Soil Community Analysis Using DGGE of 16S rDNA Polymerase Chain Reaction Products

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ABSTRACT

Separation of polymerase chain reaction (PCR)–amplified 16S rDNA products using denaturing gradient gel electrophoresis (DGGE) was tested as a means to study microbial community composition in bulk soil samples. DNA was extracted from six soils from agroecosystems in Norway and the USA under different agronomic treatments (crop, rotation, and tillage); one soil is contaminated with polyaromatic hydrocarbons (PAH, 700 mg kg^{-1}). Two sets of primers specific for Bacteria (V3 and the V6/V9 regions of 16S rRNA) and another for Archaea (V3 region of 16S rRNA) were used to determine the contribution of each domain to the microbial community. Reproducible, characteristic profiles of the communities were obtained by DGGE separation of the PCR amplification products. The number of fragments resolved by DGGE indicated bacterial diversity was far greater than that of the Archaea in the agricultural soils examined. Only the soil contaminated with PAHs had reduced bacterial diversity, evidenced by a distinct DGGE profile. The results showed that the method is useful as an initial step to discriminate among communities because it is rapid and multiple samples can be easily screened. There are some limitations, but under highly selective conditions it is possible to distinguish communities from different soils and to indicate the presence of numerically dominant populations.

Despite considerable interest in the microbiology and biogeochemistry of soils, relatively little is known about the diversity and ecology of the microbial community. Studies have demonstrated that microbial biomass is dependent on available organic matter, macroflora, and fauna; however, we understand relatively little about changes in the composition of soil microbial communities (Parkinson and Coleman, 1991; Wardle, 1992; Hopkins and Shiel, 1996). Soil community analysis has been limited in the past because only a minor proportion of the microbial population is cultivable. Recent applications of molecular biology have provided tools to determine microbial presence and diversity in the environment (Atlas et al., 1992; Head et al., 1998). A number of molecular genetic techniques, such as total DNA isolation and characterization, G + C composition, rRNA sequences, PCR amplification of rDNA, PCR amplification of functional genes, and in situ hybridization of rRNA oligonucleotide probes, are being used to study microbial communities (Akkermans et al., 1995). Comparison of nucleotide sequences has shown there are regions of rRNA sequences that are highly conserved between all organisms and other regions that vary to different degrees. The variability in these regions increases as the evolutionary distance between two organisms increases, which provides a means to determine phylogenetic relationships and to distinguish microorganisms from one another (Woese, 1987; Woese, 1992). Presently, the rRNA genes in DNA taken directly from soil can be amplified using PCR, the products cloned, and the nucleotide sequence determined. Using these techniques, a number of researchers have begun to examine the biodiversity of soil microbial communities (Ueda et al., 1995; Borneman and Triplett, 1997; Jurgens et al., 1997). It appears that microbial diversity is greater than initially realized, and in many cases new groups within the Bacteria and Archaea have been found. This approach was developed to determine biodiversity and has limitations when applied to community ecology studies, because it is labor intensive and time consuming compared with DNA fingerprinting methods. However, these studies are extremely useful because they add to the rRNA sequence database (Maidak et al., 1999), assess biodiversity, and provide information needed to determine group-specific nucleotide sequences (DeLong et al., 1989; Alm et al., 1996).

More recently, fingerprint profiles of rDNA sequences amplified by PCR have been used to study microbial communities (Muyzer et al., 1993; Muyzer et al., 1995; Ferris et al., 1996; Torsvik et al., 1998). The extent to which a DNA strand will denature depends on its nucleotide sequence composition, so PCR products with different compositions, but of the same length, will migrate different distances when exposed to a gradient of denaturing conditions. This results in distinct fingerprints when DGGE is used to separate PCR products. The method has been used successfully to compare microbial communities in different aquatic ecosystems (Ferris et al., 1996; Teske et al., 1996; Ferris and Ward, 1997; Øvreås et al., 1997), and this method is beginning to be used to study the soil microbial community (Heuer et al., 1997; Øvreås and Torsvik, 1998). The validity of this method for studying soil microbial ecology still requires further investigation. The number of phylotypes in forest soil are estimated to be in the order of 10 000 by reassociation analysis of total DNA isolated directly from the environment (Torsvik et al., 1996). This great biodiversity in soil led us to reason that fingerprinting methods such as DGGE would not yield results that can be quantitatively analyzed because of the great number of bands involved. The objective of this study was to determine if DGGE separation of 16S rDNA sequences amplified from DNA could provide qualitative and quantitative information about soil microbial community composition. Domain-specific primers for PCR amplification of 16S rDNA were used to determine

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Abbreviations: DGGE, denaturing gradient gel electrophoresis; PAH, polyaromatic hydrocarbons; PCR, polymerase chain reaction.

Table 1. Polymerase chain reaction primers specific for the amplification of 16S rRNA genes of Bacteria or Archaea.

Primer	16S rDNA target (base number)†	Primer sequence	References
PRBA338F	Bacteria V3 region (338-358)	5′‡AC TCC TAC GGG AGG CAG CAG 3′	(Lane, 1991)
PRUN518R	Universal V3 region (534-518)	5′ATT ACC GCG GCT GCT GG 3′	(Muyzer et al., 1993)
PRBA968F	Bacteria V6 region (968-983)	5′‡AA CGC GAA GAA CCT TAC 3′	(Nübel et al., 1996)
PRBA1406R	Bacteria V9 region (1406-1392)	5′ACG GGC GGT GTG TAC 3′	(Lane et al., 1988)
PRA46F	Archaea (46-60)	5′C/TTA AGC CAT GCG/A AGT 3′	(Øvreås et al., 1997)
PREA1100R	Archaea (1117-1100)	5′T/CGG GTC TCG CTC GTT G/ACC 3′	(Øvreås et al., 1997)
PARCH340F	Archaea V3 region (340-358)	5′‡CC TAC GGG GC/TG CAG/C CAG 3′	(Øvreås et al., 1997)
PARCH519R	Archaea V3 region (534-519)	5′TTA CCG CGG CG/TG CTG 3′	(Øvreås et al., 1997)

† Bases numbered relative to *E. coli* 16S rRNA sequence.
‡ GC clamp added to the 5′ end of the primer, 5′CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G 3′.

whether the method could be used to study the diversity of the Bacteria and Archaea in soils. Two different sets of Bacteria-specific primers were tested to see if the same information is obtained. The method was tested on soils under different agronomic treatments from Norway and the USA and on one contaminated with aromatic hydrocarbons.

MATERIALS AND METHODS

Soils Samples

Soils were collected from sites in the USA and Norway. The top 10 cm of soil from at least three locations at each site were collected, composited, homogenized by sieving (2-mm mesh), and stored at 4°C until processed further. Soils from Indiana were collected at the long-term tillage plots at the Purdue Agronomy Research Center in west central Indiana. The soil is Chalmers silty clay loam soil (fine-silty, mixed, superactive, mesic Typic Endoaquolls; 4% organic matter) (Griffith et al., 1988). Samples of the Chalmers silty clay loam soil were collected under different agronomic practices; no-till or plowed fields planted with either soybean [*Glycine max* (L.) Merr.] or corn (*Zea mays* L.). A coal tar–contaminated sandy loam soil (17% organic matter) was collected in Wisconsin (ID #UT710, Lee et al., 1998). It contained 700 mg kg^{-1} of known PAH (based on the USEPA priority list). The soils from western Norway were collected south of the city of Bergen at Storåkeren and Krohnestykket. The Storåkeren site was a fallow field with sandy loam soil (14% organic matter), which was previously used for intensive agricultural production of vegetable crops. The Krohnestykket site was pastureland used for grazing cattle; it had a clay loam texture (58% organic matter).

DNA Extraction

Two typically used DNA extraction methods were performed to determine whether soil volume used for DNA extraction changed the DGGE profiles. DNA was extracted from the soil samples using either a large scale method (Torsvik et al., 1990) or a small scale method. The latter method combined freezing–thawing to lyse cells (Ueda et al., 1995) with the FastDNA kit and Spin columns (BIO 101, Vista, CA) (Borneman et al., 1996) for DNA purification. At least three replicate samples of 0.5 g of homogenized soil were used per small scale DNA extraction to determine analytical variability. Two soil samples, collected at least 0.5 m apart at random locations within the same agronomic treatment were used to compare spatial variability.

Polymerase Chain Reaction Amplification

The primers used for amplification of 16S rDNA of Bacteria and Archaea are listed in Table 1. The PRBA338F and PRUN518R primers that amplify the 338- to 518-rDNA region contain one variable loop of the rRNA, whereas the PRBA968F and PRBA1406R primers that amplify the 968- to 1406-rRNA region are longer fragments that contain three variable loops (Woese et al., 1983). Amplification mixtures with Bacteria primers had a final volume of 100 μL and contained 25 pmol of each primer, 100 μ*M* dNTPs, 2% BSA, 1X PCR buffer, and 2.5 units of Taq polymerase (Promega, Madison, WI; or AmpliTaq Gold, PE Biosystems, Foster City, CA). Polymerase chain reaction was performed in automated thermal cyclers (a 2400 Perkin Elmer/Cetus, PE Biosystems; or a PTC-100, MJ Research, Waltham, MA). The reaction began with an initial 94°C denaturation for 9 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, a final extension at 72°C for 7 min, then it was held at 4°C.

Amplification of Archaea 16S rDNA sequences was carried out using a nested PCR technique. Nested PCR was used for Archaea community analysis because DGGE is limited to fragments <500 bp in length (Muyzer et al., 1996), and Archaea-specific primers that could directly amplify this size could not be found. First, the majority of the Archaea 16S rDNA fragment was amplified using the PRA46F and PREA1100R primers (Table 1). The first amplification mixture contained the same concentration of components as in the Bacteria mix with the addition of 5% acetamide (w/v, filter sterilized) to decrease nonspecific amplification (Reysenbach et al., 1992). Polymerase chain reaction began with a 92°C denaturation for 5 min, followed by 30 cycles of 92°C for 30 s, 53.5°C for 30 s, 72°C for 1 min., and a final extension at 72°C for 5 min. The second amplification with the PARCH340F and PARCH519R primers was carried out using the same mix as for Bacteria. The amplification was performed using the same settings as in the first round of amplification, except with an annealing temperature of 53°C.

Presence of PCR products was confirmed by electrophoresis on 1.5%–agarose gels stained with ethidium bromide. To confirm reproducibility of the method all amplifications were repeated at least three times at the University of Bergen and Purdue University laboratories. The same soil DNA–extract was used as the PCR template at both locations.

Denaturing Gradient Gel Electrophoresis Analysis

Denaturing gradient gel electrophoresis was carried out in a Hoefer SE600 vertical cooler system (Amersham Pharmacia Biotech, Uppsala, Sweden) or a D-Gene apparatus (BioRad, Hercules, CA) with slight modifications to a previously described method (Muyzer et al., 1996). Briefly, PCR products were resolved on 8% (w/v) polyacrylamide gels in 0.5X TAE (20 m*M* tris-Cl, 10 m*M* acetate, 0.5 m*M* Na_2EDTA) using denaturing gradients ranging from 15 to 55% (for Bacteria using the Hoefer apparatus), 30 to 50% (for Bacteria using the BioRad apparatus), or 20 to 50% (for Archaea) (where 100% denaturant contains 7 *M* urea and 40% formamide).

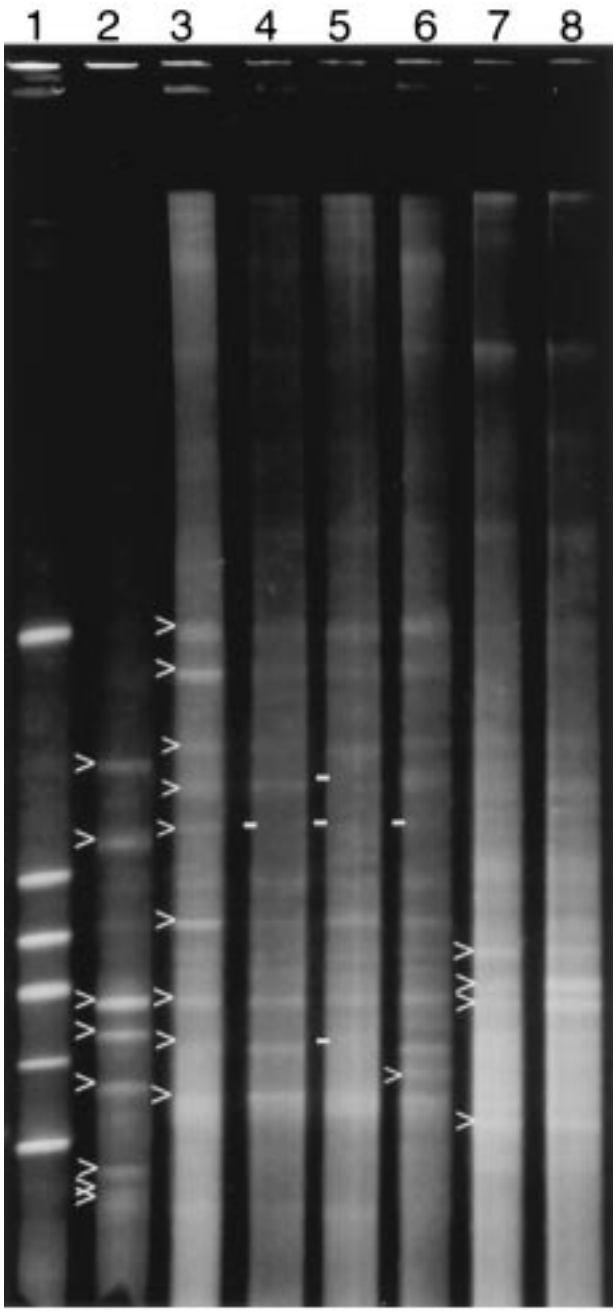

Fig. 1. Denaturing gradient gel electrophoresis gel of Bacteria 16S rDNA polymerase chain reaction (PCR) products (Bases 338 to 518 relative to the *E. coli* rRNA sequence) amplified from USA (Lanes 2–6) and Norwegian (Lanes 7–8) soil-DNA extracts. Gel gradient ranging from 30 to 55% denaturant. Lane 1, marker (PCR products from top to bottom, *Pseudomonas putida*, *Acinetobacter* sp. ADP1, *Comamonas acidovorans* ATCC 15668, *E. coli* DH5, *Alcaligenes* sp. BR40, *C. testosteroni*); Lane 2, PAH (700 mg kg^{-1})–contaminated soil; Lane 3, no-till corn field; Lane 4, no-till soybean field; Lane 5, plowed corn field; Lane 6, plowed soybean field; Lane 7, fallow field; Lane 8, pastureland. Arrows indicate the dominant populations (Lane 2) and regions of greater band intensity that characterize the community structure (Lanes 3, 4 and 7). Minus signs indicate intense bands not seen in Lanes 4–6 but present in Lane 3.

Gradients varied with the primers used for amplification, the nucleotide composition of the resultant products, and the apparatus used for DGGE. Electrophoresis was carried out at a low voltage (20 V) for 10 min, then 200 V for three to five h. Electrophoresis buffer (0.5X TAE) was maintained at 60°C. Gels were then stained with SYBR Green I or II (1:10 000 dilution; Molecular Probes, Eugene, OR) and visualized on a UV transilluminator and photographed (Polaroid MP4 Land camera, Cambridge, MA).

Statistical Analysis

Relatedness of microbial communities was determined using similarity coefficients of bands common to two samples. Our working definition was that two bands are common if they migrated the same distance on a gel. First, the total number of different bands was determined for the samples being compared. Then each sample was scored based on the presence or absence of each band in its profile when compared to the profile of each of the other samples. Sorensen's index of similarity [$C_s = 2j/(a + b)$] was used to make pairwise calculations of band sharing between samples (Sorensen, 1948). In that equation, a is the number of bands in Sample A, b is the number of bands in Sample B, and j is the number of bands common to A and B.

RESULTS

Denaturing Gradient Gel Electrophoresis Analysis using Bacteria Primers

The Bacteria primers amplified two different regions of the 16S rDNA, bases 338 (PRBA338F) to 518 (PRUN518R), and bases 968 (PRBA968F) to 1406 (PRBA1406R) relative to the *E. coli* rRNA nucleotide sequence. The DGGE profiles using both sets of primers indicate there are hundreds of different bacterial 16S rDNA nucleotide sequences amplified from agricultural soil DNA. The presence of this number of bands down the length of each lane created a smear (Fig. 1, Lanes 3–8 and Fig. 2, Lanes 1–6). In contrast, the DGGE profile of PCR products of the 338- to 518-rDNA region (primers PRBA338F and PRUN518R) amplified from PAH-contaminated soil was distinct with eight bands that were always seen (Fig. 1, Lane 2). Six bands in this profile were intense in all the amplifications performed, two varied in intensity in different trials, and three to four others were very faint and usually not observed.

The smear of bands from the bulk soils resulted in similar, but not identical, DGGE profiles from the Norwegian and Indiana soils. The patterns for the same soil differed when the two different primer sets were used (compare Fig. 1 and 2). The profiles from the Norwegian soils looked almost identical to each other using the PRBA338F and PRUN518R primers (Fig. 1) but showed many differences with the PRBA968F and PRBA1406R primers (Fig. 2). The DGGE fingerprints of Indiana soil from different agronomic treatments differed from each other and from the Norwegian soil because of differences in band intensities (arrows on Fig. 1). There were also differences within a soil treatment from Indiana if samples of it were collected 0.5 m apart (Fig. 2). For example in Fig. 2, Lanes 4a and b are identical, whereas Lanes 1a and b differ from each other, which illustrates spatial heterogeneity. However, the patterns were the same when replicate samples from homogenized soils were compared (data not shown). Basically, the same DGGE profiles were generated from

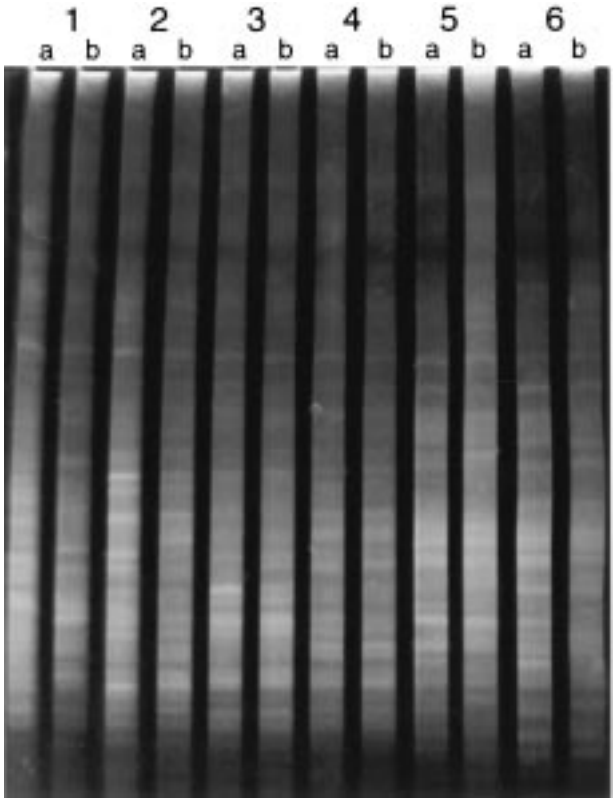

Fig. 2. Denaturing gradient gel electrophoresis gel of Bacteria 16S rDNA polymerase chain reaction products (Bases 968 to 1406 relative to the *E. coli* rRNA sequence) amplified from USA (Lanes 1–4) and Norwegian (Lanes 5–6) soil-DNA extracts. Gel gradient ranging from 30 to 55% denaturant. Lane 1, no-till corn field; Lane 2, no-till soybean field; Lane 3, plowed corn field; Lane 4, plowed soybean field; Lane 5, fallow field; Lane 6, pastureland.

each of the extracted–soil DNA samples when PCR amplification was replicated in soils from Indiana and Norway using two different polymerases, two different PCR machines, and two different DGGE apparatus (data not shown). However, the patterns were not always identical for the agricultural soils if slightly different gradients of denaturant were poured. Changes in band intensity can occur if the bands result from a clustering of populations with similar nucleotide sequence content that separate when exposed to different gradients. When denaturant gradients were varied for the less complex communities, the number of the dominant populations remained the same and only the spacing between bands changed. The results of smears are easier to interpret when profiles are run adjacently on the same gel (Fig. 2). When profiles are composed of distinct bands, comparisons can be made between gels by including molecular markers as standards.

Denaturing Gradient Gel Electrophoresis Analysis using Archaea Primers

Nested PCR amplification was used to amplify the Archaea population. After the first round of PCR amplification using the PRA46F and PREA1100R primers, no products were observed when checked for on agarose gels, indicating that the Archaea comprise a small fraction of the soil microbial community. However, products were observed after the subsequent amplification in the nested PCR protocol using primers PARCH340F and PARCH519R. The DGGE profiles of the 16S rDNA PCR products from Archaea are distinct, and the number of populations in one soil are limited to one or more that are dominant (based on band intensity) but less than ten populations in any of the soils examined (Fig.

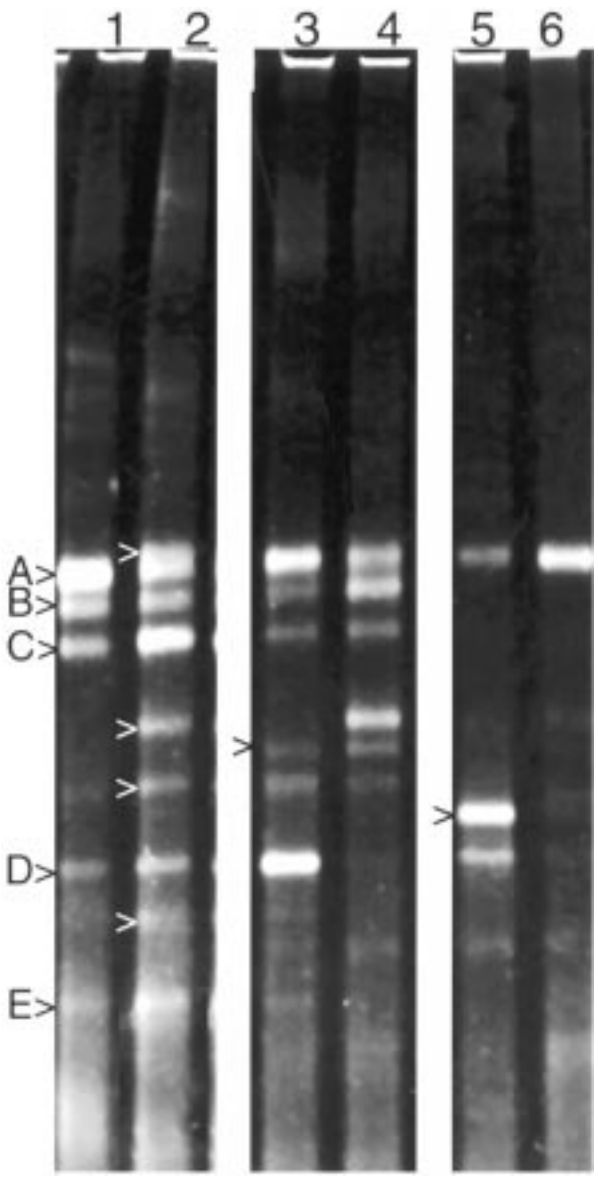

Fig. 3. Denaturing gradient gel electrophoresis (DGGE) gel of Archaea 16S rDNA polymerase chain reaction products (Bases 340 to 534 relative to the *E. coli* rRNA sequence) amplified from USA (Lanes 1–4) and Norwegian (Lanes 5–6) soil DNA extracts. Gel gradient ranging from 30 to 55% denaturant. Lane 1, no-till corn field; Lane 2, no-till soybean field; Lane 3, plowed corn field; Lane 4, plowed soybean field; Lane 5, fallow field; Lane 6, pastureland. Arrows mark each of the 11 different populations detected by DGGE, as determined by the distance the products migrated in the gel. Band A is present in all the soils. Bands B and C are common to all the Indiana soils (Lanes 1–4). Band E was unique to soils under no-till cultivation in Indiana (Lanes 1 and 2). Band D was present in some soils from Indiana and Norway, but its presence did not correlate with any agronomic treatments.

Table 2. Similarity coefficient (*C_s) of Archaea populations in agricultural soils from Indiana and Norway.

Lanes compared	1	2	3	4	5	6
1	1.00					
2	0.71	1.00				
3	0.73	0.67	1.00			
4	0.54	0.67	0.83	1.00		
5	0.50	0.33	0.44	0.22	1.00	
6	0.33	0.20	0.28	0.28	0.50	1.00

* C_s = 2 (no. of shared bands)/ total no. bands. Lanes correspond to DGGE profiles of polymerase chain reaction products amplified using Archaea-specific 16S rDNA primers as illustrated in Fig. 3.

3). Combining the results of all the soils examined, there is only a total of 11 different Archaea populations, based on the different distance PCR products migrated on the DGGE gels (marked by arrows). Some products migrated similar distances, suggesting common Archaea populations may be found in a diversity of soils. Band A (Fig. 3), which migrated a short distance on the gel and therefore had a lower GC content, was present in all the Indiana and Norway soil profiles. This was the only band observed in soil collected from Krohnestykket. All the Indiana soils had Bands B and C in common (Lanes 1–4), whereas Band E was unique to soils under no-till cultivation (Lanes 1 and 2). Band D was present in some soils from Indiana and Norway, but its presence did not correlate with any of the agronomic treatments. One band was unique to the Storåkeren soil (marked with arrow, Fig. 3 Lane 5).

Statistical Analysis

Based on the presence and absence of bands in each sample, similarity coefficients were determined for the DGGE profiles generated using the Archaea primers (Table 2). A value of 1.0 indicates all bands are shared and 0.0 indicates no bands are in common. In general, the Archaea community structure of the Indiana soils were more similar to each other (C_s ranging from 0.83 to 0.54) than the Norwegian soils (C_s ranging from 0.44 to 0.20). The community structure of the Norwegian soils were more similar to each other but the similarity was not high (C_s = 0.5). Similarity coefficients could not be determined for the bacterial community structure of the agricultural soils, because the smear indicated that a band existed at each position, resulting in all C_s equal to 1.0. Also, bands of greater intensity that were observed in these profiles were lost when replicates were run on gels with different denaturing gradients.

DISCUSSION

By using a combination of PCR amplification of 16S rDNA sequences and DGGE we were able to distinguish differences in microbial community composition in different soils (Fig. 1–3). The results of our study show that the differentiation of soil microbial communities using DGGE separation of 16S rDNA PCR products is dependent on both the soil being tested and the PCR primers chosen. Comparison of DGGE profiles of Bacteria and Archaea 16S rDNA PCR amplification products show distinct differences in soil microbial community composition because of the number and the varying distances that the PCR products migrated in the gradient gel. The method is particularly useful as an initial step in ecological studies to distinguish communities from different ecosystems and to determine if numerically dominant phylotypes, as seen with the Archaea in this study, are present. A similar approach has been used in aquatic ecosystems, in which multiple samples have been rapidly screened to obtain quantitative information about community changes through time and space (Lindstrom, 1998; Konopka et al., 1999a). However, statistical analysis was not possible with Bacteria communities in our study because dominant populations were not readily observed. Therefore, the technique is able to differentiate among most ecosystems, but there are limitations to quantifying the extent of the differences between communities with great diversity.

The DGGE profiles differed when intra- and inter-domain specific–PCR primers were tested. The domain-specific primers easily demonstrated that the Bacteria are more diverse than the Archaea in both the Norwegian and Indiana soils (Fig. 1–3). In the PAH-contaminated soils, Bacteria diversity was greatly reduced. The limited Archaea diversity in all the soils is indicated by the few products observed by DGGE (Fig. 3). Our findings illustrate a quick approach that supports other studies that found that Archaea are not a dominant component of soil ecosystems (Ueda et al., 1995; Borneman et al., 1996; Borneman and Triplett, 1997; Buckley et al., 1998). This contrasts with aquatic ecosystems in which the Archaea appear to constitute a significant proportion of the deep, subsurface water community (Fuhrman et al., 1993; Fuhrman and Davis, 1997; Massana et al., 1997). The common distances migrated by the Archaea PCR products from the different soils may result from the same organisms in the different soils. On the other hand, these bands also may consist of several different species having the same GC content in the amplified region; therefore, future studies will include sequence determination of Archaea 16S rRNA genes.

The number of bands that comprise the DGGE patterns indicated that there is a high diversity of Bacteria PCR amplification products in all soils, except the one contaminated with PAHs (Fig. 1 and 2). Nevertheless, we are still able to qualitatively distinguish between the complex DGGE profiles of the Norwegian and Indiana soils and also between the Indiana soils. The opposite is not always true; the bacterial communities from the two Norwegian soils appear to be similar because they are not readily distinguished from each other, yet this is not the case. Using DNA reassociation experiments, other studies have shown that the bacterial diversity in these two Norwegian soils are not equivalent (Øvreås and Torsvik, 1998). The Krohnestykket soil has >8800 different genome equivalents (relative to *E. coli* genomes) compared to only 350 in the Storåkeren soil. Incubation of the Krohnestykket soil for 3 wk at room temperature with methane gas as the sole C source

(Øvreås et al., 1998) reduced the community diversity to 305 genome equivalents. Although the diversity was then similar to the Storåkeren soil, the Krohnestykket DGGE profile was now distinct, showing some strong bands that indicated the presence of numerically dominant bacteria populations. These combined observations show that the presence of a few dominant species will produce DGGE profiles that can distinguish two soils with basically the same diversity, or soils with different relative proportions of populations in the two communities. However, communities differing in diversity by over 10-fold are not readily distinguished if the relative proportions of the species are equivalent, because one can not count 10^2 bands and differentiate it from 10^3 bands. Therefore, it is not possible to use DGGE to distinguish microbial communities from all ecosystems. In such complex ecosystems more information may be obtained if functional or group-specific primers can be used with the DGGE method to characterize components of the communities (Heuer et al., 1997; Kowalchuk et al., 1997).

The patterns of the DGGE profiles of Bacteria from the same soil differ when primers of two regions of 16S rDNA are used for PCR amplification (Fig. 1 vs. Fig. 2). The pattern differences are expected because the percentage of G + C content and the conservation of nucleotide sequences is not constant for the length of a gene (Clayton et al., 1995; Nübel et al., 1996). The two primer sets were chosen because they have been used in a number of studies for characterization of microbial communities (Porteous et al., 1994; Ferris et al., 1996; Muyzer et al., 1996; Nübel et al., 1996; Heuer et al., 1997; Felske et al., 1998). The 338- to 518-rDNA region is so variable that we found that pure cultures can produce several PCR products when separated by DGGE (unpublished). The multiple bands may have possibly resulted from the heterogeneity of 16S rRNA nucleotide sequences within a strain (Nübel et al., 1996), but this requires further investigation. The DGGE profiles of the bacterial communities in the Indiana soils were complex, but minor differences can be seen with the various agronomic treatments. The differences were more obvious when DGGE profiles of the 968– to 1406–16S rRNA region are compared with the 340- to 519-rRNA region. The results first suggest that the 968– to 1406–16S rRNA region is a better indicator of diversity, but alignment of the 968f primer sequence shows it is not conserved in commonly cultivated soil bacteria, such as *Burkholderia* spp. and *Ralstonia* spp. Therefore, it is possible that the communities are more readily differentiated because only a subset of the 16S rDNA from the bacterial community is being amplified. This can be beneficial if the communities are too complex and one merely wishes to see whether two soils can be differentiated.

The DGGE method is useful as the initial step in studying microbial community composition because it is not always possible to predict whether there are factors present that select for specific populations. Then, further analysis can be done using appropriate methods. For example, specific populations are not selected in Pb-contaminated soils, as seen in the PAH-contaminated soils (Konopka et al., 1999b). Our study has shown that the analysis of agricultural soils using DGGE can be done qualitatively by visually comparing the fingerprint profiles to determine that two communities differ. This is beneficial because it is a relatively rapid method of analysis that allowed us to quickly determine the major constituents of a soil using domain-specific primers. Thirty-six soil samples can be easily screened in two days using one DGGE apparatus; this includes DNA isolation and PCR amplification. This is important in ecological studies where a large number of samples often need to be screened. Quantitative analysis can not be done on all samples, because in ecosystems with high diversity the great numbers of bands are not easily resolved using the present system. Nonetheless, quantitative analysis can be done when soils are under highly selective conditions (e.g., PAH contamination) or when assessing groups with limited diversity (e.g., Archaea).

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