppression following treatment remains limited.

Inoculation of *Pseudomonas*, *Trichoderma*, *Bacillus*, or *Streptomyces*, has been explored as an alternative approach for increasing disease suppressive potential of soil microbes for many plant pathosystems [19–27]. However, most attempts at inoculative biocontrol ignore the indigenous soil community, effectively disregarding the capacities of indigenous microbes to enhance plant productivity. Moreover, despite the fact that the indigenous soil communities are often the primary factors in determining the success or failure of inoculants in reducing plant disease and enhancing plant growth, potential interactions between inoculants and indigenous soil communities in relation to plant growth or disease have received little systematic consideration [28,29]. When indigenous soil communities have been considered, the primary focus has been on the influences of the indigenous soil community on the success of inoculants rather than possible effects of inoculants on the indigenous community and its disease suppressive capacity. This leaves a substantial gap in our knowledge of the roles of indigenous microbial communities in relation to disease suppression.

Recently, a model has been developed for predicting the potential impacts of crop management approaches on indigenous disease suppressive populations in soil, with the specific goal of optimizing antagonistic phenotypes of soil communities to reduce plant disease [30]. The model builds upon the observation that plant disease severity is negatively correlated with population densities or suppressive capacities of soil microbial populations, and explores edaphic and management characteristics most likely to influence antagonistic population characteristics (population densities, pathogen-inhibitory phenotypes). The model also suggests that microbial inoculants may impose significant selection on indigenous communities, and thus offer the potential to significantly shift indigenous soil microbial functional or phylogenetic characteristics. Thus, rather than considering inoculants relative to their immediate capacities to suppress plant disease, inoculants may also be valuable for their capacities to impose selection for antagonistic phenotypes on indigenous soil microbes.

Common scab of potato is caused by thaxtomin-producing *Streptomyces scabies* [31,32]. Scab lesions on tubers severely reduce crop market value [33,34]. Common scab-suppressive soils have been documented in multiple locations [35–37], and non-pathogenic, antibiotic-producing *Streptomyces* are suggested to play a significant role in natural disease suppression [38–43]. All soils support antagonistic *Streptomyces*, and research shows these indigenous populations can be responsive to short-term selection [12,14,44,45]. *Streptomyces* spp. have also been used as inoculants in biological control of diverse plant pathogens on many plant hosts, though their effectiveness has varied. This suggests the potential for *Streptomyces* inoculants to contribute to disease suppression both via direct inhibition of plant pathogens, or as a means of imposing selection for increased population densities or inhibitory capacities of indigenous populations via competitive pressure. However, there is little information on the influences of inoculants on indigenous microbial communities, or the consequences for disease suppression, in agricultural systems.

The objective of the present study was to determine indigenous soil streptomycete community phylogenetic and functional characteristics in relation to soil characteristics, *Streptomyces* inoculation, antagonistic phenotypes, and potato common scab severity. Specifically, we inoculated *Streptomyces* with diverse characteristics into field plots at the time of planting. We measured soil characteristics (potassium, phosphorus, nitrogen, organic matter, and pH) at the time of potato planting and harvest, and measured potato common scab severity and potato yields at harvest. Population densities and inhibitory capacities of streptomycete populations were characterized over time, and communities were further analyzed using 454 sequence analyses. These data shed light on the responsiveness of indigenous antagonistic streptomycete communities to soil characteristics and inoculants, and their relationships to common scab severity. This information can help guide the development of active management approaches for enhancing the capacities of indigenous and inoculated soil microbial communities to suppress plant pathogens.

2. Materials and methods

2.1. Streptomyces spp. isolates

Four *Streptomyces* isolates exhibiting diverse phenotypes [46] were selected as soil inoculants to determine both their effects on common scab severity and on indigenous *Streptomyces* communities. Isolates were collected from agricultural soil from University of Minnesota Outreach, Research, and Education (UMore) Park (isolate 414S-4) or from native prairie soils (isolates 1231.5, 3212.4, and 3212.5) [47,48]. In addition, the pathogenic isolate *S. scabies* 87 was used in quantifying pathogen-suppressive activity of indigenous soil communities. Stock suspensions for individual antagonist isolates and pathogen isolate *S. scabies* 87 were prepared by growing each isolate on oatmeal agar (OA) [35] at 27 °C for 7–10 days. Subsequently, spores were collected using a sterile cotton applicator and placed in a 20% glycerol solution for storage at −20 °C.

2.2. Inoculum preparation and experimental field application

Streptomyces isolates were inoculated individually and in combination for a total of 11 treatments, plus a noninoculated control, into field soils (Table 1). First, the four *Streptomyces* isolates were grown on plates containing 25 ml OA at 27 °C for 14 days to achieve high numbers of spores. Oatmeal agar plates containing each *Streptomyces* isolate were then homogenized in a sterile blender with sterile distilled water at high speed for 30 s. Isolates were homogenized individually, and subsequently transferred to a sterile container and stored at 4 °C for up to 3 days. During this time, numbers of spores for each isolate were determined by dilution plating onto 1% Water Agar (WA; 1 g Bacto agar/l de-ionized water). Sterile vermiculite was prepared as a carrier for the inoculum. Briefly, 800 ml Sunshine Brand coarse vermiculite was mixed with 20 ml distilled water, and double-bagged in autoclaveable bags prior to autoclaving on two consecutive days (121 °C, 1 h liquid cycle on each day). Subsequently, inoculum slurry for an individual *Streptomyces* isolate was added at a dose of 1.44×10^{10} CFU per bag (1.8×10^{7} CFU/ml vermiculite), with each isolate maintained in separate inoculum bags. For mixed inoculations, vermiculite was mixed in equal volumes to produce the same total CFU population density, with equal population densities of each individual isolate as a fraction of the total population density. Thus, for a three-strain inoculum combination, the total population density was divided by 3 so that equal CFU population densities (4.8×10^{9} CFU) of each isolate were added to the inoculum bag. Bags were sealed and maintained on the benchtop for 24 h before incorporation into the field soil.

Field experiments were performed at the University of Minnesota Sand Plain Experiment Station, located in Becker, Minnesota, in a plot that has a long history of moderate to heavy potato common scab severities. The experiment was established as a randomized complete block design with 12 treatments (including the non-