ELSEVIER

FEMS Microbiology Ecology 35 (2001) 129–136

FEMS MICROBIOLOGY Ecology

www.fems-microbiology.org

16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and Phytophthora infected avocado roots

C.-H. Yang [b], D.E. Crowley [a,*], J.A. Menge [b]

[a] *Department of Environmental Sciences, University of California, Riverside, CA 92521, USA*
[b] *Department of Plant Pathology, University of California, Riverside, CA 92521, USA*

Received 21 April 2000; received in revised form 30 November 2000; accepted 30 November 2000

Abstract

Molecular techniques employing 16S rDNA profiles generated by PCR-DGGE were used to detect changes in bacterial community structures of the rhizosphere of avocado trees during infection by *Phytophthora cinnamomi* and during repeated bioaugmentation with a disease suppressive fluorescent pseudomonad. When the 16S rDNA profiles were analyzed by multivariate analysis procedures, distinct microbial communities were shown to occur on healthy and infected roots. Bacterial communities from healthy roots were represented by simple DNA banding profiles, suggestive of colonization by a few predominant species, and were approximately 80% similar in structure. In contrast, roots that were infected with Phytophthora, but which did not yet show visible symptoms of disease, were colonized by much more variable bacterial communities that had significantly different community structures from those of healthy roots. Root samples from trees receiving repeated applications of the disease suppressive bacterium *Pseudomonas fluorescens* st. 513 were free of *Phytophthora* infection, and had bacterial community structures that were similar to those of nontreated healthy roots. Sequence analysis of clones generated from four predominant bands cut from the DGGE gels revealed the presence of pseudomonads, as well as several previously unidentified bacteria. Differentiation of 16S rDNA profiles for healthy and infected roots suggests that rhizosphere bacterial community structure may serve as an integrative indicator of changes in chemical and biological conditions in the plant rhizosphere during the infection process.

Keywords: Plant disease; Disease suppression; Biocontrol; Bioaugmentation; DGGE

1. Introduction

Biocontrol of plant root diseases is predicated on the idea that the rhizosphere microflora can be manipulated to promote natural disease suppression, or that soils can be augmented with disease suppressive bacteria that are directly antagonistic to root pathogens [1,2]. In this manner, general disease suppression may involve competition by nonpathogenic bacteria and fungi that compete for the same growth substrates used by the pathogen. Specific bacterial strains and fungi may also promote stasis of the disease by creating an environment that is hostile for growth and survival of the pathogen. During the past few decades, this latter phenomenon has been studied particularly with respect to antibiotic and siderophore production [3], with more recent studies focusing on the role of quorum sensors in controlling the release of antibiotic substances [4].

The ability to promote and monitor disease suppressive microflora in the rhizosphere is presently hindered by lack of knowledge of rhizosphere microbial ecology and a restricted ability to identify and characterize microbial communities that are associated with healthy and infected roots. A variety of new molecular methods are available for assessing changes in community structure based on comparisons of 16S rDNA profiles [5], but have not yet been applied for this purpose. A specific advantage of these techniques is that they permit analysis of both culturable and nonculturable bacteria, and provide a rapid method for observing changes in community structure in response to different environmental factors [6].

The extent to which the general suppression by rhizosphere microbial communities actually controls disease versus the importance of specific microbial populations is

* Corresponding author. Tel.: +1 (909) 787-3785; Fax: +1 (909) 787-3993; E-mail: crowley@mail.ucr.edu

0168-6496/01/$20.00
PII: S0168-6496(00)00120-3

still a matter of speculation. Specific suppression has been shown for various strains of fluorescent pseudomonads that produce antibiotics, and field experiments have shown that the presence of certain pseudomonads above a threshold population density of 10^5 CFU g^{-1} soil can suppress *Gaeumannomyces graminis*, the causative agent of take all of wheat. In this case, disease suppression is particularly associated with strains that produce the antibiotic 2,4-diacetylchloroglucinol [7,8]. Similarly, soil or seed inoculation with *Pseudomonas cepacia* has been shown to be effective for control of *Rhizoctonia* root rot on radish [9]. Other research examining a variety of bacteria has shown that biocontrol of Phytophthora crown rot of strawberry is achieved using isolates of *Agrobacterium*, *Bacillus*, *Enterobacter* and *Pseudomonas*, with certain *Bacillus* strains being the most effective [10]. The latter study suggests that broad groups of bacteria may act in concert to help suppress root pathogens. Given the range of synergistic to detrimental interactions that might occur between soil inoculants and indigenous bacteria, there has been considerable interest in monitoring changes in rhizosphere communities during bioaugmentation with disease suppressive microorganisms [11,12]. Molecular community analysis techniques are ideally suited for this purpose.

In the research reported here, 16S rDNA community analysis techniques were applied to examine changes in the rhizosphere of avocado trees being treated with a biocontrol agent in an orchard setting. Avocados are an economically important tree fruit crop in many parts of the world, but are severely affected by *Phytophthora cinnamomi* [13,14]. This pathogen, which is only partially controlled by chemical treatments, primarily infects the feeder roots and root axes, which turn black and brittle, leading to death of the root apices distal from the infection point. Despite intensive efforts to control the disease, the pathogen has become widespread. In California, Phytophthora root rot now affects 75% of all avocado groves [13]. Ongoing studies are investigating the use of soil inoculants and mulch treatments to suppress the pathogen. In the most recent work, soil inoculants are applied in the irrigation water at approximately 10^5 CFU ml^{-1}, using an automated fermenter that cultures and injects bacteria into the irrigation water, along with substances contained in the spent medium.

To examine differences in rhizosphere bacterial community structure of healthy and Phytophthora infected avocado root tips, analyses were conducted using PCR-DGGE of 16S rDNA sampled from rhizosphere soil adhering to individual root tips. Rhizosphere communities were also examined for trees that had been continuously treated with the biocontrol agent, *Pseudomonas fluorescens* st. 513, as a soil inoculant. All of the root tips that were sampled were symptomless at the time they were collected so that communities associated with the roots during the early onset of the disease could be examined.

2. Materials and methods

2.1. Avocado root sampling

Avocado roots were sampled in August 1998 from a 30-year-old orchard located at Pomerado, San Diego, CA, USA. The experimental site was naturally infected with Phytophthora root rot and included an area that had been established as a treatment plot for a biocontrol experiment using continuous application of *P. fluorescens* st. 513 through the irrigation system. Roots were collected both in the nontreated control area and from trees that had received the biocontrol agent once a week for the preceding 4 months. The bacteria were delivered through the irrigation water using an automated 400L fermenter (BioJect, EcoSoil Systems Inc. San Diego, CA, USA) that injected the bacteria into the irrigation water applied with a minidrip sprinkler system. The cells were grown to late log phase to a density of approximately 10^8 cells per ml, and were applied at an inoculum density of 10^5 cells ml^{-1} water after dilution in the irrigation water. Twelve individual trees were sampled, including four from the biocontrol treated area, four from obviously diseased trees and four from healthy trees with no visible symptoms. Fresh roots were collected from avocado trees with a distance of 1 m away from the trunk and 10 cm deep from the soil surface. The root samples were gently shaken to remove loosely adhering soil and aseptically transferred to storage bags. The samples were maintained on ice and transported back to the laboratory where they were processed for assay of their infection status within 12 h.

Root samples consisting of 0.5-cm long root tips were dissected from the field samples and sliced longitudinally using sterile razor blades. One half of each root tip was placed onto modified PARPH selective medium [15] containing 75 mg l^{-1} hymexazol for detection of Phytophthora root rot caused by *P. cinnamomi*. The other half of the root tip was then placed into a numbered vial, corresponding to the sample used for disease analysis, and was stored at −70°C for future processing for molecular fingerprinting of 16S rDNA. A total of 240 root samples were examined, of which three random samples from each tree or 36 total samples were selected for microbial community analysis.

2.2. Bacterial community DNA extraction

For lysis of rhizosphere bacterial cells, root samples containing rhizosphere soil were placed into a beadbeater tube (BIO 101 Vista, CA, USA) and shaken in a Fastprep™ FP120 beater (BIO101) at 5.5 m/s for 30 s. Total DNA from the soil was isolated with a fast DNA kit from BIO 101, Inc. as described using the manufacturer's protocols (BIO 101, Inc., 1070 Joshua Way, Vista, CA 92038, USA).

2.2.1. DGGE analysis

Primers PRBA338f (5′-ACTCCTACGGGAGGCAG-CAG-3′) and PRUN518r (5′-ATTACCGCGGCTGCT-GG-3′) were used for eubacterial community amplification [16]. PRBA338f is located at the V3 region of 16S rRNA genes from bacterioplankton conserved among the domain *Bacteria*. A GC clamp (5′-CGCCCGCCGCGCGCG-GCGGGCGGGGCGGGGGCACGGGGGG-3′) was attached to the 5′ end of the PBRA338f primer. PCR amplification of the 16S rDNA fragments was conducted with 5 pmol of primers using ready-to-go PCR beads (Pharmacia Biotech), with a final volume of 25 μl. The PCR cycles used for amplification were as follows: 1 cycle at 94°C for 5 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final extension cycle at 72°C for 6 min.

DGGE gels were made from 8% (wt/vol) acrylamide stock solutions (acrylamide/bisacrylamide solution 37.5:1) containing 0 and 100% denaturant (7 M urea and 40% (vol/vol) formamide). DGGE was performed with acrylamide gels containing a linear chemical gradient ranging from 20 to 70% denaturant. The gels were run for 3.5 h at 200 V with a DCodeTM universal mutation detection system from Bio-Rad Laboratories (Hercules, CA, USA), and were then stained in ethidium bromide and photographed.

2.2.2. Statistical analyses

Microbial community structures associated with healthy and Phytophthora infected roots were analyzed by comparisons of 16S rDNA fingerprints of communities associated with healthy and infected roots collected from 12 avocado trees, including four diseased, four healthy, and four biocontrol treated trees. Two DGGE gels were run simultaneously with samples of 12 infected and 12 healthy roots from nontreated trees, and 12 healthy root tips from trees that had been treated with the biocontrol agent (36 samples total).

16S rDNA band profiles obtained from photographs of the DGGE gels were converted into computer digital images using an image scanner. Individual lanes of the gel images were straightened and aligned using Adobe Photoshop (Adobe Systems, Inc. Mountain View, CA, USA). The DGGE images were then transformed into line plot profiles using Scion Image (Scion Corp, Frederick, MD, USA). The 16S rDNA band profiles from each lane were then imported into Excel files and analyzed using peak analysis software to resolve individual peaks and quantify the 16S rDNA band intensities (PeakFit, SPSS, Inc.). Baselines were subtracted from each line profile using the AutoFit 2nd Deriv Zero routine with the best fit option. This baseline correction assumes baseline points tend to exist where the second derivative of the data is both constant and zero. After baseline correction, the peaks were resolved with a deconvolution curve fit, which defines a visible peak as one that produces a local maximum in the input data. In the deconvolution option, hidden peaks are detected by the 'sharpening' achieved by Gaussian deconvolution of the raw data. A standard peak width is assigned to all peaks using the default parameter 'full width at half maximum' that is utilized for fitting Gaussian curves to the peaks.

These peak analysis data were analyzed using correspondence analysis (CANOCO 4.0, Microcomputer Power, Ithaca, NY, USA) to generate ordination diagrams. Community similarities were visualized using ordination biplots scaled with a focus on intersample difference [17]. This type of diagram allows interpretation of the distances between centroid points for individual samples. Further statistical analyses were conducted using factor analysis to reduce the number of variables (16S rDNA bands) to a set of factors that described most of the variation in the data set (Minitab Inc., State College, PA, USA). Significant differences between bacterial communities between biocontrol treated, healthy (nontreated) and infected (nontreated) roots were then determined by analysis of variance using the first two factors, which described most of the variation in the data set. Additional independent analyses included cluster analysis and discriminate analysis to examine the percent similarity between bacterial communities associated with different trees and between healthy, infected, and biocontrol treated roots. Similarities in community structure quantified by cluster analysis were determined using the single linkage method with the Euclidean distance measure for determination of differences between clusters.

2.2.3. Bacterial identification

Ribosomal DNA bands separated from DGGE gels were excised and placed into sterilized vials. Twenty microliters of sterilized distilled water were added to each of the vials, which were then kept at 4°C overnight to allow the DNA to passively diffuse out from the gel strips. Using primers PRBA338f and PRUN518r (with the GC clamp attached to the 5′ end of the PRBA338f), 10 μl of eluted rDNA was used as a DNA template for PCR with conditions described above. The sizes of the PCR products were checked with an agarose gel and the DNAs were then cloned into pGEM-T easy vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* JM109. Isolation of plasmids from *E. coli* was performed using standard protocols from QIAprep Miniprep kit (Valencia, CA, USA). The purified plasmids were sequenced with a Li-COR (Lincoln, N.B., Canada) model 4000 L automatic sequencing system. The sequencing reaction was carried out by cycle sequencing with a SequiTherm Excel II long-read DNA sequencing kit-LC (Epicentre, Madison, WI, USA). Sequence analyses were made using the BLAST database (National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov). Overall similarities in 16S rDNA sequences to previously described

bacteria were determined using the programs PRETTY (Genetics Computer Group, Oxford Molecular Company, Madison, WI, USA). All the plasmid clones sequenced were further reamplified with primers PBRA338f and PRUN518r containing GC clamp. The relative migration distances between the PCR DNAs and the DGGE bands that previously sliced out were compared in a second DGGE gel.

3. Results

3.1. Root infection analysis

The infected roots showed no visible symptoms of Phytophthora root rot at the time of sampling, but were shown to carry the pathogen by plating a longitudinal section of each root on a selective medium. *P. cinnamomi* was identified by its typical fungal colony morphology after outgrowth from the root tips. Three replicate samples of root tips from four healthy trees, four biocontrol treated trees, and four Phytophthora infected avocado trees were collected for community DNA extraction. A total of 120 root tip samples were examined for Phytophthora infection from the *P. fluorescens* st. 513 treated trees and none of the root tips were found to be infected with the pathogen.

3.2. 16S rDNA community analysis

16S rDNA community profiles generated by PCR-DGGE revealed the presence of 12 to 18 bands for individual avocado roots from healthy and infected trees (Fig. 1). When analyzed on gels comparing replicate roots from individual trees, highly similar profiles were observed for

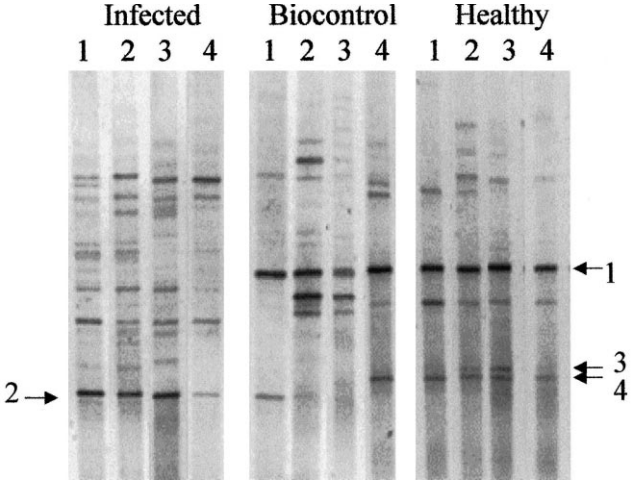

Fig. 1. PCR-DGGE 16S rDNA banding profiles of avocado rhizosphere bacteria from infected (INF1–4), biocontrol treated (BC1–4), and healthy (H1–4) roots. Lanes shown are from four separate trees for each sample group. Arrows denote bands that were cloned and sequenced for identification of bacterial species associated with these predominant DNA bands.

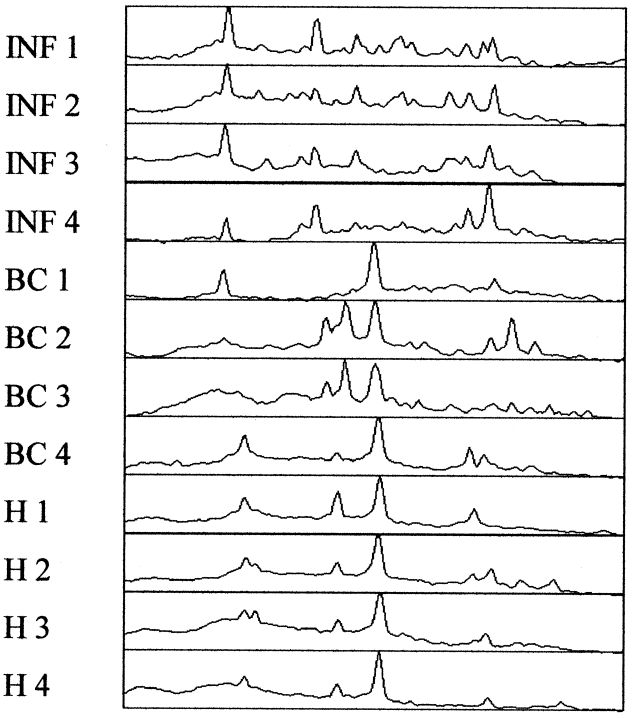

Fig. 2. Line profiles of 16S rDNA bands from healthy, biocontrol treated, and infected roots of avocado. Profiles shown here are for a reduced set of root samples with one sample from each tree of healthy, infected, and biocontrol treated roots.

replicate samples within trees, which was confirmed using several independent statistical procedures. For statistical analyses comparing the gel banding patterns, the DGGE patterns were analyzed for three replicate root samples from four different trees in each of the tree groups (healthy, infected, and biocontrol treated). The 16S rDNA banding patterns were first converted to line profiles for each DNA sample (Fig. 2). These line profiles were then exported as *x*/*y* plot values to a data spreadsheet, which was then imported into a peak analysis program to determine the number of distinct bands and the band intensity, integrated as the area under each peak. The line profiles presented in Fig. 2 are for a subsample of roots, with one replicate sample per tree from each group. DNA profiles for the infected roots (INF1–4) were considerably more complex and variable as compared to those for the healthy roots (H1–4). Banding profiles for the roots from healthy trees and those treated with the biocontrol bacteria (BC1–4) all contained one prominent peak that was also present at a much lower intensity in the infected roots. When cut from the gel, cloned and sequenced, this particular peak, AV1, was subsequently shown to represent plastid DNA with high similarity to *Nicotiana* chloroplast DNA (Table 1). This band was eliminated from all statistical analyses to focus the comparisons of community similarities only on the bacterial DNA bands.

Peak analysis revealed bands with 31 distinct R_f values in addition to the plastid DNA peak. In the statistical

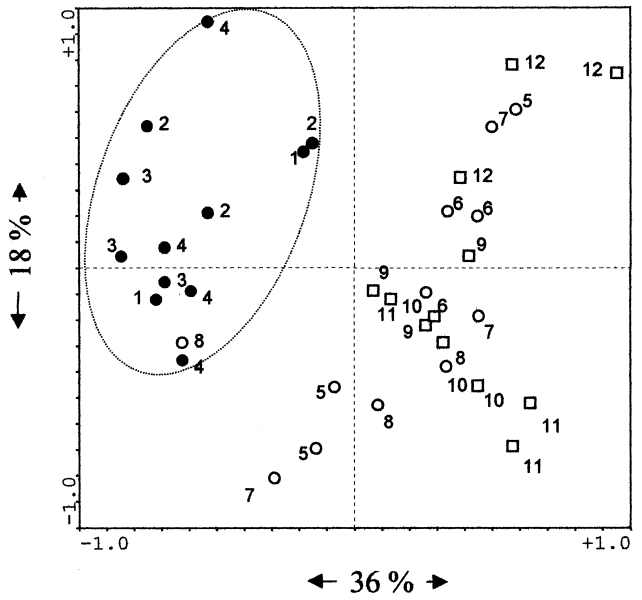

Fig. 3. Correspondence analysis of 16S rDNA banding profiles for bacterial communities associated with root tips of avocado. Ordination diagram was constructed using 16S rDNA profiles of replicate samples of healthy trees with no Phytophthora (□), biocontrol treated trees (■), and Phytophthora infected trees (○). Phytophthora infected samples formed a cluster which is circled. Numbers adjacent to each point represent individual trees, with three replicated samples per tree, and show relative similarities of bacterial communities from the same tree, as compared to variation by treatment and disease status.

analyses, each band or R_f value was denoted as a 'species' or bacterial group, with the band intensity corresponding to the abundance of that group. Within each lane, band intensity does not necessarily correspond with true species abundance, due to differences in 16S rDNA copy number and methods-related PCR artifacts [18]. However, changes in the band intensity between lanes for identically prepared samples provide a value for changes in the relative abundance of that species or group which is represented by a specific DNA band.

Several complementary statistical procedures were used to evaluate the data set. In the first analysis, principal components analysis was used to compare the similarities of the communities from the replicate tree root samples. The first two principal components described 56% of the variation in the data set and differences between the bacterial communities with respect to these two principal components are shown in Fig. 3. This analysis procedure reduced the 32 different band location variables to a smaller set of hypothetical variables, or principal components (PC), that are ranked for their importance in describing variation in the data set. The first principal component (PC1 plotted on the x axis in Fig. 3) described 36% of the variation between the community profiles. An additional 18% of the variation was described by PC2, plotted on the y axis. The ordination diagram plotted with respect to these two eigenvectors revealed the high similarities of the bacterial communities from the healthy roots of control trees and trees treated with the biocontrol bacteria. In contrast, the points representing the communities from the infected roots formed a cluster that was clearly separate from those of healthy roots. Analysis of data for Fig. 3 using a Monte Carlo permutation test showed that root infection had a statistically significant effect ($P > 0.05$) on community structure. When included as a covariable in the correspondence analysis, Phytophthora infection explained 100% of the variation described by PC1 and communities separated based on infection status were statistically different ($P > 0.05$). The statistical differences between the bacterial community structures described by PC1 and PC2 were also examined by analysis of variance. By this analysis, communities of healthy and infected roots were statistically different at the 0.0001 level of significance, whereas communities associated with the healthy roots of biocontrol and nontreated trees were not different. This analysis procedure was also used to examine differences between individual trees within each group. Variation in community structure among replicate samples with the same tree from healthy and infected trees was not statistically significant, although in some cases, the communities within certain trees were highly similar. To further study this variation within and between trees, discriminate analysis was conducted using the raw peak data rather than the principal components. This analysis re-

Table 1
Bacterial isolates identified in predominant 16S rDNA DGGE bands from the rhizosphere of healthy and *P. cinnamomi* infected avocado roots

DGGE band clones	Blast search results	Score and accession[a]
AV1-4	*N. plumbaginifolia*	99% (M82900)
	F. australis	99% (AF038624)
AV2-1	*Pseudomonas* sp.	98% (D87346)
AV2-3	clone K20-37	90% (AF145834)
AV3-6	uncultivated soil bacterium	91% (AF128659)
AV3-8	uncultivated soil bacterium	95% (AF128659)
AV4-6	Unidentified *Pseudomonas* sp.	100% (AF006502)
AV4-7	*Polyangium* sp.	84% (M94280)
AV4-8	*Cytophaga* sp.	95% (D12675)
AV4-9	unidentified eubacterium	88% (AF175632)

[a]Score values indicate the percent 16S rDNA similarity to bacteria that are referenced in the BLAST database by these accession numbers.

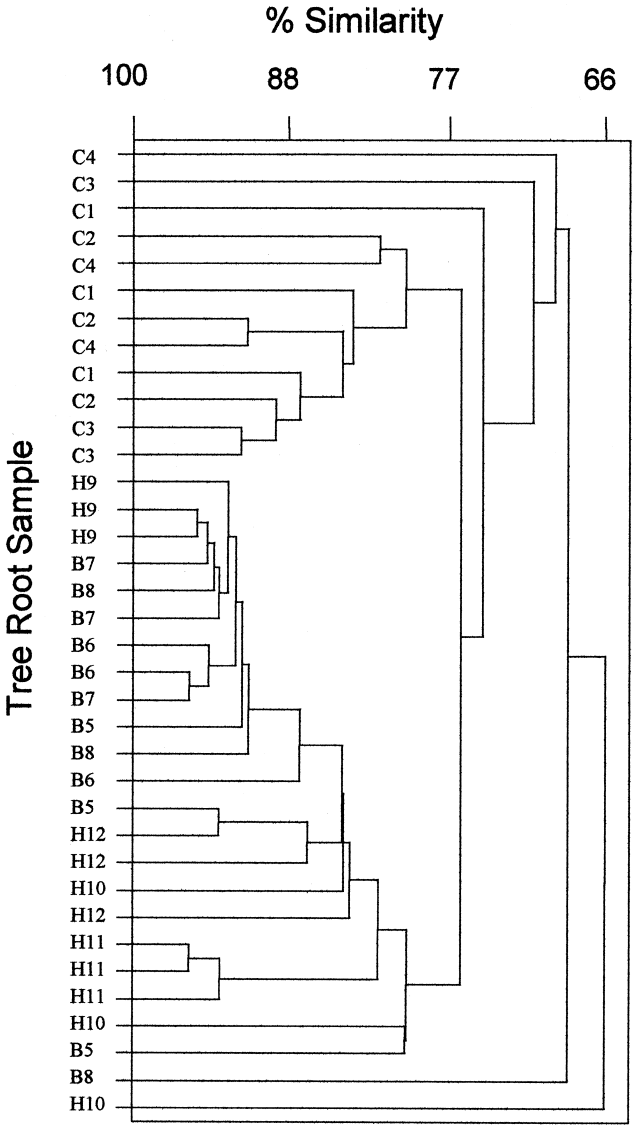

Fig. 4. Cluster analysis of 16S rDNA banding profiles for bacterial communities from healthy, biocontrol treated, and infected avocado roots.

vealed two groups of bacterial communities, which corresponded to healthy and infected trees. With the 36 samples, 35 were correctly identified with respect to disease status. In contrast, only 23 of the 36 samples were correctly identified with respect to the individual trees from which they were collected.

Similar results characterizing community similarities were obtained using cluster analysis (Fig. 4). With one exception, all of the root samples from the healthy roots were 80–90% similar to each other. Similarly, all of the infected roots were about 80% similar to each other and clustered in a separate group in the dendrogram, with a linkage of approximately 75% similarity to healthy roots. Overall, bacterial communities associated with trees receiving the biocontrol treatment had the greatest similarity, with eight of the 12 samples having greater than 90% similarity.

3.3. Sequence analysis of DGGE bands

Four major 16S rDNA DGGE bands, AV1, AV2, AV3 and AV4 amplified from healthy and infected avocado root tips were sliced out and reamplified with primers PBRA338f and PRUN518r (Fig. 1). The PCR products were further run in an agarose gel and the 220 bp fragments were purified and cloned into pGEM for sequence identification. The sequence derived from DGGE band AV1 affiliated with the chloroplast 16S rRNA genes of *Nicotiana plumbaginifolia* and *Flindersia australis* through the BLAST search analysis, with a 99% match score.

Two individual 16S rDNA clones were sequenced from band AV2, one of which was 97% similar to *Pseudomonas* sp. The second clone was unidentified, having only a 90% match to another unidentified bacterium in the database. Clones generated from band AV3 were similarly unidentified, and had match values of 91 and 95% to an unidentified, uncultivated eubacterium. DGGE band AV4 was associated primarily with healthy root tips and with one biocontrol treated root sample, but was not associated with the infected roots, and was thus relevant for identification of bacteria that might possibly be antagonistic to *P. cinnamomi*. However, this band was shown to be comprised of at least four different microorganisms, as revealed by the presence of different sequences from four individual clones that were generated (Table 1). To confirm that the sequences were not PCR artifacts, the sequenced clones were again amplified with primers PRBA338f and PRUN518r. In all cases, the same relative migration positions were observed between the PCR products from sequence clones and the bands originally sliced our from the first DGGE gel (data not shown).

4. Discussion

In this research, culture independent methods for analysis of microbial community structure were applied to examine the influence of soil bioaugmentation with a disease suppressive microorganism on rhizosphere bacterial communities and changes that occur on avocado roots during early disease infection by *P. cinnamomi*. Results of these analyses showed that there are significant changes in the rhizosphere bacterial community of avocado tree roots prior to any visually observable infection by Phytophthora root rot, and that bioaugmentation did not significantly affect bacterial community structure to a degree that could be statistically distinguished from those of healthy roots.

Characterization of microbial community structure in the rhizosphere has previously been accomplished primarily by means of culture based analyses that involve plating of microorganisms on to selective media. Techniques for rhizosphere analysis involving PCR-DGGE of 16S rDNA offer many advantages over culture based approaches, but have only begun to be utilized for studies on the plant

rhizosphere. In a recent study by Duineveld and coworkers (1998), the rhizosphere bacterial communities of Chrysanthemum were examined by 16S rDNA-DGGE assay, and were shown to be highly reproducible from the same sample with variation between replicates being relatively small [19]. In another study employing PCR-DGGE, Yang and Crowley (2000) showed that the rhizosphere of barley plants varied reproducibly for different root zones, and was strongly influenced by the plant iron nutritional status.

Examination of bacterial communities from healthy and infected root tips of avocado showed that the 16S rDNA banding patterns were relatively simple with highly distinct bands that could be identified by peak analysis, and thus suggest that the root tips are heavily colonized by only a few predominant species (Fig. 2). In this study, a relatively small number of trees from the orchard were examined, but highly significant differences were revealed in comparisons of healthy and infected roots, which could be detected using a variety of complementary statistical procedures. All of these statistical tests showed that there was a highly significant effect of disease, but that there was no significant difference between communities from individual trees. Taken altogether, these data strongly suggest that variation between communities due to individual tree and location effects is much less than that caused by the effects of disease. In future studies, it will be of interest to determine whether these same differences can be detected for orchards on different soil types or at different times of the year. Phytophthora root rot is endemic in most avocado orchards, but disease progression typically is greatest following extended wet periods that favor zoospore germination and movement toward the plant roots.

Use of DNA banding patterns for determination of community similarities proved to be a powerful technique for resolving the different communities, but also was shown to have limited resolution due to comigration of 16S rDNA of different species. More than one bacterial species was found in bands AV2, AV3, and AV4 through DNA fragment cloning and sequence analysis. Conversely, different bands, as shown here for *Pseudomonas* sp. may represent similar species, which were presented in two dominant bands AV2 and AV4 (Table 1).

Band AV4 from DGGE gel attracted interest due to its ubiquitous association with healthy root tips. This dominant band is present in most of the healthy root tips but not in the infected root tips. Upon detailed analysis, it was shown that the 16S rDNA clones from this band had the best DNA similarities to four different bacteria, including *Pseudomonas* sp. (100%), *Polyangium* sp. (84%), *Cytophaga* sp. (85%), and an unidentified eubacterium (88%). *Pseudomonas* sp. is widely used as a biological control agent for disease control due to antibiotics and pyoverdine production [7]. *Cytophaga* sp., which produce cellulases, also is potentially relevant for suppression of *Phytophthora*, which contains cellulose as cell wall component. *Polyangium* sp. is a not well studied organism and is reported to be difficult to culture [20]. Lastly, out of nine 16S rDNA fragments from four bands, four uncharacterized rhizosphere organisms were shown to occur on avocado root tips. The discovery of broader bacterial communities in this study is direct evidence of the utility of the PCR-DGGE method, which does not have the limitations of cultivation based methods and provides a rapid, reproducible method for analyzing microbial community structures of the plant rhizosphere. In this case, it is still not clear whether the communities associated with healthy roots in fact promote suppression, or instead reflect the root exudate release patterns of healthy roots as opposed to those of infected trees. Nevertheless, the observation that these communities are represented by distinct 16S rDNA profiles provides a starting point for examining changes in the rhizosphere bacterial community during the establishment and progression of root disease.

References

[1] Weindling, R. (1946) Microbial antagonism and disease control. Soil Sci. 61, 23–30.
[2] Sanford, G.B. (1946) Soil-borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Sci. 61, 9–22.
[3] Thomashow, L.S. (1996) Biological control of plant root pathogens. Curr. Opin. Biotechnol. 7, 343–347.
[4] Pierson, E.A., Wood, D.W., Cannon, J.A., Blachere, F.M. and Pierson, L. (1998) Interpopulation signaling via N-acyl-homoserine lactones among bacteria in the wheat rhizosphere. Mol. Plant-Microbe Interact. 11, 1078–1084.
[5] Van Elsas, J.D., Duarte, G.F., Rosado, A.S. and Smalla, K. (1998) Microbiological and molecular biological methods for monitoring microbial inoculants and their effects in the soil environment. J. Microbiol. Methods 32, 133–154.
[6] Yang, C.-H. and Crowley, D.E. (2000) Effects of plant iron nutritional status on rhizosphere community structure in different root locations. Appl. Environ. Microbiol. 66, 345–351.
[7] Cook, R.J., Thomashow, L.S., Weller, D.M., Fujimoto, D., Mazzola, M., Bangera, G. and Kim, D.S. (1995) Molecular mechanisms of defense by rhizobacteria against root disease. Proc. Natl. Acad. Sci. USA 92, 4197–4201.
[8] Raaijmakers, J.M., Weller, D.M. and Thomashow, L.S. (1997) Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl. Environ. Microbiol. 63, 881–887.
[9] Toyota, K., Yamamoto, K. and Kimura, M. (1994) Isolation and characterization of bacteria responsible for the suppression of *Fusarium oxysporum* f. sp. *raphani* on the host rhizoplane. Soil Sci. Plant Nutr. 40, 381–390.
[10] Hessenmueller, A. and Zeller, W. (1996) Biological control of soil-borne Phytophthora species on strawberry with bacterial antagonists: 1. Antagonistic effect of colonization of rhizoplane. Z. Pflanzenkrankh. Pflanzenschutz 103, 602–609.
[11] Naseby, D.C. and Lynch, J.M. (1999) Effects of Pseudomonas fluorescens F113 on ecological functions in the pea rhizosphere are dependent on pH. Microb. Ecol. 37, 248–256.
[12] Nacamulli, C., Bevivino, A., Dalmastri, C., Tabacchioni, S. and Chiarini, L. (1997) Perturbation of maize rhizosphere microflora following seed bacterization with *Burkholderia cepacia* MCI 7. FEMS Microbiol. Ecol. 23, 183–193.

[13] Coffey, M.D. (1992) In: Plant Diseases of International importance. Vol. III. Diseases of Fruit Crops (Kumar, J., Chaube, H.S., Singh, U.S. and Mukhopadhyay, A.N., Eds.), pp. 423–444. Prentice-Hall, Englewood Cliffs, NJ.

[14] Zentmyer, G.A., Menge, J.A. and Ohr, H.D. (1994) In: Compendium of Tropical Fruit Diseases (Ploetz, R., Zentmyer, G.A., Nishijima, W.T., Rohrback, K.G. and Ohr, H.D., Eds.), pp. 77–79. APS Press, American Phytopathological Society, St. Paul, MI.

[15] Kannwischer, M.E. and Mitchell, D.J. (1981) Relationships of numbers of spores of *Phytophthora parasitica* var. nicotianae to infection and mortality of tobacco. Phytopathology 71, 69–73.

[16] Ovreas, L., Forney, L., Daae, F.L. and Torsvik, V. (1997) Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Appl. Environ. Microbiol. 63, 3367–3373.

[17] Jongman, R.H., Braak, C.J.F.t. and Van Tongeren, O.F.R. (1995) Data Analysis in Community and Landscape Ecology. 21st Edn., 299 pp. Cambridge University Press, Cambridge.

[18] Farrelly, V., Rainey, F.A. and Stackebrandt, E. (1995) Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl. Environ. Microbiol. 61, 2798–2801.

[19] Duineveld, B.M., Rosado, A.S., Van Elsas, J.D. and Van Veen, J.A. (1998) Analysis of the dynamics of bacterial communities in the rhizosphere of the chrysanthemum via denaturing gradient gel electrophoresis and substrate utilization patterns. Appl. Environ. Microbiol. 64, 4950–4957.

[20] Balows, A. (1992) The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd, Edn. Springer, New York.