Microbial Communities Associated with Potato Common Scab-Suppressive Soil Determined by Pyrosequencing Analyses

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Abstract

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Potato common scab, caused by *Streptomyces* spp., is an annual production problem for potato growers, and not effectively controlled by current methods. A field with naturally occurring common scab suppression has been identified in Michigan, and confirmed to have a biological basis for this disease suppression. This field and an adjacent scab nursery conducive to disease were studied using pyrosequencing to compare the two microbial communities. Total DNA was extracted from both the disease-conducive and -suppressive soils. A phylogenetically taxon-informative region of the 16S rRNA gene was used to establish operational taxonomic units (OTUs) to characterize bacterial

community richness and diversity. In total, 1,124 OTUs were detected and 565 OTUs (10% dissimilarity) were identified in disease-conducive soil and 859 in disease-suppressive soil, including 300 shared between sites. Common phyla based on relative sequence abundance were Acidobacteria, Proteobacteria, and Firmicutes. Sequences of *Lysobacter* were found in significantly higher numbers in the disease-suppressive soil, as were sequences of group 4 and group 6 Acidobacteria. The relative abundance of sequences identified as the genus *Bacillus* was significantly higher by an order of magnitude in the disease-conducive soil.

Potato common scab (PCS), caused by *Streptomyces* spp., is an annual production problem for potato growers worldwide (26,43). The disease is of particular importance in Michigan and the United States North-Central region, where disease development can be severe due to favorable environmental conditions, including warm, dry seasons with soil temperatures above 22°C (44). PCS pressure can vary between years, locations, and potato varieties (52). Consistent and effective management of PCS has remained elusive (26,43). Few commercially important potato varieties show significant resistance to PCS under variable production conditions (54). PCS is of particular concern in chipping varieties where the disease impact is economic due to cosmetic symptoms on the tuber surface. Cultural practices, including planting certified disease-free tubers, rotating crops, maintaining soil moisture during tuber formation, lowering soil pH, and chemically fumigating soils, provide inconsistent results or fail to significantly reduce pathogen inoculum levels (14). Most cultural practices for disease control are related to enhancing soil microbial activities (18). Because modification of microbial communities by these methods is indirect and the mechanisms involved not well known, inconsistency in disease control is understandable. To overcome this limitation, studies of naturally occurring disease-suppressive soils may provide a baseline for characterization of important microbial populations that contribute to disease suppressiveness, and aid the development of a sustainable alternative to current control strategies.

With recent advances in culture-independent approaches to microbial community analysis, there has been renewed interest in the phenomena of endemic suppression of soilborne plant diseases, including exploration of the effects of plant species and soil management on disease suppression and the associated microbial community structure. Intrinsic disease suppression in agricultural soils has long been known and has been explored in multiple pathogen-plant systems (2,8,33,50). PCS disease suppression is found in cultivated soils (4,22) and appears to be related to changes in microbial populations. Disease control is largely attributed to biological interactions (e.g., parasitism and competition) between antagonistic microflora and pathogens mediated via antibiotic production or enzymatic activity (3). Characterization of the native potato-associated soil microbial community in disease-suppressive soils offers new opportunities for managing native soil microbes to control PCS.

The development of newer sequencing technologies means that soil microbial communities, including those of PCS disease-suppressive soils, can be studied in greater detail than was previously possible with earlier, lower-resolution culture-independent techniques. For example, identification of microorganism on selective or semiselective media can only recover less than 5% of microorganisms in the whole community (47), and a priori community nutrient-use profiling (BIOLOG plate; Hayward, CA) and fatty acid methyl ester methods are highly dependent on sample preparation and current available databases (38,40). Terminal restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis cannot give precise species and taxonomic identity (27,32). The recent accessibility of the pyrosequencing platform has also enabled relatively affordable analysis of microbial community structure of the soil (16,23). Pyrosequencing as implemented in the Roche Genome Sequencer FLX Titanium is a high-throughput next-generation platform capable of producing approximately 10⁶ sequence reads of 400 bp in a single run, and provides greater depth than traditional DNA sequencing technologies and, hence, detection of rare species (16).

We identified a field in East Lansing, MI that exhibited natural suppression to PCS after consecutive years of potato production (32). In culture-dependent studies, dilution plating showed no clear trends in differences in populations of total fungi, total bacteria,

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streptomycetes, fluorescent pseudomonads, and bacilli, but T-RFLP analysis revealed distinct microbial community structure of suppressive and conducive soils (32). With this concurrent study, it was possible to link genus-level taxonomic identity and compare microbial community structure of the suppressive soil with an adjacent nonsuppressive disease-infected field, using a pyrosequencing approach. Disease suppression is largely attributed to biological interactions (e.g., parasitism and competition) between antagonistic microflora and pathogens mediated via antibiotic production or enzymatic activity (4,22,32). We hypothesize that this disease-suppressive soil has a characteristic microbial community structure. We amplified the variable region V4-V5 because it is more conserved in length which, within the highly conserved 16S rRNA gene, aids in quality control and reduced computer processing time without a loss of taxonomic resolution of soil microbial communities.

Materials and Methods

The culture-independent approach consisted of (i) pyrosequencing of ribosomal 16S V4-V5 fragment amplicons using DNA barcoding; (ii) computational sorting of resulting bar-coded sequences into conducive soil (CS) and suppressive soil (SS) sources; (iii) clustering the resulting sequences into operational taxonomic units (OTUs); (iv) comparison of sequences using phylotype analyses based on the Ribosomal Database Project (RDP) taxonomy; and (v) statistical analysis of frequency distributions; OTU richness, diversity, and relative abundance of predominant taxa in CS and SS.

Soil sampling and DNA extraction. Soil samples were collected during tuber bulking from the root-soil interface of potato plants ('Snowden') from CS and SS in two adjacent fields (CS: 42°42'56.35"N, 84°27'56.35"W, elevation 262 m and SS: 42°43'01.31"N, 84°27'57.11"W, elevation 262 m) with a similar history of continuous potato monoculture, in East Lansing, MI. Each field measured 30 by 60 m. A composite of three soil cores within 1 m² from five random soil sampling sites were collected for each CS and SS field ($n = 10$ soil sampling sites total). Individual sampling sites were at least 3 m apart. At each sampling site, soil cores were collected with a 25-mm JMC soil corer (Clements Assoc., Newton, IA) to a depth of 100 mm. The soil corer was disinfested with 10% NaClO followed by 70% EtOH and the composite soil cores (approximately 300 g) placed into separate plastic bags, transported to the laboratory on ice, and stored at 4°C pending further analysis. Subsequently, total genomic DNA was extracted from a half-gram of soil from each composite soil sample using the FastDNA SPIN Kit for Soil (MP Biomedicals LLC, Solon, OH) according to the manufacturer's instructions. DNA samples were stored at -20°C until analysis.

Polymerase chain reaction amplification of soil sample DNA and sample pooling for pyrosequencing. DNA extracted from each soil sample served as a template in triplicate polymerase chain reactions (PCRs; 30 reactions total) performed using the Roche High-Fidelity PCR system (Roche Diagnostics GmbH, Mannheim, Germany). Each 20- μ l reaction contained 1.8 mM MgCl₂, 400 nM each primer, 200 μ M each dNTP (Invitrogen Life Technologies, Carlsbad, CA), 4 μ g of bovine serum albumin (New England Biolabs, Ipswich, MA), 1 U of Taq polymerase, and 50 ng of template DNA. The forward primer consisted of the 25-bp 454 A Adapter and a 10-bp barcode, followed by the 15-bp universal bacterial primer 16S-577F (5'-CGTATCGCCTCCCTCGCGCCA TCAGbarcodeAYTGGGYDTAAAGNG-3'). The reverse primer consisted of the 25-bp 454 B Adapter and the 18-bp universal bacterial primer 16S-926R (5'-CTATGCGCCTTGCCAGCCCGC TCAGCCGTC AATTCMTTTRAGT-3'). These primers target an approximately 331-bp region of the 16S rRNA gene containing variable regions 4 and 5. This region was chosen because it is phylogenetically informative (variable across taxa) and is a length well suited for pyrosequencing (51). Following pyrosequencing, the 10-bp barcode enabled the sorting of sequences by original soil sampling site. Amplifications were performed on an Eppendorf Mastercycler thermocycler (Eppendorf North America, Hauppauge, NY) using the following temperature program: 3 min at

94°C; 30 cycles of 45 s at 94°C, 45 s at 56°C, and 1 min at 72°C; followed by final extension for 7 min at 72°C.

PCR amplicon libraries were purified on a 1.2% agarose gel. DNA was extracted from the gel slices using a QIAquick gel extraction kit (Qiagen, Maryland) per the manufacturer's directions. DNA was eluted from the filter with 30 μ l of elution buffer and purified a second time using a Qiagen PCR purification kit. Each purified reaction sample was quantified using the PicoGreen dsDNA Assay Kit (Invitrogen Life Technologies) and the Qubit fluorometer (Invitrogen Life Technologies) according to the manufacturer's instructions. Equal DNA masses of 10 separate samples representing all 10 soil cores from each field were combined, the total concentration adjusted to 2 ng/ μ l, and the pooled sample was sent to the Research Technology Support Facility at Michigan State University (East Lansing) for emulsion PCR, GS amplicon library preparation, and pyrosequencing on a 454 Life Sciences GS-FLX instrument (Roche).

Bioinformatics and computational and statistical analyses. Bioinformatics and computational analyses were performed remotely in the Computational Genetics Laboratory at the Minnesota Supercomputing Institute for Advanced Computational Research at the University of Minnesota Microbial and Plant Genomics Institute. All statistical analyses were performed using the R-statistical package (v.2.10; <http://cran.r-project.org/>) or SAS v. 9.2 TS Level. Generated sequence data have been deposited in European Molecular Biology Laboratory European Read Archive using the RDP Short Read Archive Prekit (<http://pyro.cme.msu.edu/>).

Operational taxonomic unit analysis of richness and diversity: rarefaction, Chao1, ACE, Shannon, and Simpson indices. OTU analyses were performed using the MOTHUR software package (v.1.17.0) (39). OTU calculations were based on the genetic distance between sequences and used to estimate the richness and diversity of a sampled community. For alignment purposes only, quality-filtered unique sequences (minimum length > 150 bp) were included. Chimeric sequences were removed and a preclustering step was used to reduce noise to signal from raw pyrosequencing data prior to OTU analysis. Sequences were then aligned to the SILVA alignment database (<http://www.arb-silva.de/>) using the Needleman-Wunsch pairwise alignment method with a kmer size of 8 and a reward of +1 for a match and penalties of -1 and -2 for a mismatch and gap, respectively. Subsequently, vertical gaps were removed to minimize the alignment sequence. The reference database was used to produce an alignment to cover the entire 16S rRNA gene. A PHYLIP-formatted distance matrix was calculated for distances equal to and smaller than 0.10. Sequences were clustered and assigned to OTUs at distances between 0.0 and 0.10 in 0.01 increments. The clusters served as the OTUs for generating predictive rarefaction models and for calculating ACE (6) and Chao1 (7) richness indices. The number of OTUs and the ACE and Chao1 richness estimates for the unique, 0.03, 0.05, and 0.10 OTU definitions were calculated. Single and multiple intrasample rarefaction curves were generated using a resampling-without-replacement approach. The Shannon (41) and Simpson (42) diversity indices of each sample for unique, 0.03, 0.05, and 0.10 OTU definitions were calculated.

Phylotype and statistical analyses. Bioinformatics and computational biology approaches were used to assign taxonomy information to compare the associated soil microbial communities' composition of CS and SS. A Bayesian approach was used to assign taxonomic identity to sequences. The phylotype of individual sequence OTUs was determined using the RDP6 taxonomy at various levels of taxonomic hierarchy. Finally, the resulting matches for each set of sequence data were summarized at various levels of taxonomic hierarchical structure (e.g., phylum and class). Kolmogorov-Smirnov, analysis of variance, and Wilcoxon nonparametric analyses were performed on taxonomy results.

Results

Bacterial communities associated with CS and SS. Richness estimates of bacteria associated with CS and SS. In total, 66,308

high-quality sequences were obtained from the 10 individual soil DNA samples (Table 1). To discriminate OTUs, rarefaction curves were calculated on the complete data set using a range of dissimilarity values (unique, 3, 5 and 10%; Fig. 1). Community diversity and richness are reported for 10% similarity (Table 2). Subsequent community comparison (β -diversity) analyses focused on defining OTUs at the conservative 10% dissimilarity, because the rarefaction curves suggest the greatest potential for comprehensive sampling of the soil communities at this dissimilarity level.

Among all soil samples, there were 1,124 distinct OTUs observed at 10% dissimilarity (Table 2; Fig. 2). In all, 46% (521) of all OTUs were represented by a single sequence (singletons), and 27% (300) of OTUs were observed in both CS and SS. CS samples had a total of 565 OTUs and SS samples supported 859 OTUs in 21,110 and 23,342 sequences, respectively (Table 2; Fig. 2). On average, individual SS samples supported a greater number of OTUs than CS samples; average OTU numbers per soil type were 318 and 471 for CS and SS, respectively. The total number of OTUs represented once (singletons) + OTUs represented twice (doubletons) associated with soil type was 314 (55.6% of all OTUs on that soil type) and 531 (61.8% of all OTUs on that soil type) for CS and SS, respectively (*data not shown*).

Bacterial community richness and diversity among soil types. Mean bacterial OTU richness (number of OTUs per soil sample) and diversity were consistently higher for SS than for CS, as were both the mean ACE and Chao1 estimates of OTU richness at 3, 5 and 10% dissimilarity (Table 2; Supplementary Tables S1 and S2). Moreover, the mean Shannon and Simpson diversity indices for individual plants were higher for SS than CS at 3, 5 and 10% dissimilarity (Table 2).

Phylotype-based analysis of bacterial community composition. DNA sequence reads were compared against the RDP6 taxonomy classification to assign best-match taxonomic sequence identity using an 80% cutoff confidence interval. In total, 20 bacterial phyla, 48 bacterial classes, 86 bacterial orders, 172 bacterial families, and 334 bacterial genera were identified based on percent sequence similarity (Table 1).

Relative abundance of bacterial taxa. DNA sequence assignment was dominated by 5 of the 20 bacterial phyla recovered, comprising nearly 87% of the total bacterial community assemblage (Table

3). These phyla were present in every soil sample and consisted of major bacterial groups, including Proteobacteria (39%), Acidobacteria (14%), Gemmatimonadetes (12%), and Actinobacteria (7%), with unclassified bacteria representing 13% of the total bacterial phyla. Bacterial phyla that were unique to a field represented less than 1% of the total and were typically represented as singletons from only one sample (*data not shown*). There were significant differences in relative abundance (based on mean number of sequence reads) between the soil conditions for five bacterial phyla ($\alpha = 0.05$, Wilcoxon; Table 3). CS had a significantly greater abundance of the phyla *Deinococcus-Thermus* (CS: 5.6%; SS: 2.6%) and Firmicutes (CS: 7.3%; SS: 4.5%). Although SS had a greater abundance of the phyla BRC1 and WS3, these phyla, in

Fig. 1. Rarefaction analysis curves generated for operational taxonomic units (OTUs) that contain unique sequences and for sequence dissimilarities that do not exceed 3, 5, or 10%. Solid lines represent samples from disease-conductive soils (CS), and dashed lines represent samples from disease-suppressive soils (SS).

Table 1. Total number of sequences, average number of sequences per plant, and total bacterial phyla, families, and genera classified from disease-conductive and -suppressive soil (CS and SS, respectively)

Number of samples (soil)	Total number of sequences	Sequences/sample (range) ^b	Classification ^a				
			Phyla	Classes	Orders	Families	Genera
5 (CS)	32,174	6,438 (5,851–7,417)	19	46	66	142	256
5 (SS)	34,134	6,827 (6,154–7,246)	18	42	83	163	298
10 (Total)	66,308	6,631 (5,851–7,417)	20	48	86	172	334

^a Taxonomic classification level was determined based on the RDP6 taxonomy outline.

^b Average number of sequences/soil sample (range).

Table 2. Richness and diversity of operational taxonomic units (OTUs), which were determined based upon 10% dissimilarity of disease-conductive and -suppressive soil (CS and SS, respectively)

Sample ^a	Reads	OTU	ACE (range)	Chao1 (range)	Shannon (range)	Simpson (range)
CS1	3,376	286	535 (477, 610)	424 (372, 510)	4.08 (4.02, 4.14)	0.051 (0.046, 0.055)
CS2	3,960	206	465 (404, 545)	396 (314, 541)	2.69 (2.63, 2.76)	0.201 (0.191, 0.210)
CS3	4,878	442	970 (877, 1084)	736 (642, 873)	4.67 (4.63, 4.72)	0.024 (0.022, 0.025)
CS4	3,978	334	833 (743, 944)	558 (479, 678)	4.15 (4.09, 4.20)	0.046 (0.043, 0.050)
CS5	3,745	322	686 (612, 778)	560 (473, 698)	4.25 (4.20, 4.30)	0.037 (0.034, 0.040)
CS total	21,110	565	1,195 (1094, 1314)	992 (865, 1172)	3.82 (3.79, 3.84)	0.058 (0.056, 0.060)
SS1	4,910	502	1,177 (1070, 1304)	969 (829, 1169)	4.04 (3.98, 4.11)	0.106 (0.098, 0.114)
SS2	4,691	193	471 (406, 557)	352 (285, 471)	1.48 (1.41, 1.55)	0.608 (0.590, 0.626)
SS3	4,525	526	1,341 (1218, 1485)	894 (788, 1042)	4.90 (4.85, 4.94)	0.017 (0.016, 0.018)
SS4	4,487	513	1,260 (1145, 1396)	982 (843, 1179)	4.46 (4.40, 4.52)	0.056 (0.051, 0.061)
SS5	4,672	621	1,542 (1411, 1694)	1,152 (1009, 1348)	5.09 (5.05, 5.14)	0.014 (0.014, 0.015)
SS total	23,342	859	2,073 (1,923, 2,244)	1,512 (1357, 1714)	3.81 (3.78, 3.84)	0.097 (0.094, 0.100)

^a CS1 to -5 = CS samples 1 to 5 and SS1 to -5 = SS samples 1 to 5.

both cases and taken together, represented less than 1% of the relative abundance of the total phyla identified (Table 3).

The top 10 of the 48 classes, 86 orders, and 172 families recovered comprised nearly 85, 75, and 70%, respectively, of the total bacterial community assemblage (*data not shown*). Moreover, 24 families of bacteria differed significantly in mean relative abundance between CS and SS samples (Wilcoxon, $\alpha = 0.05$; Table 4). The most abundant families were from some phylogenetically well-established representative soil groups such as Bradyrhizobiaceae (5.4%), Gemmatimonadaceae (13%), Nocardiodaceae (2.3%), Planctomycetaceae (1.8%), Trueperaceae (3.5%), Xanthomonadaceae (23%), an unclassified Acidobacteria group 11 (4.2%), an unclassified Acidobacteria group 3 (2.5%), an unclassified Acidobacteria group 6 (12%), and an unclassified WS3 (13%) (*data not shown*). Moreover 21 families of bacteria differed significantly in mean relative abundance between CS and SS samples (Wilcoxon, $\alpha = 0.05$; Table 4). Overall, CS samples had a greater mean abundance of Acetobacteraceae (CS: 1.2%; SS: 0.5%) and Bacillaceae (CS: 1.8%; SS: 0.2%), while SS samples had a greater mean abundance of unclassified Acidobacteria group 6 (CS: 1.2%; SS: 8.6%), Nocardiodaceae (CS: 1.0%; SS: 2.6%), Pseudomonadaceae (CS: 0.04%; SS: 0.5%), unclassified Acidobacteria group 11 (CS: 0.0%; SS: 0.2%), and unclassified Bacilli (CS: 0.04%; SS: 0.4%) (Table 4). The remaining classes that were significantly different had a combined mean relative abundance of less than 1% for both CS and SS samples (Table 4).

Abundance distribution and composition of bacterial communities. The sequence abundance distributions for bacterial genera and species were compared among samples. Combining data from all samples, there was a significant difference (Kolmogorov-Smirnov test, $P = 0.01$) in the distribution of sequences among bacterial genera between soil types. In total, 10 of the 334 bacterial genera recovered comprised nearly 65% of the total bacterial community assemblage. These included taxa from some common soil groups such as *Bradyrhizobium* (2.4%), *Gemmatimonas* (12.6%), *Rhodanobacter* (19.3%), *Truepera* (2.6%), unclassified Xanthomonadaceae (2.7%), unclassified Acidobacteria group 1 (3.5%), unclassified Acidobacteria group 3 (2.3%), unclassified Acidobacteria group 6 (5.4%), unclassified Hyphomicrobiaceae 77 (1.5%), and unclassified 129 (12.6%) that was unrelated to any known bacterial lineage (*data not shown*). Moreover, 41 genera of bacteria differed significantly in mean relative abundance between CS and SS samples ($\alpha = 0.05$, Wilcoxon; Table 5). Overall, CS samples had a greater mean abundance of *Bacillus* (CS: 1.4%; SS:

0.1%), *Trupera* (CS: 5.5%; SS: 0.07%), unclassified Bacillales (CS: 1.3%; SS: 0.3%), and unclassified Bradyrhizobiaceae (CS: 2.6%; SS: 0.5%), while SS samples had a greater mean abundance of *Lysobacter* (CS: 0.04%; SS: 0.2%), *Rhizobium* (CS: 0.08%; SS: 0.2%), unclassified group 4 (CS: 0.2%; SS: 2.3%), and unclassified group 6 (CS: 1.3%; SS: 9.0%) (Table 5).

Discussion

Different soil types such as bulk and rhizosphere soils can support distinct microbial communities (12,19,24,28,45). We have found a diverse assemblage of microbial populations in the soil microbial communities associated with disease-conductive and -suppressive soils, which is supported by other work (20,57,59). There were significant differences in some of the most abundant taxa identified between CS and SS. CS and SS types were also distinguished from one another by the difference in relative abundance of key bacterial taxa, indicating differential community structure that may be linked to the soil disease profile and provide proxies indicating disease suppression.

The biological factors contributing to the development of disease-suppressive soil may vary due to the complexity of microbe-microbe interactions (1,15,33,49) and the diversity of microbes inhabiting the soil (47,48). For example, naturally occurring PCS-

Fig. 2. Venn diagram indicating shared and unique observed operational taxonomic units (OTUs) at 10% dissimilarity between disease-conductive-soil (CS) and disease-suppressive soil (SS). Numbers of OTUs in groups CS, SS, and shared between groups are shown.

Table 3. Relative sequence abundance of recovered soil bacterial classes from soil samples divided into categories of soil disease profile and combined totals

Phylum ^b	Mean relative abundance (SE) ^a		
	CS	SS	Total
Proteobacteria	34.693 (±15.515)	43.191 (±19.316)	38.942 (±17.415)
Unclassified	13.394 (±5.990)	12.716 (±5.687)	13.055 (±5.838)
Gemmatimonadetes	12.536 (±5.606)	12.231 (±5.470)	12.383 (±5.538)
Acidobacteria	12.019 (±5.375)	16.483 (±7.371)	14.251 (±6.373)
Actinobacteria	7.690 (±3.439)	7.013 (±3.136)	7.352 (±3.288)
Firmicutes*	7.339 (±3.282)	2.027 (±0.907)	4.683 (±2.094)
Deinococcus-Thermus*	5.628 (±2.517)	0.090 (±0.040)	2.859 (±1.279)
Chloroflexi	1.986 (±0.888)	2.731 (±1.222)	2.359 (±1.055)
Planctomycetes	1.826 (±0.817)	1.074 (±0.480)	1.450 (±0.648)
Cyanobacteria	1.570 (±0.702)	0.073 (±0.033)	0.822 (±0.367)
Bacteroidetes	0.597 (±0.267)	0.288 (±0.129)	0.442 (±0.198)
Verrucomicrobia	0.572 (±0.256)	1.323 (±0.592)	0.947 (±0.424)
Nitrospira	0.060 (±0.027)	0.112 (±0.050)	0.086 (±0.038)
WS3*	0.040 (±0.018)	0.464 (±0.207)	0.252 (±0.113)
OP10	0.025 (±0.011)	0.073 (±0.033)	0.049 (±0.022)
Unclassified bacteria*	0.025 (±0.011)	0.060 (±0.027)	0.043 (±0.019)
OD1	0.005 (±0.002)	...	0.003 (±0.001)
Spirochaetes	0.005 (±0.002)	...	0.003 (±0.001)
BRC1*	...	0.052 (±0.023)	0.026 (±0.012)

^a Mean relative abundance based on percentage of recovered sequences; SE = standard error of the mean.

^b Taxonomic classification level was determined based on the RDP6 taxonomy outline; * indicates a significant difference between means of disease-conductive and -suppressive soil (CS and SS, respectively) based on Wilcoxon analysis; comparisons were statistically different at $\alpha = 0.05$.

suppressive soils are generally found following long-term potato monoculture (25,33) and, in several cases, nonpathogenic *Streptomyces* spp. are a key factor for disease suppression (4,32,40,53). However, other microorganisms may also be important (4,31, 40,46).

The combined use of a curated database alignment (e.g., SILVA) and the RDP6 taxonomy enhanced the number of OTUs identified from recovered sequences while providing the added taxonomic identification of the sequences. Many sequences still remain unclassified due to database reliance on well-sampled organisms whose genomes are annotated. Moreover, based on estimates calculated by rarefaction curves (Fig. 1), it is likely that our results represent only a fraction of the wealth of bacterial diversity that exists in soil microbial communities in cultivated soils. Although an average of 6,631 sequences per soil sample were recovered, the entirety of bacterial richness at the species level within an individual sample was not achieved. Therefore, the large size (CFU per gram of soil) and high diversity and complexity of soil communities (11,48) will require future effort in more extensive sequencing to comprehensively characterize community composition, and to ultimately attribute function to distinct bacterial taxa responsible for disease suppression. In spite of this limited characterization of the microbial community, the taxa that dominate in abundance may be key in maintaining disease levels in SS systems.

Pyrosequencing was used successfully to detect a multitude of bacteria inhabiting cultivated soil, including a large proportion of unclassified taxa that are presumably unculturable to date. This is complementary to and confirmation of our results using culture-based methods (32). The two methods together reveal the soil microbial community associated with disease-suppressive soil. Pyrosequencing technology brings the advantage of depth of detection compared with the culture- and non-culture-based studies that have typically focused on specific taxa or functional groups (22,29,30,35,37,40,45,55,56). This study found major bacterial taxa that are common to most soil types at high levels of relative abundance similar to previous work (57,58): class α -Proteobacteria and phyla Actinobacteria, Acidobacteria, Verrucomicrobia, Proteobacteria, and Firmicutes. Conversely, two phyla that were poorly represented by low relative abundance were the recently recognized OD1 and the Spirochaetes. As the DNA database develops, we

anticipate that the unclassified DNA reads corresponding to different taxa, as shown with previous studies (5), will be identified.

According to results from the OUT-based analyses, 73% of the taxa were unique to CS and SS. This agrees with results from T-RFLP 16S rDNA analyses, in which distinct microbial communities were distinguished by SS and CS (32). Moreover these results are both quantitative and qualitative by identifying the taxa unique to CS and SS and, more importantly, their relative abundance, whereas T-RFLP is able to distinguish communities but not their respective constituents.

The Proteobacteria was the most abundant phylum identified. This phyla has been found previously in Brazilian Atlantic Forest soils (10) and agricultural soils (57). Within the γ -Proteobacteria, the genus *Lysobacter* belonging to the family Xanthomonadaceae was in significantly higher numbers in SS. Similar results have been found in soils suppressive to *Rhizoctonia solani* AG2.2IIIB in sugar beet and *Streptomyces scabies* in radish correlated positively with the presence of antagonistic *Lysobacter* spp. and *Streptomyces* spp., respectively (34). Moreover, these results are consistent with previous work on disease-suppressive bacteria that identified γ - and β -Proteobacteria (Pseudomonadaceae, Burkholderiaceae, and Xanthomonadales) and the Firmicutes (Lactobacillaceae) as the most dynamic taxa associated with disease suppression (31). Therefore, continued species-level and functional analyses of the genus *Lyso-* *bacter* may identify members of this group effective in biologically based plant disease management.

The Acidobacteria was the second most abundant phyla identified in this study, which is found in diverse soil types from North and South America (17,20), active in cultivated wheat (57) associated with later stages of the take-all decline disease (36), and in the rhizosphere of chestnut trees (21). Group 4 and 6 Acidobacteria were in significantly higher numbers in SS samples. These groups are linked to pH ranges (17,20,57), which are within the range of the pH of the soil in this study (32). Moreover, Acidobacteria group 4 is enhanced in wheat-soybean rotation management practices (57). Acidobacteria groups sensitive to some cultural practices may provide important indicators of disease suppression.

Members of the phyla Actinobacteria, commonly found among multiple soil samples in previous studies (20,57) and in SS (31), were among the most abundant recovered from both CS and SS

Table 4. Relative sequence abundance of significantly different bacteria from soil samples divided into categories of soil disease profile and combined totals

Family ^b	Mean relative abundance (SE) ^a		
	CS ^c	SS ^c	Total
Acetobacteraceae	1.254 (± 0.124)	0.490 (± 0.099)	0.872 (± 0.677)
Actinospicaceae	0.206 (± 0.096)	0.009 (± 0.005)	0.107 (± 0.105)
Bacillaceae	1.756 (± 0.846)	0.198 (± 0.028)	0.977 (± 0.892)
Clostridiaceae	1.079 (± 0.249)	0.391 (± 0.084)	0.735 (± 0.581)
Comamonadaceae	0.030 (± 0.020)	0.240 (± 0.067)	0.135 (± 0.049)
Deinococcaceae	0.110 (± 0.055)	0.009 (± 0.005)	0.059 (± 0.058)
Ilumatobacter	...	0.086 (± 0.018)	0.043 (± 0.009)
Incertae Sedis XVIII	0.030 (± 0.018)	0.150 (± 0.030)	0.090 (± 0.030)
Mycobacteriaceae	0.291 (± 0.049)	0.116 (± 0.023)	0.203 (± 0.157)
Nocardiodaceae	1.013 (± 0.186)	2.607 (± 0.619)	1.810 (± 0.816)
Pseudomonadaceae	0.035 (± 0.025)	0.498 (± 0.259)	0.267 (± 0.147)
Rhizobiaceae	0.100 (± 0.071)	0.339 (± 0.077)	0.220 (± 0.089)
Rhodobacteraceae	0.015 (± 0.010)	0.133 (± 0.042)	0.074 (± 0.028)
Trueperaceae	5.518 (± 4.981)	0.073 (± 0.036)	2.795 (± 2.777)
Unclassified Acidimicrobiae incertae sedis	1.690 (± 0.252)	0.571 (± 0.093)	1.131 (± 0.892)
Unclassified Actinomycetales	...	0.052 (± 0.026)	0.026 (± 0.013)
Unclassified Gp 11	...	0.125 (± 0.083)	0.062 (± 0.041)
Unclassified Nocardiodaceae	0.115 (± 0.042)	0.004 (± 0.004)	0.060 (± 0.060)
Unclassified Actinomycetales	1.124 (± 0.267)	0.279 (± 0.047)	0.701 (± 0.585)
Unclassified Solirubacterales	...	0.017 (± 0.004)	0.009 (± 0.002)
Unclassified Bacilli	0.040 (± 0.026)	0.464 (± 0.292)	0.252 (± 0.166)
Unclassified Clostridiales	0.025 (± 0.025)	0.060 (± 0.024)	0.043 (± 0.024)
Unclassified Gp6	1.259 (± 0.551)	8.967 (± 2.200)	5.113 (± 1.729)

^a Mean relative abundance based on percent of recovered sequences; SE = standard error of the mean.

^b Taxonomic classification level was determined based on the RDP6 taxonomy outline.

^c Indicates a significant difference between means of disease-conducive and -suppressive soil (CS and SS, respectively) based on Wilcoxon analysis; comparisons were statistically different at $\alpha = 0.05$.

samples. Nonpathogenic Actinobacteria (e.g., *S. diastatochromogenes* and *S. albogriseolus*) produce compounds inhibitory against *S. scabies* and contribute to disease suppression (4,25). The relative abundance of *Streptomyces* spp. was not different between CS and SS, as shown using dilution plating (32). However, SS may have higher populations of nonpathogenic *Streptomyces* spp. that are associated with less severe common scab, suggesting an interaction between host plant and *Streptomyces* microbial communities that affects disease severity of PCS (4,32,40). This population dynamic may be one of the reasons the pathogenic *Streptomyces* spp. were lower in SS (32). Due to the limitations of the present technology and databases, the resolution of species identity is difficult. Therefore, further studies on pathogenicity genes, community diversity, and composition targeting *Streptomyces* spp. may determine molecularly the relative abundance of pathogenic and nonpathogenic genera contributing to disease severity or suppression.

The phyla Firmicute, also commonly found among multiple soil samples in other work (20,57), include bacilli that were among the most abundant in both CS and SS samples. One genus of *Bacillus* was significantly higher in CS by an order of magnitude, whereas a yet-to-be-classified bacillus was found to have a higher relative

abundance in SS. Previous studies found that metabolites (iturin A) produced by *Bacillus* sp. sunhua showed antifungal activity against *Fusarium oxysporum*, the causal organism of dry rot disease in potato; *S. scabies*; and other pathogens of potato (13). *Bacillus* sp. sunhua is beneficial for potato disease control under field conditions (13). The results presented here indicate that this specific site, at the level of genus bacilli in the soil, may not necessarily reflect the individual species of functional importance to endemic soil disease suppression.

This study reveals variation in bacterial communities associated with potato fields of CS and SS. Characterizing microbial composition and diversity, spatial patterns, and the distribution of microbial diversity (9,48) in disease-suppressive soil is an important first step to understanding the potential impacts of these communities on plant fitness. This will help to develop disease management practices to reduce pathogen populations in soil and, therefore, reduce the economic and environmental impacts of plant disease control.

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Table 5. Relative sequence abundance of significantly different bacteria from soil samples divided into categories of soil disease profile and combined totals

Genus ^b	Mean relative abundance (SE) ^a		
	CS ^c	SS ^c	Total
<i>Actinospica</i>	0.206 (±0.096)	0.009 (±0.005)	0.107 (±0.105)
<i>Aeromicrobium</i>	0.030 (±0.012)	0.193 (±0.065)	0.112 (±0.047)
<i>Agromyces</i>	...	0.021 (±0.007)	0.011 (±0.003)
<i>Amaricoccus</i>	0.005 (±0.005)	0.094 (±0.038)	0.050 (±0.022)
<i>Aminobacter</i>	0.005 (±0.005)	...	0.003 (±0.003)
<i>Bacillus</i>	1.374 (±0.814)	0.112 (±0.013)	0.743 (±0.693)
<i>Cohnella</i>	0.070 (±0.023)	0.009 (±0.005)	0.039 (±0.038)
<i>Deinococcus</i>	0.110 (±0.055)	0.009 (±0.005)	0.059 (±0.058)
<i>Lysobacter</i>	0.040 (±0.029)	0.206 (±0.053)	0.123 (±0.046)
<i>Microvirga</i>	0.035 (±0.015)	0.240 (±0.083)	0.138 (±0.059)
<i>Mycobacterium</i>	0.296 (±0.049)	0.116 (±0.023)	0.206 (±0.160)
<i>Nitrobacter</i>	0.135 (±0.047)	0.017 (±0.004)	0.076 (±0.070)
<i>Novosphingobium</i>	...	0.017 (±0.004)	0.009 (±0.002)
<i>Pedomicrobium</i>	0.020 (±0.009)	0.236 (±0.085)	0.128 (±0.052)
<i>Phyllobacterium</i>	...	0.064 (±0.043)	0.032 (±0.022)
<i>Rhizobium</i>	0.075 (±0.069)	0.240 (±0.054)	0.158 (±0.065)
<i>Sporacetigenium</i>	0.080 (±0.022)	0.009 (±0.005)	0.044 (±0.043)
<i>Symbiobacterium</i>	0.030 (±0.018)	0.150 (±0.030)	0.090 (±0.030)
<i>Truepera</i>	5.518 (±4.981)	0.073 (±0.036)	2.795 (±2.777)
Unclassified Sorangiineae	0.005 (±0.005)	0.133 (±0.056)	0.069 (±0.031)
Unclassified 112	0.145 (±0.072)	0.764 (±0.281)	0.455 (±0.213)
Unclassified WS3	0.040 (±0.026)	0.447 (±0.289)	0.243 (±0.165)
Unclassified β-Proteobacteria	0.010 (±0.010)	0.060 (±0.021)	0.035 (±0.015)
Unclassified Ilumatobacter	...	0.086 (±0.018)	0.043 (±0.009)
Unclassified Gp 11	...	0.125 (±0.083)	0.062 (±0.041)
Unclassified Nocardioideae	0.231 (±0.050)	0.752 (±0.141)	0.491 (±0.186)
Unclassified Solirubacteriales	1.701 (±0.268)	0.571 (±0.088)	1.136 (±0.894)
Unclassified BRC1	...	0.052 (±0.026)	0.026 (±0.013)
Unclassified Chloroflexi	0.005 (±0.005)	0.064 (±0.018)	0.035 (±0.011)
Unclassified Thermomicrobia	0.712 (±0.222)	0.150 (±0.037)	0.431 (±0.375)
Unclassified 45	0.281 (±0.038)	0.082 (±0.022)	0.181 (±0.151)
Unclassified Planococcaceae	0.090 (±0.028)	0.013 (±0.005)	0.052 (±0.048)
Unclassified Bacillaceae	1.349 (±0.262)	0.305 (±0.042)	0.827 (±0.695)
Unclassified Clostridiaceae	0.065 (±0.017)	0.004 (±0.004)	0.035 (±0.035)
Unclassified Gp 4	0.226 (±0.114)	2.328 (±0.567)	1.277 (±0.396)
Unclassified Bradyrhizobiaceae	2.624 (±0.331)	0.515 (±0.186)	1.569 (±1.405)
Unclassified Rhodobacteraceae	...	0.030 (±0.011)	0.015 (±0.005)
Unclassified Acetobacteraceae	0.742 (±0.062)	0.344 (±0.045)	0.543 (±0.393)
Unclassified Gp 6	1.259 (±0.551)	8.967 (±2.200)	5.113 (±1.729)
Unclassified Sphingomonadaceae	0.005 (±0.005)	0.039 (±0.013)	0.022 (±0.009)
Unclassified Comamonadaceae	0.005 (±0.005)	0.103 (±0.036)	0.054 (±0.021)
Unclassified Acetobacteraceae	0.497 (±0.123)	0.060 (±0.014)	0.278 (±0.255)

^a Mean relative abundance based on percentage of recovered sequences; SE = standard error of the mean.

^b Taxonomic classification level was determined based on the RDP6 taxonomy outline; unclassified genera identities were based on the highest taxonomic level of resolution.

^c Indicates a significant difference between means of disease-conducive and -suppressive soil (CS and SS, respectively) based on Wilcoxon analysis; comparisons were statistically different at $\alpha = 0.05$.

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