Soil Suppressiveness to Fusarium Disease: Shifts in Root Microbiome Associated with Reduction of Pathogen Root Colonization

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ABSTRACT

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Soil suppressiveness to Fusarium disease was induced by incubating sandy soil with debris of wild rocket (WR; *Diptaxis tenuifolia*) under field conditions. We studied microbial dynamics in the roots of cucumber seedlings following transplantation into WR-amended or nonamended soil, as influenced by inoculation with *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. Disease symptoms initiated in nonamended soil 6 days after inoculation, compared with 14 days in WR-amended soil. Root infection by *F. oxysporum* f. sp. *radicis-cucumerinum* was quantified using real-time polymerase chain reaction (PCR). Target numbers were similar 3 days after inoculation for both WR-amended and nonamended soils, and were significantly lower (66%) 6 days after inoculation and transplanting into the suppressive (WR-amended) soil. This decrease in root colonization was correlated with a reduction in disease (60%) 21 days after inoculation and transplanting into the suppressive soil. Fungal community composition on cucumber roots was assessed using mass sequencing of fungal internal transcribed spacer gene fragments. Sequences related to *F. oxysporum*, *Fusarium* sp. 14005, *Chaetomium* sp. 15003, and

an unclassified Ascomycota composed 96% of the total fungal sequences in all samples. The relative abundances of these major groups were highly affected by root inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum*, with a 10-fold increase in *F. oxysporum* sequences, but were not affected by the WR amendment. Quantitative analysis and mass-sequencing methods indicated a qualitative shift in the root's bacterial community composition in suppressive soil, rather than a change in bacterial numbers. A sharp reduction in the size and root dominance of the *Massilia* population in suppressive soil was accompanied by a significant increase in the relative abundance of specific populations; namely, *Rhizobium*, *Bacillus*, *Paenibacillus*, and *Streptomyces* spp. Composition of the *Streptomyces* community shifted significantly, as determined by PCR denaturing gradient gel electrophoresis, resulting in an increase in the dominance of a specific population in suppressive soils after only 3 days. This shift was related mainly to the increase in *Streptomyces humidorius*, a group previously described as antagonistic to phytopathogenic fungi. Thus, suitable soil amendment resulted in a shift in the root's bacterial communities, and infection by a virulent pathogen was contained by the root microbiome, leading to a reduced disease rate.

Additional keyword: fingerprint.

Incorporation of certain organic amendments into soil may induce soil suppressiveness against soilborne as well as foliar pathogens. The process involves biological control mechanisms, which have the potential to persist in the treated soils for an extended period of time (33,41,47,64,66). Disease incidence or severity remains low in suppressive soils, even after repeated introduction of a pathogen (15).

The soil microbiome plays a significant role in natural and induced disease suppression. The possible mechanisms of induced soil suppressiveness include pathogen suppression (13,65), induced systemic resistance within the host (34,55,76), and microbial interactions which take place in the rhizosphere. The latter involve mechanisms such as competition for nutrients and antibiosis (44,58). The mechanisms of soil suppressiveness have been extensively studied by classical means, such as in culture and by direct root-microbe analysis (20,62).

Molecular methods are effective tools to characterize soil and rhizosphere microbiomes (8,9,43,46,49) for the purpose of identifying predictive microbial parameters for disease suppressiveness and potential antagonists that can be used as biological control agents (7,9,56,75). However, adoption of metagenomic ap-

proaches for soil microbial ecology have revealed the enormous complexity of the microbial interactions in such environments (5,67). Such a detailed and accurate characterization of the microbial interactions can indicate possible suppressive mechanisms and assist in identifying factors that enhance them.

Plant host, soil type, and physical and chemical environments are important factors in establishing the platform upon which microbial interactions that will exhibit soil suppressiveness occur (32). Available information clearly indicates that the microbial mechanisms of soil suppressiveness involve the activity of consortia of diverse microbial populations, rather than one specific organism population (44,49). Much effort has been invested in characterizing microorganisms and their genetic elements, which are related to disease suppression in the bulk and rhizosphere of suppressive soils (9,35,49,56,69). In contrast, less attention has been paid to microbial events at the site of the host-pathogen interaction—the root itself—during different stages of pathogen infection and disease development in disease-suppressive or -conductive soils (62,74). Early findings indicate that organisms are attracted to plant roots at early stages of seed germination and root development (4,12,52). Hence, a temporal analysis of the microbial interactions at different stages of pathogen infection is essential for understanding the mechanisms underlying soil-suppressive interactions. Data on soil microbial population do not necessarily reflect in the microbial situation in the root tissues (19). Highly sensitive methods for microbial ecology charac-

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terization, such as mass sequencing of 16S rRNA gene fragments, can increase the observational resolution of these microbial interactions in a pathosystem. In a previous work (33), we demonstrated that organic amendments, such as herb residues, induce suppressiveness in soil against *Fusarium* crown and root rot in cucumber. The objectives of the current study were to characterize the microbial events taking place in the root environment, during pathogen establishment, under conducive and suppressive soil conditions. For this purpose, we studied *Fusarium* crown and root rot establishment in cucumber roots after inoculation and at symptom appearance. The effect of induced soil suppression on the microbial colonization of roots was characterized by mass sequencing of 16S rRNA and internal transcribed spacer (ITS) gene fragments, qualitative analyses with polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), and quantitative examination of the root microbiome.

MATERIALS AND METHODS

Inducing soil suppressiveness with wild rocket residues. Soil suppressiveness to *Fusarium* wilt and crown rot of cucumber was induced as described previously (33). Briefly, ground dried leaves and stems of *Diplotaxis tenuifolia* (L.) DC. (wild rocket [WR]) were mixed with sandy soil from Rehovot (94% sand, 2% silt, 4% clay, and 0.12% organic matter, pH 7.9; field capacity of 9%) at a rate of 1% (wt/wt). The soil-WR mixture was wetted to water-holding capacity and was packed in a porous, woven plastic net bag (total 22 kg/bag). Nonamended soil was prepared and packed similarly, and served as the respective control. The bags containing the amended and nonamended wetted soils were buried in separate small field plots, mulched with a transparent polyethylene sheet, and additionally covered with a shading screen (90% shade; Polysak, Nir Itzhak, Israel) which was laid over the plastic mulch to minimize soil heating (soil temperature at a 20-cm depth reached a maximum 32 to 34°C in the shaded plots). After 28 days in the field, the soil bags were retrieved with their contents and brought to the laboratory. The soil was left to air dry at 25°C for 1 month prior to its use for assessing suppressiveness.

Soil suppressiveness to *Fusarium* crown and root rot in cucumber. *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Vakal., the causal agent of cucumber root and crown rot disease, was used for the pathosystem and suppressiveness tests. Cucumber (*Cucumis sativus* L. 'Kfir') seedlings were inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum* macroconidia obtained from the stem surface of diseased plants and transplanted in WR-

amended (suppressive) and nonamended (control) soil in pot experiments, as previously described (32). Disease symptoms in cucumber seedlings usually appear 7 days after inoculation and are manifested as plant collapse and wilt. Disease progress was expressed as percentage of diseased plants, determined by area under the disease progress curve (AUDPC). Soil suppressiveness was calculated as the reduction in disease incidence or severity in amended soil compared with the respective nonamended soil.

Root sampling. Root-microbiome analyses were conducted in cucumber transplants inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum*, planted in suppressive or control soils, and grown for 3 days (before symptom appearance) or 6 days (first appearance of light chlorosis of the leaves on inoculated control plants). On each date, cucumber transplants were removed with their entire root system from the pots (three plants per replicate, five replicates per treatment). The roots were cut and washed in 30 ml of sterile saline water, using orbital vortexing for 1 min in three intervals. The washed roots were weighed, transferred to 15-ml sterile tubes, and kept at -20°C until DNA extraction.

DNA extraction. Extraction of DNA was carried out using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) as described by the manufacturer. DNA concentrations and quality were determined with an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Real-time quantitative PCR. Real-time PCR was used for the quantification of total bacteria, plant plastid, *Massilia* spp., *Streptomyces* spp., *F. oxysporum* f. sp. *radicis-cucumerinum*, and the plant *tef* gene in root samples. The plant *tef* gene, encoding translation elongation factor 1, served for data normalization as proposed by Ruppel et al. (59). A primer pair targeting the plant plastid was applied to correct bacterial target numbers for plant plastids according to Ofek et al. (53). The primers used for the different assays are listed in Table 1.

To verify primer specificity, a plasmid standard containing the target region was generated for each primer set. For this purpose, PCR products for each primer pair were amplified from root DNA samples as template. The PCR-amplified products were examined by gel electrophoresis to confirm the specificity of the amplification. In addition, each of the products was cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) as instructed by the manufacturer. Plasmids were isolated and 10 randomly selected cloned inserts were sequenced from each primer set to confirm their identity and primer specificity. The specificity of *F. oxysporum* f. sp. *radicis-cucumerinum* primers, used following Lievens et al. (39), was also confirmed by PCR of DNA extracted from different pathogenic *Fusarium* strains, including *F. oxy-*

TABLE 1. Primers used in this study^y

Primer	Sequence	Gene	Target organisms	Reference
ITS1f	5'-CTTGGTCATTTAGAGGAAGTAA-3'	fungal rRNA ITS	Fungi	10
ITS2	5'-GCTGCGTCTTCATCGATGC-3'	fungal rRNA ITS	Fungi	10
530f	5'-GTGCCAGCMGCGGCGG-3'	16S rRNA	Bacteria	17
1100r	5'-GGGTTNCGNTCGTTG-3'	16S rRNA	Bacteria	17
341f ^z	5'-GCCTACGGGAGGCAGCAG-3'	16S rRNA	Bacteria	50
907r	5'-CCGTCAATTCMTTGTAGTTT-3'	16S rRNA	Bacteria	50
515f	5'-GTGCCAGCMGCCGCGGTAA-3'	16S rRNA	Universal	36
Plast_f	5'-GAGGCAATAGCTTACCAAGGCG-3'	16S rRNA	Plant plastid	53
Plast_r	5'-CTTGGTAGTTTCCACCGCCTG-3'	16S rRNA	Plant plastid	53
Strep661f	5'-GTAGGGGAGATCGGAATT-3'	16S rRNA	<i>Streptomyces</i>	29
Sterp1218r ^z	5'-AGCACGTGTGCAGCCCAA-3'	16S rRNA	<i>Streptomyces</i>	29
Oxalo225f	5'-GGGTTGGCGGCCCTCTG-3'	16S rRNA	Oxalobacteraceae	23
Mass656r	5'-TTCTAGCCTTGCAGTCTCCATC-3'	16S rRNA	<i>Massilia</i>	16
Tef_f	5'-ACTGTGAGTAGTACTTGGTG-3'	Translation elongation factor1	Plants	70
Tef_r	5'-AAGCTAGGAGGTATTGACAAG-3'	Translation elongation factor1	Plants	70
FORC F5	5'-TCGTACAAATGATTTCAGCAT-3'	RAPD SCAR marker	<i>Fusarium oxysporum</i> f. sp. <i>radicis-cucumerinum</i>	39
FORCR2	5'-GTGACGCAGGGTAGGCAT-3'	RAPD SCAR marker	<i>F. oxysporum</i> f. sp. <i>radicis-cucumerinum</i>	39

^y ITS = internal transcribed spacer, RAPD SCAR = random amplified polymorphic DNA sequence characterized amplified region.

^z For polymerase chain reaction denaturing gradient gel electrophoresis analysis, a 5'-GC-rich tail was added to the primer: CGCCCGCCGCGCCCGCGCGCCGTCGCCGCGCCCGCCCGCCG (49).

sporum f. sp. *melonis*, *F. oxysporum* f. sp. *radicis-lycopersici*, and *F. oxysporum* f. sp. *proliferatum*. Plasmid DNA concentrations were determined spectrophotometrically, copy numbers were calculated accordingly, and 10-fold dilution series within a range of 10^9 to 10 copies/ μ l were then prepared.

Real-time PCR assays were conducted in an Mx3000P QPCR System (Stratagene, La Jolla, CA) in 96-well polypropylene plates. Each well contained a 25- μ l mixture consisting of 12.5 μ l of Absolute Blue SYBR Green ROX Mix (Thermo Fisher Scientific, Surrey, UK), 1 μ l of each primer (10 μ M final concentration), 9.5 μ l of H₂O, and 1 μ l of template DNA. The PCR conditions were 15 min at 95°C, followed by 40 cycles of 95°C for 30 s, 60°C (or 58°C for the bacterial primer pair) for 30 s, and 72°C for 30 s. Melting-curve analysis of the PCR products was conducted following each assay to confirm specificity. The PCR products were also examined by agarose gel electrophoresis to confirm the specificity of amplification. Results were analyzed using MxPro QPCR Software analysis tools (Stratagene).

PCR-DGGE. *Streptomyces* spp.-specific population patterns were examined using the PCR-DGGE method. DNA extracted from different root samples served as the template for PCR amplification of *Streptomyces* 16S rRNA gene fragments using primers Strep661f and Strep1218r(GC) (Table 1), performed as previously described (29). DGGE was performed in 6% (wt/vol) acrylamide gels containing a linear urea-formamide gradient of 20 to 70% denaturant (with 100% defined as 7 M urea and 40% [vol/vol] formamide). Gels were run for 17 h at 100 V with the Dcode Universal Mutation System (Bio-Rad Laboratories, Hercules, CA). DNA was visualized after staining with Gelstar (Invitrogen) by UV transillumination (302 nm) and was photographed with a Kodak KDS digital camera. DGGE images were analyzed using Fingerprinting II software (Bio-Rad Laboratories), and an unweighted pair-group method with arithmetic means tree based on cosine correlation matrix was produced. Aligned densitometric curves were exported from the Fingerprint II software and multidimensional scaling analysis was performed based on 1-Pearson r distance matrix using STATISTICA software (version 7.1; Stat Soft Inc., Tulsa, OK). Specific bands were excised from the gels. Band DNA was reamplified by PCR and verified on another DGGE, analyzed, and cloned using the TOPO TA Cloning Kit. Plasmids were isolated and sequenced. Phylogenetic affiliation of the retrieved sequences was determined using ARB software (42) and National Center for Biotechnology Information (NCBI) Blast analyses (3).

Sequencing of 16S rRNA gene fragments. DNA extracted from the root samples was subjected to mass sequencing. Bacterial 16S rDNA and fungal ITS tag-encoded FLX amplicon pyrosequencing were performed by the Research and Testing Laboratory (Lubbock, TX), as described by Dowd et al. (17). Primers are listed in Table 1. The retrieved sequences were analyzed using MOTHUR (60). Five replicates were analyzed for each treatment at 3 and 6 days. For the root samples taken from inoculated WR-amended soil 3 days after transplantation, only four of the five replicates produced results in the fungal ITS assay (preparation of one sample library failed).

For fungal ITS, low-quality sequences and those shorter than 320 bases were omitted from further analyses. Then, suspected chimeras were detected using the MOTHUR chimera.check module ($\approx 10\%$ of the total sequences) and eliminated from further analysis. The sequences were aligned using ARB software (42) and the alignment was exported to MOTHUR, where a distance matrix was calculated using the MOTHUR dis.seqs module. Sequences were then classified into operational taxonomic units (OTUs) using a 99% sequence-similarity threshold. Representatives of each OTU were classified by NCBI Blast analysis.

From the bacterial sequences obtained, low-quality sequences and those shorter than 250 bases were omitted from further analyses, as were the suspected chimeras ($\approx 10\%$ of the total se-

quences), and eliminated from further analysis. Bacterial sequences were aligned using the Silva-compatible alignment database and a distance matrix was calculated using MOTHUR dis.seq module. Sequences were then classified into OTUs using a 97% sequence similarity threshold. Representatives of each OTU were classified with the MOTHUR classify.seqs module, and affiliation was verified by NCBI Blast analysis. Sequences were deposited in the GenBank SRA database under accession number SRA048248 (experiment SRX109547 for bacteria and experiment SRX109548 for fungal sequences).

Statistical analyses. Soil-suppressiveness experiments were carried out independently at least twice. Separate analyses of each experiment showed homogeneous variances of the experimental error between the replicates. All treatment means were compared simultaneously by Tukey's protected test for significant differences. Statistical analyses in the presented figures show data from one representative of two experiments. Analyses were performed with SAS software (release 8.01 for PC; SAS Institute, Cary, NC) at $P \leq 0.05$. Sample coverage was estimated according to Good (22). For each sample, the Chao1 richness estimate (11) and the dominance estimate were calculated. After classification into OTUs, the count data from the different samples were Hellinger transformed, to reduce bias related to differences in the number of sequences per sample (38). The compositions were compared by non-metric multidimensional scaling based on pairwise 1-Pearson r distance matrix using STATISTICA (version 7.1) software.

RESULTS

Cucumber crown and root rot progress in suppressive versus control soils. Soil amendment with WR induced soil suppressiveness, manifested as delayed and reduced seedling wilt (Fig. 1). Crown and root rot incidence in cucumber seedlings which were inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum* before transplanting in the WR-amended soil was significantly lower compared with nonamended soil (Fig. 1), thus confirming previous results (33). Disease symptoms (chlorosis) initiated in

Fig. 1. *Fusarium* crown and root rot incidence in inoculated cucumber transplants planted in wild rocket (WR)-amended soil or control, nonamended soil. Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml and transplanted in the soil. Means and standard deviations are presented ($n = 5$). Area under the disease progress curve in the WR-amended soil (129.3) was significantly different from that of control soil (1,019.3) according to Tukey's honestly significant difference test at $P < 0.0001$.

transplants in nonamended soil 6 days after inoculation and transplantation whereas, in WR-amended soil, first symptoms appeared only after 14 days.

Real-time PCR quantification of *F. oxysporum* f. sp. *radicis-cucumerinum* in the roots 3 days after inoculation (in plants with no expressed symptoms) showed similar target numbers for both WR-amended and nonamended soils (Fig. 2A), indicating similar inoculum density in the root and infection potential. In contrast, target numbers of *F. oxysporum* f. sp. *radicis-cucumerinum* were significantly lower (by 66%, $P < 0.01$) 6 days after inoculation and transplanting in the suppressive soil, compared with the non-amended one (Fig. 2B). No *F. oxysporum* f. sp. *radicis-cucumerinum* targets were detected in control, noninoculated plants.

Impact of root inoculation by *F. oxysporum* f. sp. *radicis-cucumerinum* and of transplantation in suppressive soil on the composition of root fungal community. Fungal colonization of cucumber roots following transplantation in the conducive or in the suppressive soils was assessed using mass sequencing. A set of 182,204 high-quality fungal ITS sequences was analyzed (Table 2). These sequences were grouped into 33 OTUs, using a 99% sequence-similarity threshold. The fungal community was characterized by low species diversification in roots of all treatments (Table 2). This was also demonstrated by the low number of OTUs which were represented by one (singletons) or two (doubletons) sequences in all the samples examined (Table 2). Non-metric multidimensional scaling analysis (NMDS) revealed a strong effect of root inoculation with *F. oxysporum* f. sp. *radicis-*

cucumerinum on fungal community composition in both suppressive and control soils, 3 and 6 days after inoculation and transplanting (Fig. 3). Whereas fungal communities in samples from inoculated roots were closely clustered, samples from noninoculated roots were scattered (regardless of the soil), indicating that *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation was the major factor affecting the composition of the root fungal community. Neither soil type nor day of sampling was indicated as an influential factor according to the NMDS analysis, regardless of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation (Fig. 3).

Of the 33 OTUs found, 13 OTUs included over 99.7% of the sequences. These OTUs were affiliated with *F. oxysporum*, *Fusarium* spp., *Chaetomium* sp., *Sodaria tomento-alba*, *Plectosphaerella* sp., *Alternaria* sp., *Thielaviopsis basicola*, and uncultured Ascomycota (Table 3). As expected, the relative abundance of sequences affiliated with *F. oxysporum* increased 10-fold following root inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum*, whereas the relative abundances of the other major groups (*Fusarium* sp. 14005 and *Chaetomium* sp. 15003) were reduced. Factorial analysis demonstrated that the relative abundances of these major groups, which were highly affected by root inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum* ($P < 0.001$), were not affected by WR amendment or day of sampling. The trend of reduction in relative abundance following *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation was similar for additional detected fungal OTUs (Table 3). A significant effect of WR amendment on relative abundance was

Fig. 2. Quantification of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) in roots of cucumber grown for **A**, 3 or **B**, 6 days in wild rocket-amended, suppressive soil (S) or control soil (C). Seedlings were inoculated with macroconidia of *F. oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml (+), or noninoculated, before transplanting. *F. oxysporum* f. sp. *radicis-cucumerinum* was quantified using a specific primer pair and normalized using the reference plant *tef* gene. ND: not detected. Different letters indicate significant differences between the means according to Kruskal-Wallis analysis of variance at $P < 0.01$.

TABLE 2. Analysis of fungal community structure based on mass sequencing of fungal internal transcribed spacer gene fragments^w

Day	Soil	<i>Fusarium</i> ^x	Σ Sequences ^y	Number of 99% OTUs ^z	Singletons	Doubletons
3	Control	–	9,112	9 ± 3	0.6 ± 0.5	1.0 ± 1
3	Suppressive	–	11,315	9 ± 3	1.2 ± 1.6	1.0 ± 0.7
3	Control	+	17,962	9 ± 2	1.4 ± 1.1	0.6 ± 0.5
3	Suppressive	+	19,989	7 ± 1	1.25 ± 1.5	0.75 ± 0.5
6	Control	–	17,071	14 ± 2	2.2 ± 0.8	1.2 ± 0.8
6	Suppressive	–	22,325	11 ± 2	1.4 ± 0.9	1.2 ± 1.1
6	Control	+	46,100	8 ± 2	2.2 ± 1.8	0.8 ± 1.3
6	Suppressive	+	38,330	7 ± 2	2.0 ± 2.0	0.2 ± 0.4

^w Coverage and diversity indices were calculated for the different samples after classification of the sequence data at the genus level. Means and standard deviations ($n = 5$) are presented.

^x Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml (+), or noninoculated (–), before transplanting.

^y Number of sequences. Coverage estimates, calculated after Good (21), were >99.9% for all samples. $C_{\text{Good}} = 1 - F1/N$, $F1$ = number of singletons, and N = number of sequences.

^z Operational taxonomic units (OTUs); classification using 99% similarity threshold.

observed for less-abundant OTUs ($P < 0.05$), where either WR-related reduction or increase were found.

Impact of root inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum* and transplantation in suppressive soil on root colonization by bacteria. Real-time quantitative PCR revealed no significant differences in the abundance of total bacteria associated with cucumber roots 3 and 6 days after transplantation in suppressive or control soils, regardless of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation (Table 4).

Fig. 3. Comparison of root-associated fungal community based on mass sequencing of ribosomal internal transcribed spacer by 454-pyrosequencing. Fungal community composition was determined for cucumber roots grown for 3 (open symbols) or 6 (filled symbols) days after inoculation with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml or noninoculation, and transplantation in ■, control soil; ▲, wild rocket-amended soil; ◆, control soil + *F. oxysporum* f. sp. *radicis-cucumerinum*; and ●, wild rocket-amended soil + *F. oxysporum* f. sp. *radicis-cucumerinum*. Sequences were classified into operational taxonomic units using a 99% similarity threshold. After Hellinger transformation of the count data, a 1-Pearson r distance matrix between the different samples was calculated. The matrix was used for nonmetric multidimensional scaling analysis by STATISTICA. The first and second dimensions are presented for the complete data set (Stress = 0.065).

Mass sequencing of bacterial 16S rRNA gene fragments showed a high diversity of bacteria associated with the roots. Based on a data set of 81,731 high-quality sequences, 3,490 OTUs were defined using a 97% sequence-similarity threshold, which were affiliated with 792 genera. Based on genus classification, root inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum* significantly increased the taxonomic richness (Chao1 estimate) and moderately reduced community dominance levels 3 days after transplantation in both nonamended and suppressive soils (Table 5). This effect continued to 6 days after transplantation in the non-amended soil. In contrast, a sharp decrease in the dominance levels of bacterial communities was evident 6 days after transplantation in the suppressive soil, regardless of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation (Table 5). Comparative analysis of root bacterial community compositions revealed divergence in root assemblages from nonamended and suppressive soils 6 days after transplantation (Fig. 4).

The effect of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation on bacterial community composition only became apparent 6 days after transplantation. Proteobacteria were the dominant bacterial phylum colonizing the cucumber roots (75 to 93% of the sequences in all treatments) (Table 6). Among the Proteobacteria, β -proteobacteria and α -proteobacteria were the most dominant (19 to 73% and 11 to 46%, respectively). Suppressive soil significantly altered the relative abundance of the dominant bacterial groups due to a reduction in *Massilia* spp. (β -proteobacteria), the single most dominant genus in the data set ($P < 0.05$), compared with the root-associated bacteria from nonamended soil (Table 6). After 6 days, the relative abundance of root-associated *Massilia* spp. was four to five times lower in the WR-amended, suppressive soil relative to nonamended soils. The relative abundance of *Massilia* spp. in the *F. oxysporum* f. sp. *radicis-cucumerinum*-inoculated roots was further reduced in the suppressive soil plants ($P < 0.05$). In contrast, the relative abundance of *Rhizobium* and *Devosia* spp. and other α -proteobacteria in the roots significantly increased in suppressive soil compared with the nonamended one ($P < 0.05$).

Quantitative analysis of root-associated *Massilia* spp. revealed one order of magnitude lower abundance on roots of suppressive soil, compared with nonamended soil, after 6 days of growth (Table 4). These quantitative real-time PCR results were consistent with those obtained by mass sequencing.

The relative abundance of root-associated actinobacteria, including *Streptomyces* spp., was significantly higher in suppressive

TABLE 3. Composition and relative abundance of fungi associated with cucumber roots, in control (C) and suppressive (S) soils, as affected by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* inoculation at 10^5 CFU/ml (+)^x

Taxonomy ^y	NCBI relative ^z	3 days				6 days			
		C	S	C+	S+	C	S	C+	S+
<i>F. oxysporum</i> (Hypocreales) a	JF440593; 99%	15.9	2.4	82.7	89.6	11.0	3.5	95.3	87.1
<i>Fusarium</i> sp. 14005 (Hypocreales) a	EU750680; 99%	40.8	64.2	2.5	1.9	51.8	71.7	4.3	10.0
<i>Fusarium</i> sp. (Hypocreales) a,b,d	AY729051; 99%	ND	2.3	ND	>0.1	0.3	3.8	>0.1	>0.1
<i>Thielaviopsis basicola</i> (Microascales)	GQ131878; 99%	1.9	>0.1	>0.1	>0.1	1.8	>0.1	>0.1	>0.1
<i>Chaetomium</i> sp. 15003 (Sordariales) a,c	EU750691; 98%	25.9	22.6	12.6	5.9	16.3	9.2	0.2	0.4
<i>Sordaria tomento-alba</i> (Sordariales)	AY681195; 100%	6.3	1.6	>0.1	>0.1	11.1	ND	ND	ND
<i>Plectosphaerella</i> sp. (Phyllachorales)	FJ196603; 99%	ND	ND	ND	>0.1	0.9	>0.1	>0.1	>0.1
<i>Alternaria</i> sp. (Pleosporales)	FJ210489; 100%	3.0	>0.1	>0.1	ND	0.2	>0.1	ND	ND
Uncultured Ascomycota a,b	EU437434; 99%	2.9	6.3	1.2	2.2	4.1	10.1	0.1	2.3
Uncultured endophytic fungus	FJ524302; 88%	ND	0.2	0.4	ND	>0.1	0.8	>0.1	>0.1
Uncultured Ascomycota a,b,d	EU489987; 93%	0.7	>0.1	>0.1	ND	1.3	>0.1	>0.1	>0.1
Uncultured Ascomycota	HM162273; 96%	0.6	ND	ND	ND	ND	ND	ND	ND
Unclassified fungus	GU078645; 94%	1.4	>0.1	>0.1	>0.1	0.3	0.5	>0.1	>0.1
Others	...	0.7	0.3	0.3	0.4	0.8	0.3	>0.1	>0.1

^x Composition was determined by mass sequencing of the fungal internal transcribed spacer genes. Data from seedlings 3 and 6 days after inoculation and transplantation is presented. Mean relative abundances of the dominant operational taxonomic units (99% similarity threshold) are presented. Symbols: + = *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation at 10^5 CFU/ml, C = control soil, S = suppressive soil, and ND = not detected.

^y For each indicated taxon, the mean relative abundances were compared by factorial analysis of variance followed by Tukey's honestly significant difference test. Letters indicate significant differences ($P < 0.05$) between the factors: *F. oxysporum* f. sp. *radicis-cucumerinum* (a), soil (b), day (c), *F. oxysporum* f. sp. *radicis-cucumerinum* $\times$ soil (d).

^z NCBI = National Center for Biotechnology Information.

sive versus nonamended soil, with no significant effect of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation (Table 6). Three days after transplantation, the number of *Streptomyces* spp., which was the dominant taxon in the actinobacteria, was significantly higher in the suppressive versus nonamended soil (Table 4). However, its quantity in the roots of *F. oxysporum* f. sp. *radicis-cucumerinum*-inoculated transplants decreased significantly after 6 days compared with the noninoculated transplants in the nonamended soil (Table 4).

The relative abundance of members of the Firmicutes, namely, *Bacillus* and *Paenibacillus* spp., also increased in the suppressive soils compared with the nonamended soils for both 3- and 6-day-old transplants.

Root infection with *F. oxysporum* f. sp. *radicis-cucumerinum* significantly affected the relative abundance of some bacterial taxons. For example, the genus *Mitsuaria* (β-proteobacteria) was detected almost exclusively in *F. oxysporum* f. sp. *radicis-cucumerinum*-inoculated nonamended soil and the relative abundance of *Methylophilus* spp. (β-proteobacteria) increased following *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation, regardless of soil type (Table 6).

Composition of root-associated *Streptomyces* spp. The relative abundance of root-associated *Streptomyces* spp. increased in the suppressive soils compared with the nonamended soils as early as 3 days post *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation and transplantation (Table 6). A significant shift in composition of the root-associated *Streptomyces* populations between control and suppressive soils was found 6 days after transplantation according to PCR-DGGE analysis (Fig. 5). In contrast, *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation had no visible effect on the community fingerprint, in either suppressive or nonamended soils. Similar results were found in roots 3 days after transplantation (Fig. 6). The difference between root *Streptomyces* community fingerprints in WR-amended and non-amended soils was mainly attributed to a dominant band (Figs. 5 and 6, marked with arrow) that appeared in all samples from WR-amended soil. This band also appeared in some samples from nonamended soil but, in most of them, at much lower intensity. Sequence analysis of this band was performed for eight excised bands representing all of the treatments. All obtained sequences were identical and were 99.9% similar to the *Streptomyces humifusus*-related population (HQ607425) according to NCBI Blast analysis.

DISCUSSION

Soil suppressiveness to root diseases evolves following amendment with WR and is sustainable, even after repeated inoculations with the pathogen in the plant–soil system (33). This suppressiveness was further verified and validated in the current study (Fig. 1). Disease suppression was maintained for 21 days after *F.*

oxysporum f. sp. *radicis-cucumerinum* inoculation of cucumber seedlings and transplanting into the WR-amended soil. Delayed disease onset and reduced symptom expression in the suppressive soil were noticeable as early as 6 days postinoculation. Therefore, we hypothesize that the impact of soil suppressiveness on root infection by *F. oxysporum* f. sp. *radicis-cucumerinum* and disease development begins after root inoculation by the pathogen. This impact may involve direct suppression of fungal survival and growth, or an indirect effect via shifts in the microbial population and assembly in the rhizosphere and roots.

Quantitative assessment of *F. oxysporum* f. sp. *radicis-cucumerinum* in the cucumber roots indicated that the initial infection units of the pathogen were not affected by the suppressive soil 3 days after seedling inoculation and transplanting (Fig. 2). However, after an additional 3 days, the buildup of pathogen population in the roots of plants in control, nonamended soil was

Fig. 4. Comparison of the bacterial community composition on roots based on mass sequencing of bacterial 16S rRNA gene fragments. Bacterial community composition was determined for cucumber roots grown for 3 (open symbols) or 6 (filled symbols) days after inoculation with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10⁵ CFU/ml, or noninoculation, and transplantation in ■, control soil; ▲, suppressive soil; ○, control soil + *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation; and ●, suppressive soil + *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation. Sequences were classified into operational taxonomic units using a 97% similarity threshold. After Hellinger transformation of the count data, a 1-Pearson *r* distance matrix between the different samples was calculated. The matrix was used for nonmetric multidimensional scaling analysis by STATISTICA (Stress = 0.038). The first and second dimensions are presented.

TABLE 4. Quantification of total bacteria (*Streptomyces* and *Massilia* spp.) on cucumber roots 3 and 6 days after inoculation with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*Fusarium*) and transplantation^a

Day	Soil	<i>Fusarium</i>	Log targets/plant <i>tef</i>		
			Total bacteria	<i>Streptomyces</i>	<i>Massilia</i>
3	Control	–	2.14 ± 0.16	–0.18 ± 0.18 b	1.40 ± 0.20
3	Suppressive	–	2.20 ± 0.29	0.20 ± 0.22 a	1.09 ± 0.25
3	Control	+	1.96 ± 0.37	0.08 ± 0.11 ab	1.41 ± 0.43
3	Suppressive	+	1.88 ± 0.78	0.28 ± 0.29 a	0.96 ± 0.79
6	Control	–	2.25 ± 0.79	1.79 ± 0.44 a	2.20 ± 0.45 a
6	Suppressive	–	2.24 ± 0.16	1.42 ± 0.13 ab	0.78 ± 0.36 b
6	Control	+	2.54 ± 0.14	1.53 ± 0.22 ab	2.06 ± 0.23 a
6	Suppressive	+	2.36 ± 0.19	1.23 ± 0.09 b	0.58 ± 0.45 b

^a Quantification based on real-time quantitative polymerase chain reaction using the plant *tef* gene for normalization of the data. The means (*n* = 5) are presented. Means were compared by factorial analysis of variance. Only statistically significant sources and interactions are indicated. Different letters in a column indicate significant differences at *P* < 0.05.

TABLE 5. Analysis of bacterial community structure based on mass sequencing of 16S rRNA gene fragments^q

Day	Soil	<i>Fusarium</i> ^r	Σ Sequences ^s	97% OTUs ^t	Genera	Coverage ^{u,v}	Diversity indices ^w		
							Chao1 ^{v,x}	Dominance ^y	H' ^z
3	Control	–	11,260	524 ± 54	116 ± 16	0.979	173	0.285	2.38
3	Suppressive	–	7,967	466 ± 63	122 ± 16	0.968	189	0.205	2.79
3	Control	+	15,300	628 ± 99	168 ± 26	0.979	239	0.185	2.94
3	Suppressive	+	11,872	472 ± 36	145 ± 17	0.977	200	0.129	3.13
6	Control	–	12,246	572 ± 54	158 ± 13	0.975	222	0.266	2.67
6	Suppressive	–	5,917	429 ± 76	135 ± 10	0.974	206	0.071	3.52
6	Control	+	10,637	499 ± 78	142 ± 22	0.975	206	0.155	3.06
6	Suppressive	+	6,496	415 ± 36	137 ± 10	0.958	216	0.059	3.66

^q Coverage and diversity indices were calculated for the different samples after classification of the sequence data at the genus level. Means and standard deviations ($n = 5$) are presented for coverage, richness, dominance, and Shannon H' diversity estimators.

^r Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10⁵ CFU/ml (+), or noninoculated (–), before transplanting.

^s Number of sequences.

^t OTUs = operational taxonomic units.

^u Sample coverage estimate calculated after Good (22): $C_{\text{Good}} = 1 - F1/N$; F1 = number of singletons and N = number of sequences.

^v No significant differences according to analysis of variance and Tukey's honestly significant difference test ($P = 0.05$).

^w Chao1 taxa richness estimate (11).

^x Neuman-Keuls critical range: Chao1 = 43, Dominance = 0.071, and H' = 0.37.

^y Dominance $D = \Sigma[(n_i/N)^2]$; n_i = number of sequences assigned to the i th genus.

^z Shannon H' index of diversity $H' = -\Sigma p_i \times \ln(p_i)$; p_i = relative abundance of the i th genus.

TABLE 6. Composition and relative abundance (%) of the major taxonomic groups identified in cucumber root bacterial communities by mass sequencing of 16S rRNA gene fragments by 454-pyrosequencing^x

Taxonomy ^y	3 days				6 days			
	Null		<i>Fusarium</i>		Null		<i>Fusarium</i>	
	C	S	C	S	C	S	C	S
Proteobacteria	93.4	85.6	90.2	86.5	89.2	74.8	86.2	76.7
β-Proteobacteria	69.0	56.7	64.3	45.2	73.3	21.9	61.3	19.3
<i>Massilia</i> a,b,c,d	51.9	42.5	41.0	28.3	50.7	12.5	34.3	6.7
<i>Janthinobacterium</i> a,b,d	1.7	0.8	2.1	0.3	4.2	0.3	4.5	0.5
<i>Methylophilus</i> c	1.6	1.8	2.5	2.8	1.5	0.9	3.2	4.0
<i>Methylobacillus</i> a,b,d	ND	0.2	ND	0.4	<0.1	1.64	ND	1.6
<i>Mitsuaria</i> b,c,e	ND	ND	1.6	<0.1	ND	ND	3.1	ND
<i>Thauera</i> a,b,c,d,f,g	0.1	0.3	0.1	0.2	<0.1	0.2	0.7	1.5
α-Proteobacteria	20.8	23.3	21.1	33.3	10.7	43.2	19.6	45.8
<i>Rhizobium</i> a,b,d	7.2	9.8	6.0	10.3	4.3	17.1	7.0	16.6
<i>Brevundimonas</i> b,c,g	4.2	2.9	3.4	6.1	0.9	4.4	4.2	6.1
<i>Devosia</i> a,b,d	0.7	2.3	0.6	2.4	0.3	6.0	0.3	4.9
<i>Shinella</i> genera a,b,d	0.4	1.5	0.5	0.9	0.3	4.5	0.4	5.3
<i>Mesorhizobium</i> b,d	1.3	1.5	1.1	1.4	0.9	1.9	0.8	1.9
<i>Sphingopyxis</i> a,b,d	0.1	0.4	<0.1	0.3	<0.1	1.3	0.2	1.5
<i>Phenylobacterium</i> b,c	0.2	0.3	0.4	0.7	0.2	0.7	0.4	1.0
γ-Proteobacteria	3.5	5.4	4.8	7.7	4.7	9.5	5.2	11.5
<i>Pseudomonas</i>	1.9	2.0	2.7	3.2	1.9	1.6	2.4	3.4
<i>Cellvibrio</i>	<0.1	0.1	<0.1	0.4	0.2	2.8	<0.1	2.3
<i>Colwellia</i> a,b,d	0.2	0.6	0.2	0.6	<0.1	1.5	0.2	1.8
<i>Stenotrophomonas</i> c,f	0.2	0.3	0.1	0.4	0.2	0.2	0.7	1.1
Bacteroidetes	2.5	2.8	4.7	2.7	4.7	4.2	10.8	5.6
<i>Chryseobacterium</i> b,c,e	0.1	<0.1	0.7	<0.1	0.2	ND	5.5	ND
Chitinophagaceae a,b	1.7	0.7	2.3	0.8	3.4	1.0	4.6	2.0
<i>Flavisolibacter</i> a,b,d	0.1	<0.1	0.2	<0.1	1.2	0.3	0.8	0.4
<i>Sphingobacterium</i> b	0.5	0.9	0.9	0.8	0.4	1.1	0.3	1.0
Actinobacteria	2.6	4.6	3.4	4.3	3.5	9.6	1.4	4.8
<i>Streptomyces</i> a,b,d	0.4	2.0	1.2	2.4	1.9	6.2	0.6	2.6
Firmicutes	1.1	5.7	1.4	4.3	2.1	8.5	1.4	9.7
<i>Bacillus</i> b	<0.1	1.53	<0.1	1.2	ND	1.0	ND	1.0
<i>Paenibacillus</i> a,b,d	0.5	2.0	0.8	1.8	1.6	5.9	0.7	7.6
Planococcaceae b	<0.1	1.1	<0.1	0.5	ND	0.9	ND	0.7
Other phyla ^z	0.4	1.5	0.3	2.5	1.1	3.2	0.3	3.2

^x Noninoculated (Null) and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*Fusarium*) inoculation at 10⁵ CFU/ml; C = control soil and S = suppressive soil. ND = not detected.

^y For each indicated taxon, the mean relative abundances were compared by factorial analysis of variance followed by Tukey's honestly significant difference test. Letters indicate significant differences ($P < 0.05$) between the factors day (a), soil (b), *F. oxysporum* f. sp. *radicis-cucumerinum* (c), day × soil (d), soil × *F. oxysporum* f. sp. *radicis-cucumerinum* (e), day × *F. oxysporum* f. sp. *radicis-cucumerinum* (f), and day × soil × *F. oxysporum* f. sp. *radicis-cucumerinum* (g).

^z Combination of phyla in which the total taxonomic groups detected had relative abundance <1%.

three times higher than in the suppressive soil. This suggests that the suppressive soil itself does not initially directly reduce the number of pathogen propagules or their capacity to colonize the roots. It also emphasizes that disease suppressiveness is associated with the root zone, with the presence of both pathogen and host, under suppressive conditions (58,61).

Soil suppressiveness to root diseases may result from specific antagonism to the pathogen. For example, soils that are naturally suppressive to *Fusarium* wilt have been well documented (64,71): they are characterized by competition for niche and nutrients that is closely related to saprophytic, nonpathogenic species of *Fusarium* (71) and the activity of native protective *F. oxysporum* strains and *Pseudomonas* spp. (2,18). In the roots of cucumber transplants, native *Fusarium* spp. constituted the dominant root fungal population in all tested soils, regardless of previous soil treatment (suppressive or not) or transplant inoculation with *F. oxysporum* f. sp. *radicis-cucumerinum* (Table 3). This dominance of saprophytic *Fusarium* spp. did not, however, interfere with soil or root receptivity to *F. oxysporum* f. sp. *radicis-cucumerinum*, as reflected by its pronounced colonization of the roots and disease severity of plants in the nonamended soil (Fig. 1). Moreover, the root-associated fungal community composition was not affected by the suppressive soil during the time intervals examined ($P > 0.05$). However, *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation increased the relative abundance of *F. oxysporum* in roots from 3 to 14 to 88 to 89% in WR-amended as well as nonamended soils (Fig. 3; Table 3). Hence, mechanisms other than the presence of protective nonpathogenic *Fusarium* spp. play a more important role in disease suppressiveness in WR-amended soil.

Fig. 5. Community fingerprint of root *Streptomyces* spp. in cucumber seedlings 6 days after transplantation into suppressive soil (S) compared with control soil (C). Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml (+), or noninoculated before planting. *Streptomyces* community compositions were compared based on polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) patterns of 16S rRNA gene fragments amplified with a *Streptomyces*-specific primer pair. PCR-DGGE patterns were analyzed using Fingerprint II software. An unweighted pair-group method with arithmetic means tree was calculated from cosine correlation distance matrix. The arrow indicates bands corresponding to *Streptomyces* populations identified by sequence analysis of excised bands.

The evolution of soil suppressiveness has been related to shifts in the soil- and root-associated microbiome (18,47). In many cases, soil suppressiveness is biologically derived from shifts in the bacterial balance in the rhizosphere, including its density and functional diversity (41,58,75). Indeed, the composition of the fungal community in the root zone was not affected in the suppressive soils during our study ($P > 0.05$). Moreover, it has been suggested that consortia of microorganisms and mechanisms, rather than a single group, are involved in disease suppressiveness (44,49,63). Therefore, the WR-mediated suppression of *F. oxysporum* f. sp. *radicis-cucumerinum* development observed in infected roots 6 days post inoculation seems to be associated with changes in root bacterial community properties.

Quantitative analysis and mass-sequencing methods indicated that the shift in bacterial communities in suppressive soil is qualitative (i.e., a shift in the composition of the bacterial community rather than in the total number of bacteria) (Table 4; Fig. 4). Previous studies have positively correlated density of cultivable heterotrophic bacterial populations with soil suppressiveness (7). Our finding, however, is based on a quantitative assessment that was not affected by the bias of cultivable heterotrophy; therefore, the suppression mechanism may stem from the interaction with more groups of microorganisms.

Bacterial community composition and structure in the rhizosphere and root have been previously associated with suppressiveness (6,9,25). In our study, an increase in diversity of the root bacterial communities was evident in the suppressive soil (Table 5). However, an increase in the diversity of the bacterial community also characterized roots as a response to *F. oxysporum* f. sp. *radicis-cucumerinum* inoculation, prior to the appearance of disease symptoms (3 days). Postma et al. (57) found that quali-

Fig. 6. Community fingerprint of root *Streptomyces* spp. in cucumber seedlings 3 days after transplantation into suppressive soil (S) compared with control soil (C). Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU/ml (+), or noninoculated before planting. *Streptomyces* community compositions were compared based on polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) patterns of 16S rRNA gene fragments amplified with a *Streptomyces*-specific primer pair. PCR-DGGE patterns were analyzed using the Fingerprint II software. An unweighted pair-group method with arithmetic means tree was calculated from cosine correlation distance matrix. The arrow indicates bands corresponding to *Streptomyces* populations identified by sequence analysis of excised bands.

tative rather than quantitative shifts in the bacterial community correlate with disease suppressiveness. Moreover, disease suppression has been correlated with increased soil (21,43,68) and endophytic (62) bacterial diversity. Yang et al. (74) also reported increased bacterial diversity on *Phytophthora* spp.-infected avocado roots compared with healthy ones. Therefore, it is still unknown whether an increase in root community diversity is, in itself, indicative of a suppressive process.

A sharp reduction in the population of *Massilia* spp. (β -proteobacteria) in the root bacterial community (Tables 4 and 6) was the key determinant of the observed structural changes in root bacterial communities in suppressive soil (Table 5). Members of this genus, first isolated from clinical samples, are characterized as gram-negative, aerobic, flagellated, and non-spore-forming rods (30,37). *Massilia* spp. have been detected in the rhizosphere of a variety of plant species (1,14,24,27), including cucumber (23,53,54). *Massilia* spp. have been found to exhibit exceptional dominance in cucumber root-associated communities at early stages of plant development and to be sensitive to increased microbial competition (27,53).

The reduction in the *Massilia* population's size and root dominance in the suppressive soil was accompanied by significant changes in the relative abundance of additional bacteria (Table 6). Most notable was the increase in relative abundance of specific populations, namely the genera *Rhizobium*, *Bacillus*, *Paenibacillus*, and *Streptomyces*, members of which are frequently linked to plant health, biological control, and disease suppression (9,31,34,47,49,51,56,63,73,75). Among these, the genus *Streptomyces* seems to be an important component in the disease-suppression process in the cucumber-*F. oxysporum* f. sp. *radicis-cucumerinum* system (26,63). The composition of the *Streptomyces* community, determined by PCR-DGGE examination, shifted significantly, resulting in a strong increase in the dominance of a specific population in suppressive soils after 3 days (Fig. 6) and on day 6 (Fig. 5). This shift was related mainly to an *S. humidorum*-related population (99.9% similarity according to sequence analysis of excised bands). Reports have indicated that some strains of *S. humidorum* are antagonistic to different phytopathogenic fungi (40), and suppress *Phytophthora capsici* in vivo through direct antibiosis (phenylacetic acid and sodium phenylacetate) (28).

In naturally developed suppressive soils, abundance of the native antagonistic microorganisms may increase in the presence of a particular pathogen (45,71,72). Therefore, the increase in populations of potential antagonists, such as *Streptomyces* spp., in suppressive soil is restricted to specific pathosystems. However, in the system studied here, in which suppressiveness was induced, the presence of *F. oxysporum* f. sp. *radicis-cucumerinum* triggered little change in the root bacterial community (Table 5). Acquisition of *Pythium* spp.-suppressive microbial consortia by cucumber seed in suppressive compost has been shown to be independent of the pathogen's presence (48,54). It is possible that the change in root bacterial composition and the increased diversity could result in enhancement of competition for nutrients or in specific antagonism which may take place in the root zone following the pathogenic infection.

Manipulation of soil microbiota to create soil suppressiveness has more chances of success than the introduction of a biocontrol agent into a hostile and competitive environment. In this study, generating an appropriate soil environment with organic amendments resulted in a shift in root bacterial communities. Under the new microbial equilibrium, root infection by a virulent pathogen was contained by the root microbiome and disease severity was reduced.

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