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Extraction of DNA from Soil for Analysis of Bacterial Diversity in Transgenic and Nontransgenic Papaya Sites

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The influence of transgenic crops on the soil diversity of microorganisms is one of the major risk assessments being conducted in Taiwan since 2007, and a reliable soil DNA extraction method for denaturing gradient gel electrophoresis (DGGE) is required. Six soils of different type, organic matter content, cation exchange capacity, and pH were tested, and four previously reported soil DNA extraction methods were applied to these soils. Soil DNA extracts by Zhou's CS method plus QIAquick gel was recommended in our laboratory for DGGE to monitor the microbial diversity in soil. There were some differences on the bacterial diversity based on DGGE patterns at the beginning of planting, and the difference decreased after six months. The results also indicated that clay content (10.8−25.0%) and pH (4.4−6.9) of different soil samples we tested did not affect the DNA extraction efficiencies, but positive correlations were found between the organic matter content (1.2−3.9%) of soils and the DNA yields in Widmer's GS method ($r = 0.93$, $p = 0.005$) and the MoBio UC method ($r = 0.92$, $p = 0.007$). Coefficient of determinations between organic matter content and DNA yield were higher than those between clay content, CEC, and pH, indicating that organic matter content was more correlated with DNA yield than that clay content, CEC, and pH in our soil samples tested.

KEYWORDS: Bacterial 16S rDNA; DGGE (Denaturing gradient gel electrophoresis); PCR inhibition; Soil DNA extraction

INTRODUCTION

Root exudates produced by plants may influence the growth of microorganisms by altering the chemistry of soil; thus, soil microbial communities may vary in structure and species composition. Soil from field plots where lignin peroxidase-producing transgenic alfalfa (*Medicago sativa* L.) was grown over two years had significantly higher population levels of culturable, aerobic spore-forming and cellulose-utilizing bacteria compared with that of the parental nontransgenic lines (*1*). Saxena and Stotzky observed no apparent effects of Bt toxin from Bt corn through root exudates on bacteria and fungi (*2*). However, they suggested that more detailed research needs to be conducted to determine the effects of the toxin on soil biodiversity. Donegan et al. found a transient but significant increase in culturable aerobic bacteria and fungi with two of three transgenic Bt cotton lines, which was attributed to unexpected changes in plant characteristics as a result of genetic manipulation or tissue culture (*3*). Current studies on the field-released transgenic papaya showed that soil microbial communities were affected, and there were significant differences in the total number of colony forming units (CFUs) (*4*). Consequently, the influence of transgenic crops on the soil microbial diversity is one of the major public concerns in Taiwan, and the risk assessment of the impact of genetically modified crops on soil microorganisms has been conducted in Taiwan since 2007.

Traditionally, the culture method is used to study the structure and diversity of the microorganisms and their relationship on ecosystem. However, the number of microorganisms typically cultured from soil represents 1% or fewer of the total microbial community, and uncultured microorganisms comprise the majority of the soil microbial diversity; thus, culture-based methods miss much of the soil microbial diversity information in environments. Recently, culture-independent molecular methods based on electrophoresis of PCR-amplified 16S rDNA fragments in DGGE are most commonly used to study the bacterial community diversity (*5*−*9*). Molecular analyses of soil communities by PCR−DGGE depend on the extraction of DNA directly from soils, and it is first necessary to set a standard method of isolating DNA from soil samples. However, it is difficult to suggest such a standard protocol for soil DNA extraction because of the complex matrix of soil.

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Table 1. Soil Textures and Crops of Soil Samples Tested

crop	texture (sample code)	sand (%)	silt (%)	clay (%)	H_2O (%)	organic matter (%)	organic carbon (%)	total Kjeldahl nitrogen (%)	cation exchange capacity, CEC (cmol/kg)	pH
corn	silty loam (S005)	19.0	56.0	25.0	9.9	2.0	1.1	0.13	10.5	5.2
blank	silty loam (S028)	28.3	54.0	17.7	16.0	1.2	0.6	0.11	7.0	6.5
nontransgenic papaya	clay loam (S036)	25.5	46.5	27.7	18.0	3.9	2.1	0.17	15.0	5.2
transgenic papaya	sandy loam (S026)	53.2	36.0	10.8	12.8	1.5	0.6	0.08	6.5	5.5
nontransgenic papaya	Sandy loam (S068)	61.0	23.0	16.0	6.5	1.9	1.0	0.11	9.0	4.4
nontransgenic papaya	loam (S040)	33.3	44.0	22.7	16.0	1.5	0.7	0.11	4.8	6.9

Soil DNA extraction is usually started by mixing water with soil samples to obtain good soil dispersion and homogeneity, and then soil supernatant is suspended in a buffer and lysed either mechanically (bead-beating) or chemically (SDS, sodium dodecyl sulfate; CTAB hexadecylmethylammonium bromide), or by a combination of above treatments. SDS and CTAB are used to disturb cell walls, and CTAB can also be used to remove some polyphenolic and other organic molecules such as polysaccharides. Adding detergents can help to release adsorbed DNA from soil particles. Lysoenzyme and proteinase K are often added in cell breakage steps. Proteinase K is used to digest contaminating proteins that interfered with the extraction of DNA. After the lysis, DNA is extracted by organic solvent phenol and chloroform and then precipitated from the aqueous phase of the organic extractions by addition of alcohol (2-propanol or ethanol). This crude soil DNA extract needs to be further purified, and there are many purification steps reported. Many of the protocols are effective on the soil types that were tested. The use of agarose gel electrophoresis to separate DNA from contaminated humic materials is a popular method (*10, 11*). Adding CTAB and increasing the salt concentration could also help remove humic materials (*11, 12*). PEG 8000 (9−11), polyvinylpolypyrrolidone (PVPP) (*14, 15*), CsCl−ethidium bromide density gradient centrifugation (*12, 16*), hexadecylmethylammonium bromide (CTAB) (*11*), and guanidine dodecyl sulfate (GTC) (*17*) were also be used for soil DNA purification.

The main purposes of the present work were: (1) to select a soil DNA extraction protocol used in our laboratory by comparing the ability of various methods previously described, in order to conduct PCR amplification from a wide range of soils, and (2) to apply PCR−DGGE for investigating the influence of virus-resistant transgenic papaya (expressing *nptII*, *pldmv*, and *prsv* genes) on the soil microbial community in an experimental confined field.

MATERIALS AND METHODS

Soil samples. Soil samples for DNA extraction studies were collected from six sites of different farm lands (S005, S026, S028, S036, S040, and S068) in Taiwan (**Table 1**). S005 soil was collected from a corn (*Zea mays*) field at Wufong. S028 soil was collected from a blank field (no crop was planted) at Wufong. S036 soil, S040 soil, and S068 soil were collected from three different fields where traditional papaya were planted at Beil-lian, Tai-mar-li of Taidong, and Tou-wu of Miaoli, respectively. S026 soil was collected from the experimental confined field at Bei-goul where virus resistant transgenic papayas were planted.

The experimental confined field was fenced and planted with 3 month-old different transgenic papaya (TP, lines 10−4, 12−4, and 14−3) and nontransgenic papaya (NTP) in four replicates. Only soil samples collected from experimental confined fields were used to study the influence of transgenic papaya (TP) on soil bacterial communities.

Soil Sample Preparation. Soil samples were randomly collected from each site. The upper 15 cm of soils were transferred to plastic bags by sterile garden trowels and were immediately transported to the laboratory for analysis. For each site, approximately three 500 g samples of the upper 15 cm of soil, 10−15 cm away from the trunk, were collected, and 25 g of each was thoroughly mixed by sieving (<2 mm) to obtain a single representative composite sample (subsample). Soil textures were determined by standards methods at the Soil Analysis Center in the Department of Soil, National Chung Hsing University.

DNA Extraction. *Extraction of Genomic DNA*. Genomic DNA from fresh leaves of transgenic papaya (*Carica papaya* L) (Line: 18−2−4) (*18*) was extracted by Lipp's CTAB-based method (*19*), and genomic DNA was amplified to yield a 398-bp PCR fragment, which was added to the soil DNA extracts to compare the PCR inhibition of different soil DNA extraction methods.

Extraction of Soil DNA. The six soils tested differed in type, organic matter content (OM), cation exchange capacity, and pH (**Table 1**), and four previously reported soil DNA extraction methods were applied to these soils: (1) the MoBio UltraClean Soil DNA kit (MoBio UC method, MoBio Laboratories, Inc., Solana Beach, CA); (2) Widmer's GS method (*14*); (3) Zhou's CS method (*11*), (4) Miller's BS method (*20*). Fresh soil samples of 0.1 g (in triplicates) were used to extract DNA (crude DNA) and was further purified by QIAquick gel before PCR amplification. QIAquick Gel extraction kit (Germany) uses a microcentrifuge method which is designed to purify DNA of 70-bp to 10 kb from standard or low-melt agarose gels. When the MoBio UC method was applied, the manufacturer's procedures were followed, and the DNA extracts obtained from the MoBio UC method were directly used as template DNA without gel purification for PCR amplification.

GTC-SDS Method (Widmer's GS method). Procedures developed by Widmer et al. (*14*) were followed, excepted for the addition of proteinase K and RNase A. RNase A was used to digest contaminating RNA that coextracted with DNA. Soil samples were added to 2-mL screw-cap plastic vials, mixed with 700 μL of extraction buffer (250 mM NaCl, 100 mM EDTA [pH 8.0], 2% SDS) and 2 μL of proteinase K (10 mg/mL) for 15 s, and 50 μL of 5 M guanidine isothiocyanate (GTC) was added, vortexed 15 s, heated at 68 °C for 1 h with vigorous shaking every 15 min, and centrifuged for 5 min (13 500*g*, room temperature). Supernatants were collected and 10 μL of RNase A (10 mg/mL) was added for 30 min. Then DNA was precipitated with 750 μL of 2-propanol at −20 °C overnight. After centrifugation for 30 min (13 500*g*, 4 °C), pellets were washed with 70% ethanol and dissolved in 100 μL of TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0) (crude DNA).

CTAB-SDS Gel Method (Zhou's CS method). Procedures developed by Zhou et al. (*11*) were followed. Soil samples were mixed with 0.27 mL of DNA extraction buffer (100 mM Tris-HCl [pH 8.0], 100 mM sodium EDTA [pH 8.0], 100 mM sodium phosphate [pH 8.0], 1.5 M NaCl, 1% CTAB), and 2 μL of proteinase K (10 mg/mL) was added to 1.5-mL Eppendorf tubes, followed by horizontal shaking at 225 rpm for 30 min at room temperature. After shaking, 0.03 mL of 20% SDS was added and incubated in a 65 °C water bath for 2 h with gentle inversions every 20 min. The supernatants were collected after centrifugation at 6000*g* for 10 min at room temperature and transferred into 1.5-mL centrifuge tubes. The soil pellets were extracted two more times by adding 0.09 mL of the extraction buffer and 0.01 mL of 20% SDS, vortexing for 10 s, incubating at 60 °C for 10 min, and centrifuging as before. Supernatants was collected, and 10 μL of RNase A (10 mg/mL) was added to 1.5 mL tubes, reacted at room temperature for 30 min, and then mixed with an equal volume of chloroform−isoamyl alcohol (24:1, vol/vol). The aqueous phase was recovered by centrifu-

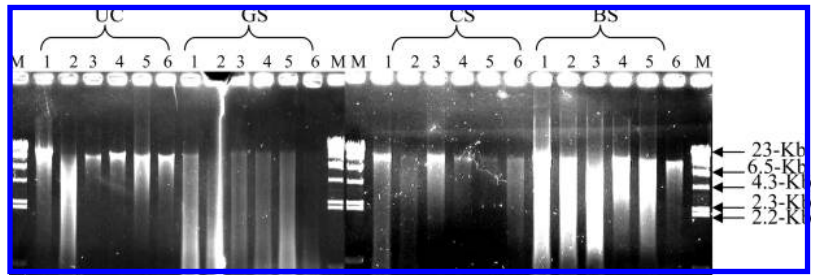

Figure 1. Comparison of soil DNA sizes obtained from the MoBio UC method (UC), Widmer's GS method (GS), Zhou's CS method (CS), and Miller's BS method (BS). Shearing of the DNA extracts were observed in all four methods, and DNA sizes were all less than 2.3 kb. Lanes 1, 2, 3, 4, 5, 6 were DNA from soil of S005, S026, S028, S036, S040, S068, respectively. Marker: Lambda/Hind III Digestion Ladder.

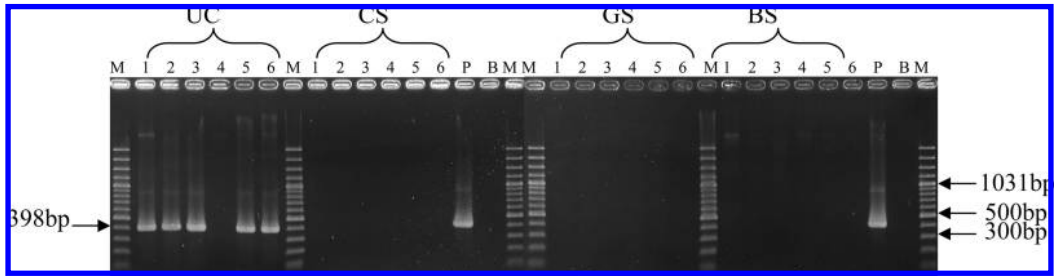

Figure 2. Crude soil DNA extracts mixed with the 398-bp fragment were tested for PCR inhibition. DNA obtained by the MoBio UC method (UC) could be used directly without gel purification, except for the soil S036 sample (lane 4). Soil DNA obtained form Zhou's CS method (CS), Widmer's GS methods (GS), and Miller's BS method (BS) failed to amplify 398-bp. Lanes 1, 2, 3, 4, 5, 6 were S005, S026, S028, S036, S040, S068, respectively. Marker: GeneRuler™ 100bp DNA Laddre plus.

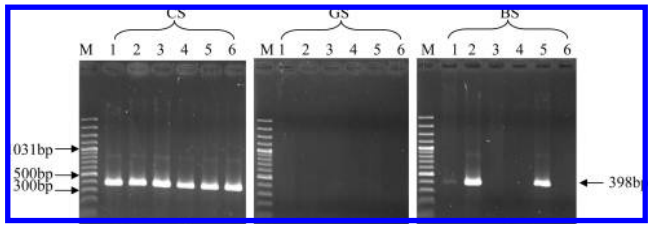

Figure 3. Purified soil DNA extracts mixed with the 398-bp fragment were tested for PCR inhibition. The results showed that purified DNA extracts from Zhou's CS method (CS) amplified successfully in all six soil samples, but PCR inhibitions were found in all DNA extracts obtained from Widmer's GS methods (GS), and some DNA extracts obtained from Miller's BS method (BS). Lanes 1, 2, 3, 4, 5, 6 were S005, S026, S028, S036, S040, S068, respectively. Marker: GeneRuler 100bp DNA Laddre plus.

gation and precipitated with 0.6 volume of 2-propanol (360 μL) at −20 °C overnight. The pellet obtained by centrifugation at 16 000*g* (20 min, room temperature) was washed with ice-cold 70% ethanol and dissolved in 100 μL of TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0) (crude DNA).

Bead-SDS Method (Miller's BS method). Procedures developed by Miller et al. (*20*) were followed, and the homogenization device tested was a Mini Bead Beater (BioSpec Product, Bartlesville, OK). Soil samples were added to 1.5-mL screw-cap plastic vials containing 2 g of sterile 0.1-mm (diameter) zirconium-silica beads. After treatment, 300 μL of phosphate buffer (100 mM NaH_2PO_4, pH 8.0), 300 μL of the SDS solution (100 mM NaCl, 500 mM Tris [pH 8.0], 10% [*w*/*v*] SDS), 300 μL of chloroform−isoamyl alcohol (24:1), and 2 μL of proteinase K (10 mg/mL) were added. Bead mill homogenization speeds were set at 2500 or 4200 rpm (2 min). Beads and soil were separated from suspensions by centrifugation (10 000*g*, 10 s), and the liquid extract was transferred to a sterile 2-mL Eppendorf tube. The liquid remaining in the interstices of the bead bed was collected by piercing the bottom of the tube with a hot needle and placing the pierced tube into a 15-mL screw-cap polypropylene tube containing a 1.5-mL Eppendorf tube with its top removed.

The nested tubes were then centrifuged for 15 min (1400*g*), the liquid drained into the lower 1.5-mL tube was pooled with the previously collected liquid from the sample, 10 μL of RNase A (10 mg/mL) was added (reaction 30 min, room temperature), and an equal volume of chloroform−isoamyl alcohol (24:1, *v*/*v*) was added for precipitation. The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of 2-propanol (360 μL) at −20 °C overnight. The pellet obtained by centrifugation at 16 000*g* (20 min, room temperature) was washed with ice-cold 70% ethanol and dissolved in 100 μL of TE (10 mM Tris HCl, 1 mM EDTA, pH 8.0) (crude DNA).

DNA Quality. DNA purity was determined by the absorbance ratios of A_{260}/A_{230} and A_{260}/A_{280} with an UV−vis diode array spectrophotometer (S2100, WPA, UK), and soil DNA yield was expressed as μg/g dry soil. Extracted DNA size was determined by electrophoresis (100 V, 23 min) on 1.5% (*w*/*v*) agarose gel (Amresco, OH). All determinations were performed in triplicate.

PCR Inhibition Assay. Crude soil DNA extracts and purified soil DNA extracts were added to PCR product of 398-bp fragments, respectively, and were tested for PCR inhibition. The PCR assay (50 μL) was conducted by adding soil DNA extracts and 398-bp fragment (total volume 5 μL) into a centrifugation tube followed by 34.5 μL of distilled deionized water, 5 μL of PCR buffer (10X buffer, PROtech Technologies, Inc.), 4 μL of dNTPs (2.5 mM), 0.5 μL of *Taq* DNA polymerase (2 units/μL, PROtech Technologies, Inc.), and 0.5 μL of each primer (MB Mission Biotech., 10 μM) (pBI 3677-F (CTGCCATCACGAGATTTCGAT)/pBI 4074-R (CCGAAGCCCAACCTTTCA)). Mixtures of template DNA were initially denatured at 94 °C for 3 min. In subsequent cycles, denaturation was carried out at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min for 35 cycles. PCR products were analyzed by electrophoresis using 1.5% (*w*/*v*) agarose gels for visualization of DNA with reaction of ethidium bromide under 527-nm UV transillumination.

DGGE Analysis of 16S rDNA. Soil DNA extracts obtained from soil samples where different transgenic papaya and nontransgenic papaya were grown at the experimental confined field were analyzed for bacterial diversity by the DGGE method. Bacterial 16S rDNA genes were amplified using the primer 341fGC (GC clamp + CCTACGGGAGGCAGCAG)/534r (ATTACCGCGGCTGCTGG) (234bp, *7*). The PCR reaction mixture (50 μL) was composed of 0.5 μL of *Taq* DNA polymerase (2 units/μL, PROtech Technologies, Inc.), 4 μL of dNTPs (2.5 mM), 5 μL of PCR buffer (10X buffer, PROtech Technologies, Inc.), 0.5 μL of each primer (MB Mission Biotech., 10 μM), 5 μL of soil DNA extract, and 34.5 μL of distilled deionized water. PCR

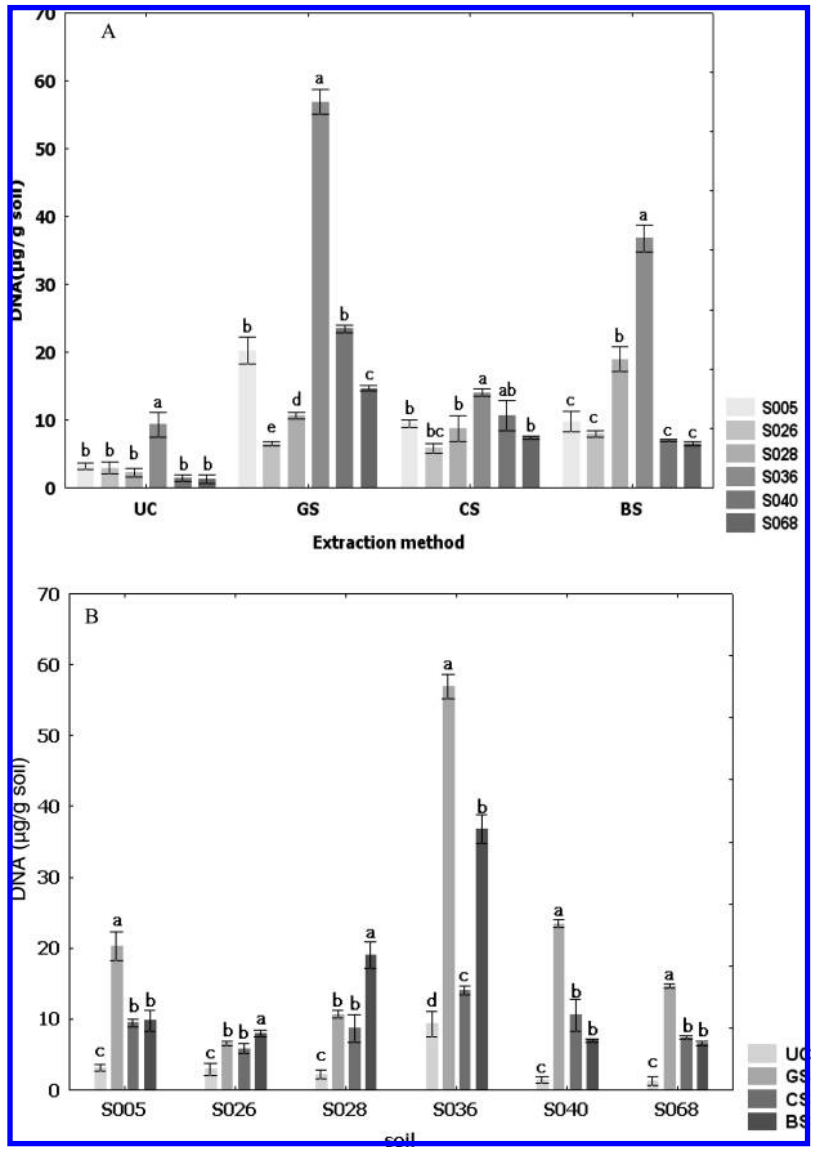

Figure 4. Purified soil DNA yields (μg/g soil) obtained from different extraction methods (A) in different soil samples (B). Values represented the means of three replicates with associated standard errors. Different letters indicate a significant difference at $p < 0.05$.

amplification conditions for 234-bp were as follows: 94 °C for 5 min; 30 cycles of 94 °C, 45 s; 54 °C, 45 s; 72 °C for 1 min followed by a final extension at 72 °C of 5 min.

Denaturing gradients were prepared in accordance with the method of Muyzer et al. (*7*) performed with the DCode Universal Mutation Detection System (Bio-Rad). PCR samples were loaded onto 8% polyacrylamide gels containing a denaturing gradient ranging from 30 to 70% (where 100% denaturant corresponds to 7 M urea and 40% (*v*/*v*) formamide, and the gels were run in 0.5x TAE buffer (40 mM Tris base, pH 7.4, 20 mM sodium acetate, 1 mM EDTA). Electrophoresis was performed at constant voltage (70 V) and temperature (60 °C) for 15 h. After electrophoresis, the gels were stained with SYBR Green I, dried, and photographed. The banding patterns were analyzed by the NT-SYS program (Exeter Software, NY) by using the unweighted pair group with the mathematical averages method (UPGMA).

Statistical Analysis. Statistical analysis was performed using SAS statistical software (Statistica, V.7.0, SAA Institute). The difference between treatment means was analyzed using the least significant difference (LSD) test at the 5% level. A p value of <0.05 was considered statistically significant. Linear and multiple regression analysis between soil DNA yields and selected soil properties were applied, and the regression correlations between extraction methods and soil properties were conducted.

RESULTS

DNA Yields and Quality for PCR. Extracted soil DNA sizes were all less than 2.3 kb, and DNA sheared severely in Miller's BS method (**Figure 1**).

The crude DNA extracts obtained from Zhou's CS method, Widmer's GS method, and Miller's BS method were inhibitory to PCR, because no 398-bp product was generated (**Figure 2**). Only the DNA extracts obtained from MoBio UC method were not inhibitory to PCR, because positive PCR reactions which yielded 398-bp fragment were found (**Figure 2**). DNA extracts from the MoBio UC kit method could be used directly for PCR amplification, except for the soil DNA extracts from S036 soil sample, because the PCR reaction failed to amplify successfully (**Figure 2**, lane 4), and a successful PCR reaction was obtained when the DNA was further purified by gel method (data not shown). The UC method is also good for DNA extraction, but the cost may be higher than the other methods if a large amount of routine DNA extraction is involved. Successful PCR amplifications were also detected when crude soil DNA extracts obtained from Zhou's CS method were purified by QIAquick gel (**Figure 3**), but the effects of gel purification were limited on Widmer's GS method and Miller's BS method (**Figure 3**).

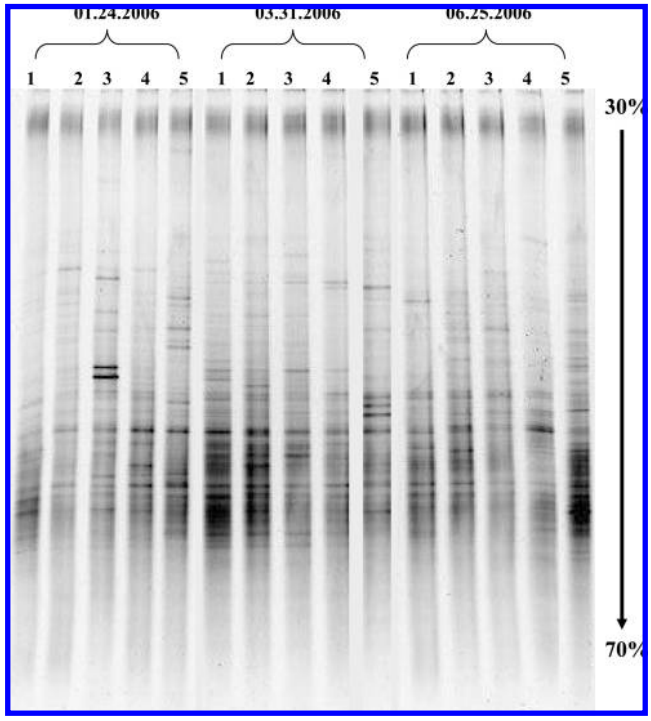

Figure 5. DGGE profiles of bacterial16S rDNA fragments amplified with primer pair 341fGC/534r (234-bp). Soil samples were taken from the experimental confined field where three different virus-resistant transgenic papaya and nontransgenic papaya were planted. Lane 1: nontransgenic papaya; lane 2: transgenic papaya of line 10−4, lane 3: transgenic papaya of line 12−4, lane 4: transgenic papaya of line 14−3; lane 5: control soil (CK), no papaya was planted.

The DNA yields obtained from Widmer's GS method and Miller's BS method were higher than the yields from the other two methods, and the lowest DNA yields were observed in the MoBio UC method (**Figure 4A**). The purified DNA yields varied with extraction method and soil samples. The lowest DNA yield (0.6 μg/g dry soil) was found in S068 soil extracted by the MoBio UC method, whereas the highest DNA yield (55.1 μg/g dry soil) was found in S036 soil extracted by Widmer's GS method (**Figure 4B**).

Although soil DNA extracted by Zhou's CS method resulted in a lower yield than that from Widmer's GS method and Miller's BS method (**Figure 4A**), the DNA quality was better than the other methods, as PCR amplification was of concern (**Figure 3**). Therefore, Zhou's CS method plus QIAquick gel purification was recommended in our laboratory for extracting soil DNA for investigating the change in the bacterial community by PCR−DGGE.

DGGE. DGGE analysis based on PCR amplification of bacterial 16S rDNA fragments (234-bp) revealed that the bacterial communities in different soils collected from the experimental confined field could be distinguished (**Figure 5**). The bacterial communities in each soil where different papaya were planted did change over time; however, at the end of the experiment (June, 2006) no overall large bacterial difference existed in soils where different papaya were planted (**Figures 5** and **6**). DGGE patterns showed that the levels of similarity of soil bacteria in different soil samples ranged from 0.563 to 0.896 (**Figure 6**). DGGE patterns and the dendrogram also depicted that there were some differences in bacterial composition at the beginning of planting (Jan 2006), but the differences were reduced after six months (**Figure 6**). For example, the similarities of bacterial communities in soils between nontransgenic papaya and transgenic papaya were in the range of 0.771 to 0.857 at January, decreased to the range of 0.708 to 0.750 at March, and then increased to the range of 0.750 to 0.896 at June (**Figure 6**). This result indicated that the influence of transgenic papaya on the soil bacterial community was limited (similarity >70%) at the beginning of planting, and the differences of bacterial communities were reduced (similarity >75%) in soils collected from transgenic papaya sites and nontransgenic papaya sites after six months.

DISCUSSION

Several studies have indicated that the soil DNA extracts were shearing in the agarose gel (**Figure 1**) (*11, 13, 14, 20−23*), and severe shearing of DNA was attributed to the harsh lysis procedure (*20*). However, the sheared DNA obtained from the MoBio UC method did not affect the PCR assays (**Figure 2**), and the sheared DNA obtained from Zhou's CS method was also suitable for the PCR reaction (**Figure 3**).

The color of S036 soil sample was dark, and it consisted of the highest amounts of organic matter (3.9%), organic carbon (2.1%), and CEC (15.0 cmol/kg), more than the other five soil samples tested. This indicated that high organic matter in S036 might induce high microbial activities in S036, which would result in the increase of total DNA in the soil. This might also be the reason for the unsuccessful PCR amplification of DNA extracts of S036 by the MoBio UC kit method. We do not know the capacity of the MoBio UC kit to remove humic acids; however, based on this study, use of the MoBio UC kit to extract DNA from soil of high organic matter should be evaluated by PCR amplification. Ikeda et al. (*24*) reported the same conclusion, that the UltraClean soil DNA isolation kit (MoBio UC kit method) failed to yield detectable amounts of DNA.

DNA yields in our studies compared favorably with the values obtained from other reports. Berthelet et al. (*25*) reported that DNA yields ranged from 4.6 to 33.0 μg/g soil, Blum et al. (*26*) reported that the total soil DNA extracted ranged from 50 to 207 μg/g soil, and Arlene and Armstrong (*27*) reported that DNA concentrations in soils ranged from 9 to 25 μg/g soil.

The purified DNA yields of Miller's BS method ranged from 6.8 μg/g soil for S068 soil to 38.3 μg/g soil for S036 (**Figure 4A**). The yield of DNA was comparable to that previously reported (*20, 28*). The DNA yields obtained by the bead beating method with agricultural soil and forest soil were 14.7 and 75.6 μg/g soil, respectively (*20*), and the beat beating direct lysis method extracted soil DNA between 15 and 23.5 μg/g soil (*28*).

Extraction of soil DNA from soil environments always resulted in coextraction of humic substances which inhibited the activity of *Taq* DNA polymerase in the PCR reaction (*29, 30*). The SDS-based method is reported to be a good soil extraction method (*11*), and our data also indicated that SDS-based methods with CTAB treatment (Zhou's CS method) plus gel purification and the SDS-based method of bead treatment (Miller's BS method) plus gel purification produced a higher DNA yield than the MoBio UC method (**Figure 4A**). Zhou's CS extraction method plus gel purification yielded strong PCR products of 398-bp (**Figure 3**), and soil DNA extracts by Zhou's method achieved strong bands for 16S rDNA amplification (*31*).

The effect of gel purification on DNA quality was good but still not an effective method to remove all contaminants, because the ratios of A_{260}/A_{230} ranged from 0.2 to 1.3, and the ratios of A_{260}/A_{280} ranged from 0.9 to 1.4 (data not shown). Although the removals of the dark brown color from the DNA extracts were obtained, gel purified soil DNA extracts probably still contained greater amounts of high-molecular-weight humic acids that could

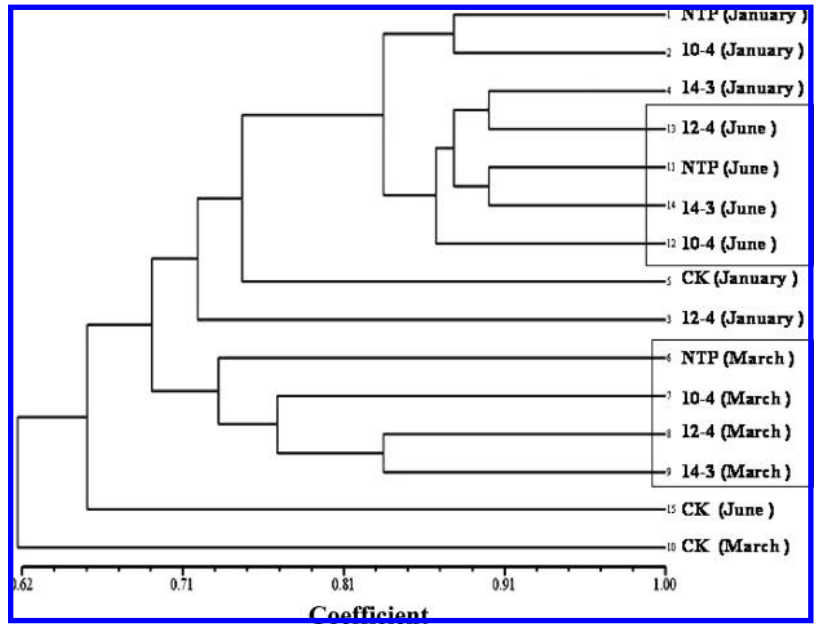

Figure 6. Dendrogram of PCR−DGGE profiles based on bacterial 16S rDNA sequence (234-bp). The bacterial similarities were low between the soil from transgenic papaya and the soil from nontransgenic papaya at the first month of planting (Jan, 2006), but the similarity increased six months later (June, 2006). NTP: nontransgenic papaya; 10−4, 12−4, and 14−3: three transgenic papaya lines; CK: control soil, no papaya was planted.

Table 2. Multiple Regression Analysis between Soil DNA Yields and Soil Characteristics Selected ($P < 0.05$)

method	regression	R^2
UC	$Y_{DNA} = -7.49 - 0.28X_{Clay} + 0.67X_{OM} + 0.42X_{CEC} + 0.35X_{pH}$	0.99
BS	$Y_{DNA} = -53.29 - 0.28X_{Clay} + 0.17X_{OM} + 0.97X_{CEC} + 0.65X_{pH}$	0.94
CS	$Y_{DNA} = -11.32 + 0.31X_{Clay} + 0.69X_{OM} + 0.41X_{CEC} + 0.63X_{pH}$	0.99
GS	$Y_{DNA} = -60.01 + 0.15X_{Clay} + 1.12X_{OM} - 0.05X_{CEC} + 0.30X_{pH}$	0.99

not be washed through filters with lower molecular-weight cut offs in gel method (70 kb−10 kb). Yeates et al. (*28*) reported that the purified DNA extracts were still brown in color (ratios of A_{260}/A_{230} and A_{260}/A_{280} were 1.82 and 1.69, respectively), and Porteous and Armstrong (*12*) reported similar result that the ratios of purified soil DNA extracts were 0.6 to 0.8 for A_{260}/A_{230} and 1.2 to 1.3 for A_{260}/A_{280}. Although each of the four methods of soil DNA extraction has advantages and disadvantages, our data indicated that Zhou's CS method would be a better choice than the other three methods if the PCR reaction and the cost of material are of concern.

The results from soil DNA extraction indicated that clay content and pH of different soil types tested did not affect the DNA extraction efficiencies, because the linear relationship ($Y = \beta_0 + \beta_1 X$) between soil DNA yields (Y) and selected soil properties (X) showed that the coefficient of determinations was very low ($R^2 < 0.1$). The organic matter content of soil showed significant positive correlations with the DNA yield in Widmer's GS method ($r = 0.93$, $p = 0.005$) and the MoBio UC method ($r = 0.92$, $p = 0.007$); however, nonsignificant but positive correlations were observed in the treatments of Zhou's CS method ($r = 0.78$, $p = 0.067$) and Miller's BS method ($r = 0.78$, $p = 0.05$).

When multiple soil characteristics (X_n) were included for analysis the correlation with DNA yield ($Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4$), the effects of selected soil characteristics were not significant ($p > 0.05$) in all four methods (**Table 2**), although the coefficient of determinations were high ($R^2 = 0.94-0.99$) (**Table 2**). A high coefficient of determination of the DNA yields from four soil DNA extraction methods indicated that the interactions of the soil properties selected were strongly connected with DNA yields, but no significant overall correlations were observed ($p > 0.05$) (**Table 2**). The organic matter content (%) in soils showed positive correlations and high coefficients of determinations to the soil DNA yields in all four extraction methods (**Table 2**). Soil organic matter content (%) was more correlated with DNA yield in the MoBio UC method ($r = 0.67$, $p = 0.20$), Widmer's GS method ($r = 1.12$, $p = 0.67$), and Zhou's CS method ($r = 0.69$, $p = 0.17$), CEC was more correlated with DNA yield in Miller's BS method ($r = 0.97$, $p = 0.43$), but all these correlations were not significant ($p > 0.05$) (**Table 2**).

DGGE Profiles. The DGGE profiles and dendrogram showed some differences in soil bacterial composition between the soil samples collected from transgenic papaya sites and nontransgenic papaya sites during the test period. The differences reached the highest level three months after planting, and then the differences decreased after six months. The reason for the high difference in the first three months after planting is not clear, but the influences on soil microorganisms are transient and temporary; therefore, the soil bacteria could recover from its initial impact in several months. The similarities of bacterial community were in between 0.563 and 0.896 in our studies (**Figure 6**), and Hsieh and Pan (*32*) also reported results similar to ours; they reported that the similarity of soil microorganisms of upper layer soils around the transgenic papaya planting area and around nontransgenic papaya planting area was about 80%.

Wei et al. (*4*) reported that there were significant differences in the total number of colony forming units (CFUs) of bacteria, actinomycetes, and fungi between soils planted with RP-transgenic and nontransgenic plants. It indicated that further studies on the influences on the CFUs of bacteria, actinomycetes, and fungi between soils planted with transgenic crops and nontransgenic crops are important.

Conclusion. We evaluated four soil DNA extraction methods, and soil DNA extracts by Zhou's CS method with gel purification was recommended for DGGE to monitor the microbial diversity

in soil. The results showed that there were some differences in the bacterial diversity between the soil collected from transgenic papaya sites and from nontransgenic papaya sites at the first month of planting, but that the differences decreased after six months. The results also indicated that clay content (10.8−25.0%) and pH (4.4−6.9) of different soil samples we tested did not affect the DNA extraction efficiencies, but positive correlations were found between the organic matter content of soil and the DNA yield in the MoBio UC method, Widmer's GS method, and Zhou's CS method. The coefficient of determination (R^2) between organic matter content and DNA yield was higher than that between clay content, CEC, and pH, indicating that organic matter content was more correlated with DNA yield than the clay content, CEC, and pH in the soil samples we tested.

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