Multiple statistical approaches of community fingerprint data reveal bacterial populations associated with general disease suppression arising from the application of different organic field management strategies

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Abstract

Multiple statistical analyses of terminal restriction fragment length polymorphism (T-RFLP) data were used to screen and identify bacterial populations involved in general disease suppression in an organically managed soil. Prior to sampling three different management strategies (i.e. mixed hay (H), tilled fallowing and open-field vegetables production) were used during the transition from conventional to organic farming, with and without compost amendment. The H transition strategy consistently led to the lowest damping-off disease incidence on two different crops in separate greenhouse and field experiments. Bacterial population structure in bulk soil and the rhizosphere of both crops was characterized using T-RFLP analyses of amplified 16S rDNA sequences. First, principal component analysis (PCA) revealed changes in the relative abundance of bacterial terminal restriction fragments (TRF) in response to transition strategy and/or compost amendment in eight different experimental contexts. In each context, a different subset of TRF substantially contributed to the variation along the first two principal components. However, terminal restriction fragment M148 contributed significantly to the observed variation in 6 out of the 8 experiments, and moderately in the remaining 2 experiments. As a second approach, nonparametric analyses of variance revealed that the relative abundance of TRF differed among treatments. While the responsive subsets identified varied somewhat by experimental context, M137, M139 and M141 were more abundant in samples from the H transition strategy in multiple experimental contexts. Subsequent correlation analyses revealed that TRF associated with disease suppressive treatments (i.e. H with and without compost) were frequently negatively correlated with damping-off disease incidence. As a group, these TRF were disproportionately associated with lower disease levels further indicating their role in disease suppression. Interestingly, *in silico* analysis of the bacterial 16S rDNA sequence database revealed that the TRF identified in this study (e.g. M137, M139, M141, and M148) might correspond to well-characterized genera of bacterial biological control agents.
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1. Introduction

Agricultural management practices impact soil and rhizosphere microbial diversity and community structure.

The interactions between crop species, management strategy, and soil type, affect soil microbial communities (Garbeva et al., 2004a). For example, Berg et al. (2002) observed differences in bacterial communities in the rhizosphere of different crop species: potato, oilseed rape, and strawberry. In contrast, Hiddink et al. (2005), reported no significant differences in soil microbial communities between single and mixed crop cropping systems, and Girvan et al. (2003) described differences in communities associated to soil types compared to crop species. Tillage

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(Feng et al., 2003), rotation (Lupwayi et al., 1998; Larkin, 2003), use of mulches (Tiquia et al., 2002), cover crops (Schutter et al., 2001; Schutter and Dick, 2002) and amendments (Parham et al., 2003; Pérez-Piqueres et al., 2006) are also known to influence the structure and activity of microbial communities. The effects of different farming practices in the abundance of rhizosphere colonizing biocontrol *Pseudomonas* specifically, those producing the antibiotic 2,4-diacetylphloroglucinol (DAPG), were described by Rotenberg et al. (2007). The abundance of DAPG-producing *Pseudomonas*, which have previously been implicated in soilborne disease suppression (Weller et al., 2002, McSpadden Gardener, 2007), in the rhizosphere of corn were positively correlated with stand and yield and predictably responded to rotation sequence, tillage, compost amendments and seed treatments.

Soils differ in their ability to suppress plant disease development. Disease-suppressive soils are soils in which pathogens fail to establish or to produce disease (Baker and Cook, 1974). Pathogen survival and growth is often limited due to a variety of biological parameters of soil. Two types of biologically based disease suppression have been described. General suppression, which occurs as an overall effect of the microbial community, principally through resource competition differs from specific suppression, which relates to a specific mode of action against pathogen populations. However, it seems likely that both general and specific suppressive activities occur to varying degrees in most soils. It is well established that particular farm management practices can be used to promote disease suppression. For example, the use of compost for the control of soilborne pathogens and the characteristics of these organic amendments contributing to suppressiveness has been widely studied (Hoitink and Fahy, 1986; Hoitink and Boehm, 1999). Similarly, Larkin and Honeycutt (2006) demonstrated the importance of rotation sequence on the buildup of soil microbial communities suppressive to *Rhizoctonia* diseases of potato; and Berg et al. (2002) described the importance of host species for bacterial antagonists to *Verticillium dahliae*.

Microorganisms associated with disease suppressiveness may represent useful biological control agents. After soils with various disease suppressive levels have been recognized, a fingerprint of the microbial community can provide information about candidate microorganisms involved in this function (Borneman et al., 2004). Over 10 years ago, Tunlid et al. (1989) and Boehm et al. (1993) used multivariate analyses of fatty acid profiles to demonstrate that multiple bacterial populations contributed to general suppression of *Rhizoctonia* and *Pythium* damping-off of cucumber. Specific microbial populations have also been implicated in disease suppression. For instance, populations of DAPG-producing *Pseudomonas* are more abundant in long-term wheat monoculture systems exhibiting suppression to take all disease of wheat (Weller et al., 2002). Similarly, the fungus *Dactylella oviparasitica*, identified through rRNA gene analysis, is involved in the suppression of the beet-cyst nematode by specific California soils (Olatinwo et al., 2006).

Culture-independent studies of microbial communities rely on the analysis of conserved DNA sequences. Terminal restriction fragment length polymorphism (T-RFLP) is a PCR-based technique, which can be used to create community profiles based on differences in restriction fragment length of a specific DNA region (Marsh, 1999). T-RFLP has been used to study differences in community structure and diversity in different systems, at the domain level (based on ribosomal DNA sequences) and for specific functional groups, such as denitrifiers (Braker et al., 2001).

This study was encompassed in a multi-disciplinary research project aimed to evaluate economic, environmental and biological impacts of management strategies to be used during the transition from conventional to organic farming. In the United States, a 3-year period without synthetic chemical inputs is required to obtain official organic certification. During this transition period growers typically experience yield reductions due to insect, weed and disease pressure, and do not always provide adequate fertility (Liebhardt et al., 1989; Tu et al., 2006). Growers may approach this prescribed transition period in several ways; and, their choices are driven by economic as well as agronomic concerns (MacRae et al., 1990; Tu et al., 2006).

In this work, T-RFLP analyses were used to study differences in bacterial community structure in soil and rhizosphere samples taken from plants grown in soils previously experiencing different organic transition strategies. Plants grown in soils from different treatments varied in health and vigor, in both greenhouse and field bioassays (Baysal-Tustas et al., 2006). Based on these results, we hypothesized that the different management treatments modified microbial community structure so as to alter the level of suppression to soilborne diseases, such as pre-emergence damping-off. Soil and rhizosphere microbial communities are known to be complex, and information obtained from molecular fingerprinting analyses could be limited. Hence, we used a variety of statistical approaches to mine community fingerprint data to identify associations between different microbial populations and soilborne disease suppression. The approach consisted of a combination of multivariate and nonparametric univariate procedures that provide different perspectives on the extensive data sets generated by microbial community fingerprinting techniques.

2. Materials and methods

2.1. Field site description

An organic transition field experiment was established at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. Soil at this site is a moderately well-drained Wooster silt loam with approximately 2.2% organic matter, $40\,mg\,kg^{-1}\,P$, and $95\,mg\,kg^{-1}\,K$. The field was previously under a conventional corn and soybean