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METHODS

Comparison of methods for total community DNA extraction and purification from compost

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Abstract The differences on DNA yield and purity of three different DNA extraction protocols were compared with regard to the use for PCR and other molecular analyses. Total DNA was extracted from compost by the three protocols, and then was purified by spin-bind cartridges after being precipitated by PEG8000. The detection performed on a nucleic acid and protein analyzer showed that all three methods produced high DNA yields. The agarose gel electrophoresis showed that the fragments of crude and purified DNA had a length of about 23 kb. A eubacterial 16S rRNA gene-targeted primer pair was used for PCR amplification, and full length 16S rDNAs were amplified from all the purified DNA samples. After being digested by restriction endonucleases, the restriction map of amplified rDNA showed identical genetic diversity. The products of PCR using primer pair GC341F and 907R were also used for denaturing gradient gel electrophoresis analysis. The results indicated that high-quality DNA was extracted from compost by the three protocols, and each of the protocols is adapted to extract microbial genome DNA from compost expediently and cheaply.

Keywords Molecular ecology · Vegetable waste compost · 16S rDNA · DNA extraction · ARDRA · DGGE

Introduction

Composting is the biological conversation of biodegradable solid waste into usable end products such as fertilizers, substrates for mushroom production, or biogas (methane) (Goyal et al. 2005; Peters et al. 2000). Traditional approaches for studying compost microbial communities are cultivation-dependent techniques. However, it is generally accepted now that most microbes in nature are uncultivatable (Amann et al. 1995; Torsvik and Øvreås 2002; Ward et al. 1992), the microbial diversity in compost is abundant and variable (Ishii et al. 2000), and the traditional cultivation-dependent approaches to analyze the microbial diversity could not show the real-time succession of the microbial community, so that it could not satisfy us for compost microbe studies (Burtscher and Wuertz 2003). Molecular ecology introduces new techniques to microbiology ecologists for studying microbial communities, especially unculturable microbes in various environments. Molecular ecological analysis of microbial diversity in complex environmental samples, such as soil and compost (Cahyani et al. 2004; Dees and Ghiorse 2001; Franke-Whittle et al. 2005; Peters et al. 2000; Schloss et al. 2000; Tiquia et al. 2005), has been proven to be a powerful technique.

Molecular ecological analysis of a microbial community in compost requires efficient and unbiased DNA extraction and purification methods. Direct extraction of DNA from environmental samples yields more DNA, contains less humic acids, requires shorter time, and introduces less bias than methods in which cells are separated from the sample matrix before DNA extraction (von Wintzingerode et al. 1997). A large number of methods have been published for the extraction and purification of total community DNA from environmental samples such as soils and sediments for molecular ecological analysis of microbial communities (Bürgmann et al. 2001; Jiang et al. 2005; Martin-Laurent et al. 2001; Porteous et al. 1997; Tsai and Olson 1991; Zhou et al. 1996). Because of the variety of microbes species and

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the enzyme inhibitors such as humic acids in compost, it seems to be much more difficult for high-quality microbial DNA extraction and purification from compost than that from other environmental samples, so that only few methods have been developed for extracting and purifying total DNA from compost (Howeler et al. 2003; LaMontagne et al. 2002).

In this study, total DNA was extracted from vegetable waste (VW) compost by three different protocols (LL, UL and PL) and then was purified with PEG8000 and spin-bind cartridges. We evaluated these three DNA extraction methods and the purification procedure based on total DNA yield, DNA purity, DNA recovery, humic acids concentration, and PCR amplifiability using universal eubacterial 16S rDNA primer pair. We also used the amplified ribosomal DNA restriction analysis (ARDRA) and denaturing gradient gel electrophoresis (DGGE) analysis to compare the genetic diversity in the amplified 16S rRNA genes to make sure that there was no bias introduced by different methods. Based on the results of the comparison, we found that all three extraction methods were efficient and could be used for compost DNA extracting.

Materials and methods

Compost samples

The compost samples were taken from a 20-l VW composting reactor charged with nonsterile tap water, dried VW, and garden soil. In the reactor, about 10 kg dried VW, 3 kg garden soil, and 7 kg water were mixed to obtain a C/N ratio of 18 and initial moisture content of 55% wet basis (mass of water/mass of wet solids) (Schloss et al. 2005). For microbiological analysis, a 20-g of sample was taken from the VW composting reactor at 22°C, after reaching a peak of 46°C, and immediately stored at −20°C until the sample was used for sample pretreatment, cell lysis, and DNA extraction (see below).

Sample pretreatment

Equal weights (1 g) of compost sample and 4 ml phosphate buffer (0.12 mol l^{-1}, pH 8) (LaMontagne et al. 2002) were added sequentially to 9–5-ml polypropylene tubes and shaken at room temperature on an orbital shaker for 5 min at 150 rpm. After centrifugation at 4°C for 10 min at 6,000×*g*, the pellets were washed once again.

DNA extraction

The microbial cells in the washed compost samples were lysed with three different protocols designated LL, UL, and PL.

1. Protocol LL. This protocol had some modification to that which LaMontagne et al. had reported (LaMontagne et al. 2002). Three pellets were resuspended in 1.5 ml of lysozyme solution (0.15 mol l^{-1} NaCl, 0.1 mol l^{-1} Na_2EDTA, 10 mg ml^{-1} lysozyme, pH 8), 0.5 ml lysis buffer [10% sodium dodecyl sulfate (SDS), 0.1 mol l^{-1} NaCl, 0.5 mol l^{-1} Tris–HCl, pH 8], and 0.5 ml phosphate buffer (0.1 mol l^{-1}, pH 8) and shaked at 37° C on an orbital shaker at 225 rpm for about 30 min, and 0.5 ml chloroform/isoamyl alcohol (24:1) was added into the tubes. Then, the tubes were homogenized at 2,800 rpm for 10 min and centrifuged (6,000×*g*, 3 min). The upper layers were transferred to fresh tubes and 0.5 ml sterile deionized water was added into the former tubes to wash the pellets with centrifugation (6,000×*g*, 1 min). Both the upper layers were mixed and centrifuged at 12,000×*g* for 5 min, and then were treated with 0.6 vol of isopropanol for 1 h at room temperature. The mixtures were centrifuged at 16,000×*g* for 10 min, and the precipitated crude DNA was washed twice with 0.7 ml ice-cold 70% ethanol. The crude DNA was dissolved in 600 µl TE buffer (10 mmol l^{-1} Tris–Cl, 1 mmol l^{-1} EDTA, pH 8) after being dried under vacuum.
2. Protocol UL. This protocol was modified from a previously reported method for soil DNA extraction (Krsek and Wellington 1999). Three washed samples were added into 10 ml isopropanol and then lysed by ultrasonic with a Model 450 sonifier (Branson, Danbury, CT, USA) for 10 min. Then, the mixtures were centrifuged at 16,000×*g* for 5 min and the pellets were transferred to fresh 5-ml tubes and were mixed with 0.5 ml lysis buffer (10% SDS, 0.1 mol l^{-1} NaCl, 0.5 mol l^{-1} Tris–HCl, pH 8), 0.5 ml phosphate buffer (0.1 mol l^{-1}, pH 8), 0.5 ml chloroform/isoamyl alcohol (24:1), and 1.0 g glass beads (0.2 mm). The mixtures were homogenized at 2,800 rpm for 10 min and then were centrifuged and washed with 0.6 vol isopropanol as described in protocol LL.
3. Protocol PL. This method was widely used for soil DNA extraction according to previous reports (Zhang et al. 2003; Zhou et al. 1996) and was modified to extract compost genomic DNA in this study. Three washed samples were mixed with 1.5 ml extraction buffer (0.1 mol l^{-1} Tris–Cl, 0.1 mol l^{-1} EDTA, 0.1 mol l^{-1} sodium phosphate, 1.5 mol l^{-1} NaCl, 1% cetyltrimethylammonium bromide, pH 8) and 10 µl proteinase K solution (10 mg ml^{-1}). After shaking at 37°C on an orbital shaker for 30 min at 225 rpm, 200 µl of 10% SDS was added into the tubes and then the mixtures were incubated in a 65°C water bath for 1 h with agitation at 15- to 20-min intervals. Then, the mixtures were centrifuged at 6,000×*g* for 5 min at room

temperature. After the upper layers were transferred to fresh tubes, 0.5 ml extraction buffer and 50 μl of 10% SDS were added into the primary tubes to wash the pellets with homogenizing at 2,800 rpm for 30 s, and then the mixture was incubated in a 65°C water bath for 10 min. The mixtures were centrifuged at 6,000×*g* for 5 min and both the upper layers were mixed, and the pellets were treated once again. All the upper layers were mixed with 1× volume of chloroform/isoamyl alcohol (24:1) by shaking gently by hand. The aqueous layers were transferred to clean tubes after centrifugation (6,000×*g*, 5 min) and were precipitated with 0.6 vol of isopropanol for 1 h. The pellets of crude DNA were washed twice with 0.7 ml ice-cold 70% ethanol and dried under vacuum after centrifugation (16,000×*g*, 5 min, 4°C). The crude DNA was dissolved in 600 μl TE buffer and stored at −20°C for future use.

DNA purification

The crude DNA was precipitated by adding 0.5 vol of 50% (w/v) PEG8000 and 0.1 vol of 5 mol l^{-1} NaCl. The samples were mixed by inverting gently and were incubated for more than 1 h or overnight at 4°C. Then, the precipitated DNA was added into a spin-bind DNA purification cartridge. In a spin-bind cartridge, the DNA bound to a microporous membrane in the presence of chaotropic salts buffer, and after the cartridge within the crude DNA was spun for 1 min at 12,000×*g* at 4°C and the cartridge was washed twice with 0.7 ml ice-cold 70% ethanol, the DNA could be eluted from the air-dry cartridge with 200 μl heat TE buffer (65°C). After being added 10 μl RNaseA (10 mg ml^{-1}) and incubated in 37°C for at least 2 h, the DNA was stored in −20°C for future use.

DNA recovery

Nine 1-g VW compost samples were washed with phosphate buffer (120 mmol l^{-1}, pH 8) and sterilized at 121°C for 20 min. After incubating with DNaseI (Worthington, Freehold, NJ, USA) and RNaseA (Amresco, Solon, OH, USA) at 37°C for 24 h, the samples were sterilized at 121°C for 20 min once again. Then, nine 20-μg pure DNA (Sangon, Shanghai, China) were added into the sterile samples, and each of the three samples was treated with protocol LL, UL, or PL before purification. DNA concentrations were quantified on a DU640 Nucleic Acids and Protein Analyze (Beckman Coulter, Chaska, MN, USA).

Humic acids and DNA concentration measurements

Humic acid amounts were tested by detecting absorbance read at 340 nm, and a standard curve was created by making serial dilutions (0.1–100 ng μl^{-1}) of commercial humic acids mixture (Aldrich Chemical, Milwaukee, WI, USA). The effects of DNA and protein contaminants on humic acids measurements were checked by adding either 10 ng μl^{-1} DNA (Sangon) or 2 μg μl^{-1} bovine albumin (Amresco) to samples of 50 ng μl^{-1} of humic acids. Absorbance measurements were made on a UV spectrophotometer. After the DNA samples had been diluted with TE buffer until an absorbance at 340 nm between 0.01 and 0.1 was obtained, the $A_{260/280}$ ratios and the concentrations of DNA were quantified on a DU640 Nucleic Acids and Protein Analyze (Beckman Coulter).

Microscopic evaluation of cell lysis

Intact VW compost samples or samples treated with phosphate buffer and subjected to the lysis procedures were stained with 0.01% acridine orange and examined with either a Zeiss (Oberkochen, Germany) Standard 18 microscope under phasecontrast and epifluorescence viewing or a Zeiss laser scanning microscope (model LSM-10) equipped for fluorescence, phase, and differential interference contrast imaging under 488-nm light from an argon laser (Moré et al. 1994). The LSM-10 is configured so that a single field of view can be examined by conventional transmitted and epifluorescence illumination or by comparable laser-scanning illumination. Both microscopes are fitted with ×100 oil immersion objective lenses with numerical apertures of 1.3 or 1.4. An acridine orange direct count agar-smear procedure was used to assess the extent of lysis of the endospores and enumerate the total number of cells in the intact VW compost samples. The computerized imaging and analysis systems of the LSM-10 were used to document the size distribution of microbial cells surviving the various lytic procedures. In enumerating cells in the compost prior to implementing lytic procedures, the average count and standard deviation were computed from duplicate smears prepared from three independent subsamples of the compost as described previously (Beloin et al. 1988). In lysis experiments, the same general procedure was followed.

PCR amplification

Bacterial 16S rDNA presented in the community for amplified ribosomal DNA restriction analysis (ARDRA) was PCR-amplified using the universal eubacterial primers: 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) forward (Adachi et al. 2001; Hoti et al. 2003) and 1495R (5′-CTA CGG CTA CCT TGT TAC GA-3′) reverse (Hoti et al. 2003), and the primer pair 341F+GC (5′-CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′) and 907R (5′-CCG TCA ATT CCT TTG AGT TT-3′) (Muyzer et al. 1998)

was used for amplifying partial 16S rDNA fragments for DGGE analysis. Each 50-μl PCR reaction mixture contained 1 μl of compost DNA, 5 μl of 10×buffer, 5 μl of 25 mmol l^{-1} deoxyribonucleotide triphosphate mixture, 1 μl of 10 μmol ml^{-1} each primer, 1 μl of 2.5 U μl^{-1} Taq DNA polymerase, 0.6 μl of 10 mg ml^{-1} bovine serum albumin (BSA) (Sangon), and 35.4 μl of sterilized ultrapure water. PCR amplification was run using the following cycling conditions: 5 min at 94°C; 30 cycles with each cycle consisting of 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C; followed by a final 10-min extension at 72°C. Samples were stored at −20°C before being analyzed. PCR samples were visualized on 1% agarose gel run at 10 V cm^{-1} for 30 min before being stained with 0.5 μg ml^{-1} ethidium bromide (EtBr) for 20 min, destained in distilled water for 10 min, and visualized under a Gel Doc 2000 System (BioRad, Hercules, CA, USA).

Amplified ribosomal DNA restriction analysis

Aliquots (12 μl) of purified 16S rDNA of VW compost were digested with 2 U restriction endonucleases *Alu*I or *Hae*III for more than 4 h at 37°C to produce a mixture of variable-length 16S rDNA fragments. Restriction digestion fragments were separated by gel electrophoresis for 1 h at about 5 V cm^{-1} using 3% agarose, and a digital image of the EtBr-stained gels was recorded and compared using the Gel Doc 2000 System (BioRad). Band sizes in the pattern were estimated by using a 100-bp ladder as a size standard.

Denaturing gradient gel electrophoresis analysis

The DGGE was carried out using a DCode™ Universal Detection System instrument and gradient former model 475 according to the manufacturer's instructions (Bio-Rad). The acrylamide concentration in the gel was 6% and the denaturing gradient was 35 to 70%. The 100% denaturant solution contained 7 M urea, 40% (v/v) formamide, 6% acrylamide/bis-acrylamide (37.5:1) and 0.5×Tris-acetate-EDTA (TAE) buffer (pH 8) in ultrapure water. The 0% denaturant solution contained 6% acrylamide/bis-acrylamide (37.5:1) and 0.5×TAE buffer (pH 8) in ultrapure water. Twenty-microliter purified PCR products were mixed with loading buffer and transferred to the bottom of the gel. Gels were run in 0.5×TAE buffer at 55°C for 14 h at 120 V (Muyzer et al. 1998). Gels were stained with SYBR™ Green I and digitized in UV light with the Gel Doc 2000 System (Bio-Rad).

Results

Humic acids concentration

The absorbance read at 340 nm yielded accurate results (data not shown) over the humic acids concentration range of 0.1–100 ng μl^{-1}. Because samples containing 50 ng μl^{-1} of humic acids and either 10 ng μl^{-1} DNA or 2 μg μl^{-1} BSA were indistinguishable from samples containing only humic acids concentrations, humic acid concentrations were quantified by the method of absorbance at 340 nm on a UV spectrophotometer in the later experiments. The data shown in Table 1 indicate that all three protocols had lower yields of humic acids than those that had been reported (Howeler et al. 2003; LaMontagne et al. 2002) and that the purification for humic acids removal was of high efficiency.

Cell lysis efficiency

Direct microscopic count of microorganisms with a Zeiss Standard 18 microscope, before and after the samples were lysed, was an effective approach for evaluating the efficiency of different lysis methods. Direct microscopic counts of microorganisms before or after cell lysis showed that the DNA extraction protocols LL, UL, and PL had cell lysis efficiencies of (94.1±0.68), (90.0±0.66), and (96.3±0.84) percent, respectively (Table 2). Compared with the diverse cell morphologies observed in the prelysis samples, the surviving cells were predominantly small, round individual cells.

DNA yield and recovery efficiency

Crude or purified total VW compost DNA was visualized under a Gel Doc 2000 System (BioRad) after electrophoresis and being stained with EtBr. The visualization indicated that most of the crude DNA and the purified

Table 1 Concentrations and amount of humic acids in crude or purified DNA based on spectrophotometry

Protocol	Concentration in crude DNA (ng μl^{-1})	Total humic acids in crude DNA (mg)	Concentration in purified DNA (ng μl^{-1})	Total humic acids in purified DNA (mg)	The efficiency of purification for humic acids removal (%)
LL	72.5±6.25	17.4±1.50	15.2±2.2	0.30±0.04	98.3±0.3
UL	90.0±7.00	21.6±1.68	18.0±2.8	0.36±0.06	98.3±0.2
PL	75.0±8.75	18.0±2.10	16.0±3.1	0.32±0.06	98.2±0.2

Table 2 Direct counts of microorganisms with a Zeiss microscope for cell lysis efficiency evaluation

Protocol	Before lysis ($\times 10^9$ cells g^{-1} compost)	After lysis ($\times 10^9$ cells g^{-1} compost)	The efficiency of cell lysis (%)
LL	8.26±0.85	0.49±0.27	94.1±0.68
UL	8.73±0.91	0.87±0.31	90.0±0.66
PL	8.60±0.93	0.32±0.15	96.3±0.84

DNA obtained from different procedures had a length of about 23 kb with the range from 6–23 kb (Fig. 1).

Because the crude DNA yields related to the cell lysis efficiencies and the number of microbial cells in VW compost samples, protocol PL yielded the most amount of DNA when protocol UL yielded the least DNA (Table 1). Additionally, data shown in Table 3 indicate that all three protocols produced good DNA yields and that the purification was efficient. Data shown in Table 3 also indicated that the procedures were efficient on DNA recovery with DNA recovery efficiencies of (94.0±1.5), (93.0±1.5), and (94.5 ±2.0) percent for protocols LL, UL, and PL, respectively.

DNA purity

The $A_{260/280}$ ratios of 1.7–1.8 of purified DNA, determined by the DU640 Analyze, showed that the purification yielded very pure DNA. The purity of the final purified VW compost DNA was also checked by the ability of PCR to amplify a region of the 16S rDNA with primers of 27F and 1495R and the restriction map of two different restriction enzymes to cleave the PCR amplified 16S rDNA. The EtBr stained gel profile showed the PCR-amplified 16S rDNA for ARDRA analysis to be between 1,430 and 1,500 bp in size, consistent with the expected PCR products of 1,468 bp in size. Additionally, nonspecific PCR amplification was not detected in any lanes.

Genetic diversity

The restriction enzyme digestion of PCR amplified 16S rDNA is showed in Fig. 2, and it is indicated that the PCR products, which were amplified from compost DNA samples that extracted with different protocols, had the same profiles after being digested with the same restriction enzyme. Figure 3 showed nearly the same profiles of DNAs extracted from the same compost with different methods.

Discussion

The cell lysis efficiencies of the three protocols were similar to the (95.3±2.3) percent reported by Howeler (Howeler et al. 2003), but higher than the value of 81% obtained by Miller (Miller et al. 1999) using soil as material. Although the microbial cells in VW compost samples treated with protocols LL and PL were less than those in the samples treated with protocol UL, these two procedures yielded more DNA than that from protocol UL with their greater efficiencies on cell lysis. Enzymatic extraction in high-salt buffer (protocols LL and PL) was approved to be a more effective method for cell lysis, and mechanical lysis (protocol UL) was insufficient for lysing the cells in a complex system such as compost. The reason might be that the enzymes in the high-salt buffer could meet cells better than glass beads, and therefore, they could fully come into play while the mixture was being incubated and shaken on an orbital shaker. This could also explain why the cells that survived the protocol UL tended to be small, round individual cells.

These DNA extraction protocols yielded more than (55± 2.9) (μg crude DNA g^{-1} compost) and (48±1.8) (μg purified DNA g^{-1} compost), and the yields were higher than other DNA yields from environmental samples that had been reported in literature (Bürgmann et al. 2001; Howeler et al. 2003). The DNA yields in the crude fraction, adjusted for the cell lysis efficiency, were (66±3.8), (61± 3.2), and (86±4.3) (μg g^{-1} compost) for protocols LL, UL, and PL, respectively. Assuming that bacteria were the predominant source of DNA in the compost, and that each bacterium had a single stationary-phase genome weighing 5×10–15 g (based on data for *Escherichia coli*), then 1 g of VW compost had theoretical DNA yields of (41.3± 4.25), (43.7±4.55), and (43.0±4.65) μg in protocols LL,

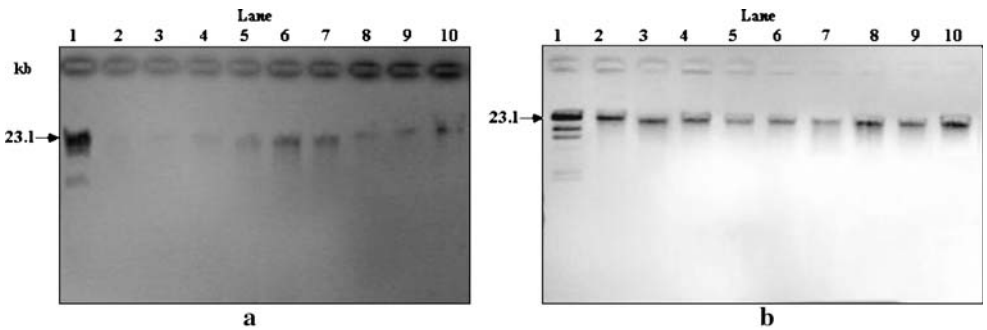

Fig. 1 Crude DNA (**a**) and purified DNA (**b**) extracted from different protocols. *Lanes*: *1*, lambda phage DNA digested with *Hind*III; *2*, *3*, and *4*, DNA treated by protocol UL; *5*, *6*, and *7*, DNA treated by protocol LL; *8*, *9*, and *10*, DNA treated by protocol PL

Table 3 Amount of crude or purified DNA from VW compost and efficiency of DNA recovery

Protocol	Crude DNA yield (μg DNA g^{-1} compost)	Purified DNA yield (μg DNA g^{-1} compost)	Pure DNA after cell lysis (μg)	Pure DNA after purification (μg)	DNA recovery efficiency (%)
LL	62±3.6	53±1.5	19.2±0.5	18.8±0.3	94.0±1.5
UL	55±2.9	48±1.8	19.0±0.3	18.6±0.3	93.0±1.5
PL	83±4.1	69±2.6	19.5±0.5	18.9±0.4	94.5±2.0

UL, and PL, respectively. These values were smaller than the DNA yields obtained in the crude fraction because there was more DNA in most microbial cells, especially those actively growing cells and eukaryotic cells, than in *E. coli* cells. The pure DNA recovery efficiencies of the three protocols in the purification procedure were higher than that of crude DNA purification. This was perhaps due to the fact that the lengths of the fragments in crude DNA were in a range between 6 and 23 kb when the pure DNA had a length of about 23 kb, and smaller fragments could be removed by the purification procedures more easily.

PEG8000 precipitation of DNA, vs isopropanol precipitation used in many other purification methods (Blanc et al. 1999; Tebbe and Vahjen 1993), may account for lower humic acid contamination. Some reports (Purdy et al. 1996) showed that PEG8000 precipitation could reduce humate contamination, although it should have removed some DNA at the same time so that it had a lower DNA yield than that obtained with an alcohol precipitation step (Krsek and Wellington 1999). The spin-bind cartridge had a powerful effect on distinctively absorbing DNA while the crude DNA solution was centrifugated so as to yield very pure DNA. Although about 15% of crude DNA was lost during purification, more than 98% of the humic acids were also removed, and there was no obvious decrease in DNA yield compared with other reports (Bürgmann et al. 2001; Howeler et al. 2003). Furthermore, the use of cartridge shortened the procedures of purification using PEG8000 for more than 3 h compared with other methods (Bürgmann et al. 2001; Howeler et al. 2003), so that pure DNA could be extracted from complex compost within 4.5 h for protocol LL, 4 h for protocol UL, and 6 h for protocol PL without special equipment and expensive commercial kits.

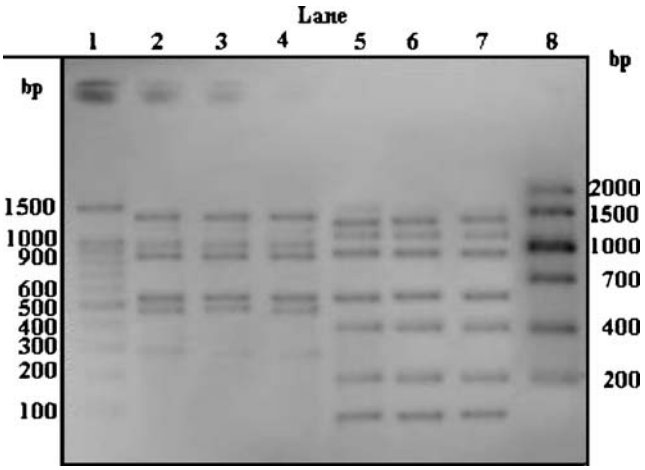

Fig. 2 Restriction enzyme digest of PCR-amplified products. *Lanes*: *1*, DNA ladder; *2*, PCR products from protocol LL digested with *Hae*III; *3*, PCR products from protocol UL digested with *Hae*III; *4*, PCR products from protocol PL digested with *Hae*III; *5*, PCR products from protocol LL digested with *Alu*I; *6*, PCR products from protocol UL digested with *Alu*I; *7*, PCR products from protocol PL digested with *Alu*I; *8*, DNA ladder

Obtaining high yield and high pure DNA from compost is, of course, very important for molecular ecological analysis, and of more importance is that all the genomic DNA in the complex community should be extracted and it, therefore, could roundly display the microbial diversity in the system. The PCR amplification of purified DNA, which was tested using eubacterial 16S rDNA primer pair of 27F and 1495R in the presence of BSA, as BSA was shown to improve PCR amplification of DNA (Al-Soud and Rådström 2000), showed that DNA extracted by the three different protocols was amplifiable for PCR. Several methods (Krsek and Wellington 1999; Stach et al. 2001) had been used for comparing DNA quality obtained by different procedures in terms of the microbial sequence diversity present in the extract. In this study, ARDRA analysis with two different restriction endonucleases (*Alu*I and *Hae*III) and DGGE analysis were used to actualize the same task. Our results showed that there were similar digestion bands in length and number on the ARDRA map after different purified PCR amplified products were digested by the same restriction endonuclease and the

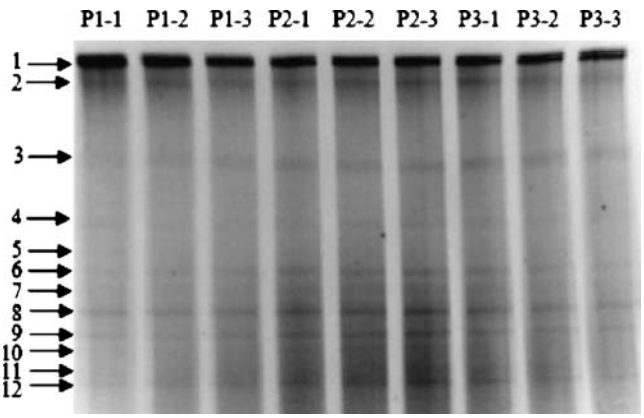

Fig. 3 DGGE profile of PCR amplified products

digestion products were electrophoresed. Although different DNA yields and purities were obtained by the three different protocols, LL, UL, and PL, they shared similar PCR amplifiabilities and DNA sequence diversities. The same conclusion was summarized based on the fact that there were the same bands, both amount and location, in different lanes in the DGGE profile.

In summary, the results of the study suggest that the direct DNA extractions with enzyme yielded more DNA than those with physical treatments, and the purification procedures with PEG8000 precipitation and spin-bind cartridge elution removed most humic acids and recovered DNA efficiently. Moreover, all the purified DNA samples could be used for PCR amplification directly and shared similar genetic diversity, as the restriction map showed. Thus, each of the protocols, LL, UL, and PL, is adapted to extract microbial genome DNA from compost expediently and cheaply.

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