

Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis

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Abstract

Analysis of microbial community structure in complex environmental samples using nucleic acid techniques requires efficient unbiased DNA extraction procedures; however, humic acids and other contaminants complicate the isolation of PCR-amplifiable DNA from compost and other organic-rich samples. In this study, combinations of DNA extraction and purification methods were compared based on DNA yield, humic acid contamination, PCR amplifiability, and microbial community structure assessed by terminal restriction fragment length polymorphisms (TRFLP) of amplified 16S rRNA genes. DNA yield and humic acid contamination, determined by A_{230} , varied significantly between extraction methods. Humic acid contamination of DNA obtained from compost decreased with increasing salt concentration in the lysis buffer. DNA purified by gel permeation chromatography (Sephacrose 4B columns) gave satisfactory PCR amplification with universal eubacterial 16S rRNA gene primers only when A_{260}/A_{280} ratios exceeded 1.5. DNA purified with affinity chromatography (hydroxyapatite columns), and showing A_{260}/A_{280} ratios as high as 1.8, did not show consistently satisfactory PCR amplification using the same 16S rRNA primers. Almost all DNA samples purified by agarose gel electrophoresis showed satisfactory PCR amplification. Principal components analysis (PCA) of TRFLP patterns differentiated compost types based on the presence/absence of peaks and on the height of the peaks, but differences in TRFLP patterns were not appreciable between extraction methods that yielded relatively pure DNA. High levels of humic acid contamination in extracted DNA resulted in TRFLP patterns that were not consistent and introduced a bias towards lower estimates of diversity. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Compost; DNA extraction; Environmental; Humic; PCR; RFLP

1. Introduction

Molecular techniques such as PCR amplification of ribosomal rRNA genes or other genes of ecological significance yield relatively less biased information about microbial communities than traditional culturing approaches. Composting is an aerobic thermophilic process widely used to recycle organic residues (Fogarty

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and Tuovinen, 1991; Michel et al., 1995). Potential use of composting for bioremediation of soils and sediments contaminated with xenobiotics has been recognized (Williams et al., 1992; Laine and Jørgensen, 1997). Gradients of oxygen, nutrients, and temperature in compost support diverse microbial populations, which degrade organic matter and humify complex polymers. Optimization of the composting process depends on optimization of environmental conditions that promote the development and activity of these microbial communities.

Molecular analyses of microbial communities in complex environmental samples such as compost require efficient unbiased DNA extraction procedures. Direct extraction of DNA from environmental samples yields more DNA, requires shorter time, and introduce less bias than methods in which cells are separated from the sample matrix before DNA extraction (von Wintzingerode et al., 1997). Detergent lysis and freeze–thaw procedures (Tsai and Olson, 1991; Zhou et al., 1996; Porteous et al., 1997) are widely used for direct extraction of total DNA from soils and sediments for molecular analysis of community structure. Bead mill homogenization, with and without detergent lysis, effectively extracts but shears template DNA (Moré et al., 1994; van Elsas et al., 1997; Kuske et al., 1998), which may affect PCR-based estimates of community diversity (von Wintzingerode et al., 1997). Hot-detergent lysis and freeze–thaw procedures for DNA isolation typically yield unsheared high-molecular weight DNA but co-extract humic acids which can inhibit DNA-modifying enzymes (Tsai and Olson, 1991; Tebbe and Vahjen, 1993). Hence, additional purification steps such as agarose gel electrophoresis, gel permeation chromatography, or affinity chromatography are usually needed to obtain PCR-amplifiable DNA (Tsai and Olson, 1991; Zhou et al., 1996; Porteous et al., 1997; von Wintzingerode et al., 1997).

Composts contain particularly high levels of organic matter (30–70%) that are much higher than the levels in most soils and pose a particular challenge in obtaining PCR-amplifiable DNA. Methods designed to extract DNA from soils and sediments have been adapted to obtain PCR-amplifiable DNA from composts (Wikström et al., 1996; Blanc et al., 1999; Kowalchuk et al., 1999). However, the relative effectiveness of extraction and purification methods for

isolating compost DNA of sufficient purity for PCR amplification has not been examined. Also, potential bias introduced by different extraction protocols has not been investigated. In this paper, we evaluated three widely used DNA extraction methods (Tsai and Olson, 1991; Zhou et al., 1996; Porteous et al., 1997) and three purification procedures (Purdy et al., 1996; Zhou et al., 1996; Jackson et al., 1997) based on yield, humic acid contamination, and PCR amplifiability. We also used principal components analysis (PCA), which has been applied to PCR-based studies of microbial communities (McCaig et al., 2001), to compare terminal restriction fragment length polymorphisms (TRFLP; Liu et al., 1997) of 16S rRNA genes amplified from DNA obtained from two composts. PCA suggested that variance introduced by extraction protocols in the analysis of microbial community structure was less than variance between compost types. Based on this comparison and an optimization of salt concentrations in extraction buffer, we report a modified protocol to obtain high-molecular weight DNA from compost suitable for molecular analyses.

2. Methods

Compost samples were collected from five compost piles designated A to E. Compost piles A and D contained only yard trimmings (mixture of leaves, fresh grass, and brush in a ratio of 4:2:1 by volume); pile B contained soil and yard trimmings (in a ratio of 1:2); pile C contained soil, yard trimmings, and mint leaves (in a ratio of 1:1:1); pile E contained livestock manure and sawdust. Each pile contained about 31 m³ of material and achieved typical composting conditions (Michel et al., 1995) as evidenced by temperatures greater than 55 °C for 60–90 days and rapid organic matter degradation. Samples (1 kg) were collected from 6 to 10 different parts of piles A, B, C, D, and E on days 64, 100, 100, 100, and 90, respectively. Subsamples (100 g) from each sample were pooled, homogenized using a Waring blender, and stored at 4 °C. Composts F and G represent composts A and C after storage for 1 year at 4 °C. For comparison, a soil sample was collected from the 0- to 15-cm surface horizon of grassland soil, after removing surface litter, at the Lisque Valley in the Sedgwick Reserve in Santa ynez, CA.

2.1. Comparison of DNA extraction methods

Triplicate 2.25-g subsamples of composite samples A–C were washed twice to reduce extracellular DNA and soluble organic contaminants by shaking for 15 min at 150 rpm with 20 ml of 120 mM phosphate buffer (pH 8.0) and centrifuging for 10 min at $6000 \times g$. The pellet was resuspended in 20 ml (15 mg ml^{-1}) of lysozyme solution (Tsai and Olson, 1991) for 30 min at 37°C . Treated samples were divided into three 6.25-ml aliquots. DNA was extracted from aliquots by three protocols designated FT, HS, or GP (Table 1). Crude DNA was recovered from these extractions by precipitation (Table 1) and resuspended in $500 \mu\text{l}$ 10 mM TrisCl/1 mM EDTA pH 8 (TE).

To quantify DNA yield, crude DNA extracts were agarose (1%) gel electrophoresed, stained with $0.5 \mu\text{g ml}^{-1}$ ethidium bromide (Sambrook et al., 1989), digitized using a Gelprint 20001 system (Biophotonics, Ann Arbor, MI), and compared to *Hind*III-digested λ DNA (GibcoBRL, Gaithersburg, MD) using the Gel-Compar 4.0™ program (Applied Maths, Kortrijk, Belgium). DNA was also quantified with the Pico-green™ reagent (Molecular Probes, Eugene, OR). UV spectra were obtained using either a Cary 1-Bio (Varian Australia, Mulgrave, Victoria) or a λ -II (Perkin Elmer, Gaithersburg, MD) spectrophotometer using a 50- μl quartz cuvette with a 1-cm path length. To estimate humic acid contamination, the A_{230} of crude DNA extracts was compared to the A_{230} (0.5) of a $19\text{-}\mu\text{g ml}^{-1}$ humic acid sodium salt solution (Aldrich, Milwaukee, WI).

2.2. Effect of buffer salt

Three NaCl concentrations (0.1, 0.6, and 1.1 M) in the lysis buffer were used to extract DNA from two

composts (D and E above) and the soil sample. Replicate samples (0.3 g in 2-ml screw cap tubes) were amended with $850 \mu\text{l}$ lysis buffer (150 mM TrisCl pH 8.0, 3 mM EDTA, 1.5% CTAB, and NaCl as above), $10 \mu\text{l}$ of 20 mg ml^{-1} Proteinase K, and 0.3 g 0.1-mm glass beads (acid washed and baked 3 h at 250°C). After incubation for 30 min at 37°C , $55 \mu\text{l}$ of 20% SDS was added and the tubes were heated at 65°C for 60 min. After cooling to room temperature, $700 \mu\text{l}$ of chloroform was added. The tubes were then vortexed briefly and centrifuged at $10,000 \times g$ for 4 min. The aqueous layer was removed and extracted with a second $700 \mu\text{l}$ of chloroform. The organic layer was amended with $700 \mu\text{l}$ lysis buffer and $45 \mu\text{l}$ 20% SDS agitated for 30 s at 2000 rpm with a Mini Beadbeater-8 (Biospec, Bartlesville, OK) and centrifuged as above. The recovered aqueous layer was used to extract the second organic layer and the aqueous layers were pooled. The pooled aqueous layers ($\sim 1.4 \text{ ml}$) were centrifuged (2 min, $700 \times g$) through a 1.5-ml bed of PVPP packed in a 3-ml syringe. Eluate from the column was amended to 1.1 M NaCl (if necessary) and 10% polyethylene glycol-8000 (PEG), incubated overnight at 4°C , centrifuged (15 min, $16,000 \times g$), and resuspended in $40 \mu\text{l}$ TE.

2.3. DNA purification

Crude DNA preparations were purified using either Sepharose 4B gel permeation chromatography (Jackson et al., 1997), hydroxyapatite (HTP) affinity chromatography (Purdy et al., 1996), or agarose gel electrophoresis followed by agarase digestion (Zhou et al., 1996). DNA purification by gel electrophoresis was modified as follows: crude DNA extract was loaded on a 1% SeaPlaque™ GTG low-melting point

Table 1
Key differences in DNA extraction protocols

Protocol	Important buffer components	EDTA (mM) ^a	Lysis ^b	Precipitant ^c	Reference
HS	1.5 M NaCl	100	Hot 2% SDS	Isopropanol	Zhou et al. (1996)
GP	5 M Guanidine thiocyanate/0.25 M NaCl	100	Hot 4% SDS	PEG 8000	Porteous et al. (1997)
FT	0.1 M NaCl/0.25 M Tris	50	Freeze–thaw/5% SDS	Isopropanol	Tsai and Olson (1991)
SP	0.1–1.1 M NaCl/0.05 M Tris	3	Hot 1% SDS/Bead beating	PEG 8000	This study

^a Concentration of disodium ethylenediaminetetraacetic acid (EDTA) in lysis buffer. EDTA and all other chemicals listed above were obtained from Sigma (St. Louis, MO).

^b Hot SDS lysis was conducted by heating at 65°C for 60 min after adding sodium dodecyl sulfate (SDS) as indicated.

^c Precipitation by isopropanol and polyethylene glycol 8000 (PEG) as per Zhou et al. (1996) and Porteous et al. (1997), respectively.

agarose gel and electrophoresed per manufacturer's instructions (FMC, Rockland, ME). After visualization by ethidium bromide staining (Sambrook et al., 1989), the DNA band was excised, treated with agarase (Boehringer Mannheim, Indianapolis, IN), concentrated by centrifugation for 15 min at $500 \times g$ in a Microcon™-100 microconcentrator (Amicon, Beverly, MA), washed once with 450 μ l TE and collected by reversing the concentrator.

2.4. PCR-TRFLP

16S rDNA from purified DNA samples was PCR amplified using universal eubacterial primers 8F hex (fluorescently labeled forward primer) and 1392R (reverse primer). The sequence (5'–3') of the forward and reverse primers (Liu et al., 1997), respectively, were: **AGAGTTTGATCCTGGCTCAG** and **ACGGGCGGTGTGTRC**. Each 50- μ l PCR reaction contained 50 ng template, 2.5 mM MgCl₂, 2.5 units Taq polymerase (GibcoBRL), 0.24 mM each dNTP, 0.5 μ M each DNA primer, and 1 \times PCR buffer (GibcoBRL). Reaction mixtures were held at 94 °C for 9 min, and cycled 28 times through three steps: denaturing (94 °C, 60 s), annealing (58 °C, 45 s), and primer extension (72 °C, 90 s). The final primer extension step was for 7 min. PCR products were purified with Wizard™ PCR preps (Promega, Madison, WI) or Qiagen PCR preps (Qiagen, Chatsworth, CA), and then digested with *Hha*I (10 μ l of the purified PCR product with 1 unit of *Hha*I for 2 h at 37 °C). Length of fluorescently labeled fragments was determined with an Applied Biosystems Instruments model 373A automated sequencer (Foster City, CA) as per Liu et al. (1997).

2.5. Statistical analysis

Principal components analyses (PC) were calculated with Systat™ 10 (SPSS, Chicago, IL) from either similarity indices or peak heights. Similarity indices were calculated with the Ribosomal Database Project TRFLP Profile Matrix program (Maidak et al., 1997) based on presence/absence of peaks. Length uncertainties for peak matching were set to 1 bp from 1 to 200 bp, 2 bp from 200 to 400 bp, 3 bp from 400 to 600 bp, and 4 bp from 600 to 650 bp. To conduct PCA on peak heights, TRFLPs were aligned and non-reproducible

peaks that did not appear in the three replicate profiles were discarded. Shared peaks were compared using pairwise deletion to generate a correlation matrix, from which PC were calculated. Shannon–Weaver indices of diversity (H) were calculated by adapting the index formula (Atlas and Bartha, 1993) so that $H = C/N$ [$M \log_{10} N - \sum (n_i \log_{10} n_i) \ i = 1 \text{ to } N$], where $C = 2.3$, N equals the number of TRFLP peaks and n_i equals the magnitude of the i th peak.

3. Results and discussion

Chemical/enzymatic extraction in high-salt buffer (protocol HS) yielded significantly ($P < 0.02$) more DNA than protocols GP and FT (Table 2). Ineffective lysis by freeze–thaw cycling (Moré et al., 1994; Leff et al., 1995) and inclusion of guanidine thiocyanate in the lysis buffer (Miller et al., 1999) could explain the lower yields obtained with protocols FT and GP, respectively. When DNA was extracted using protocols HS and GP, the DNA yield from compost pile A (which contained yard trimmings alone) tended to be higher than that from compost pile B (which contained a mixture of soil and yard trimmings in a ratio of 1:2) and compost pile C (which contained soil, yard trimmings, and mint leaves in a ratio of 1:1:1). However, DNA yields did not differ between piles when protocol FT was used. DNA yields from compost piles (Table 2) were generally similar to that reported for litter (54 μ g DNA g⁻¹ dry weight; Wikström et al., 1996), which had comparable organic matter content (85%) to the compost piles studied herein. Also, van Elsas et al. (1997) obtained a comparable DNA yield (35 μ g DNA g⁻¹ dry weight) from a soil containing 30% organic matter. In contrast, agricultural soil with comparatively low organic matter content (4%) yielded 14 and 3 μ g DNA g⁻¹ dry weight using the protocols HS and GP, respectively (Fig. 1).

HS and FT protocols yielded crude DNA samples showing appreciably higher contamination with humic acids (Table 2). DNA extracted using the GP protocol contained significantly ($P = 0.01$) less humic acid contamination. This is perhaps due to the fact that the GP protocol contains a step in which the crude DNA is resuspended in a high salt/CTAB buffer, which appears to reduce humic acid contamination

Table 2
Comparison of crude^a DNA obtained from compost by three protocols

Compost	Percentage OM ^b	DNA extraction protocol ^c					
		HS		GP		FT	
		DNA ^d	Humic ^e	DNA ^d	Humic ^e	DNA ^d	Humic ^e
A	56	90 ± 11	36.9 ± 1.6	32 ± 8	0.35 ± 0.14	26 ± 1	74.8 ± 11.4
B	31	60 ± 7	4.7 ± 0.5	13 ± 4	0.50 ± 0.05	30 ± 10	15.0 ± 0.9
C	44	59 ± 8	7.8 ± 0.1	20 ± 4	0.63 ± 0.04	41 ± 1	23.6 ± 1.3

^a Crude DNA is defined as the extract obtained after concentration by either PEG or isopropanol precipitation (see Table 1) but before purification by either spin-columns or agarose gel electrophoresis. Crude DNA obtained by using protocol GP was purified by CTAB/chloroform extraction after concentration by PEG precipitation.

^b Loss on ignition (Nelson and Sommers, 1996).

^c See Table 1.

^d Mean (± S.D.) DNA yield ($\mu\text{g DNA g}^{-1}$ dry weight) in triplicate crude DNA preparations was estimated by comparing band intensity in an agarose gel as described in Methods.

^e Mean (± S.D.) humic acid content ($\text{mg humic acid g}^{-1}$ dry weight) in triplicate crude DNA preparations was measured as A_{230} .

(Blanc et al., 1999). PEG precipitation of DNA in protocol GP, versus isopropanol precipitation in the other methods, may also account for lower humic contamination. PEG precipitation is known to reduce humate contamination of DNA (Purdy et al., 1996), although DNA yield may be lower than that obtained with an alcohol precipitation step (Krsek and Wellington, 1999). Cullen and Hirsch (1998) observed little difference in humic acid contamination of DNA using PEG precipitation versus isopropanol precipitation of extracts obtained from soil; however, when we substituted PEG precipitation for the terminal isopropanol precipitation step in protocol HS, the compost DNA obtained had a fourfold less humic acid contamination but there was no decrease in yield (Table 3).

In soil samples, increasing the salt concentration of the lysis buffer increases humic acid contamination (Krsek and Wellington, 1999); however, optimum extraction buffer salt concentration may be higher for samples rich in organic matter (Wikström et al., 1996). To determine the optimum salt concentration for extracting DNA from compost, we tested a range of salt concentrations on compost E (see Methods). We also took advantage of reports which indicated that chloroform aids in the cell lysis step (Miller et al., 1999), low EDTA and SDS concentrations minimize humic acid contamination (Krsek and Wellington, 1999), and that combined chemical/mechanical lysis enhances yield (Kuske et al., 1998). We designate this salt/PEG method (SP protocol). Humic acid contam-

ination, as measured by A_{230} , correlated inversely with salt concentration for the compost E (Fig. 1), which is consistent with the observation that humic acids aggregate at ionic strengths greater than 500 mM (Bainois et al., 1999). Also, extraction of compost DNA using low-salt buffer (as in Malik et al., 1994) yielded DNA with a relatively high humic acid contamination (27.7 and 6.5 mg humic acids g^{-1} dry weight for composts A and C, respectively) but the DNA yield was similar to that obtained using protocol HS (Fig. 1). Molecular size of DNA obtained using protocol SP was >10 kb but also contained some lower sized DNA which may reflect the failure of the relatively low EDTA concentration used in SP protocol to inactivate nucleases. After washing compost samples D and E twice with 100 mM phosphate buffer (pH 8) containing 100 mM Na_2EDTA before extraction using protocol SP, DNA isolated was mostly >10 kb. EDTA washing also increased DNA yield from compost E to 44 $\mu\text{g DNA g}^{-1}$ dry weight.

The relationship between organic matter content and DNA yield depended upon the extraction method. DNA yield, obtained by extraction protocols HS and SP, peaked for samples with 30–60% organic matter (Fig. 1). For comparison, composts D and E, as well as the grassland soil, were extracted with the Ultra-Clean™ Kit (MoBio, Solana Beach, CA). Yields obtained with the commercial kit were similar or less than that obtained with protocol SP (Fig. 1).

Purification of crude DNA using Sepharose 4B column yielded relatively pure DNA. There was a

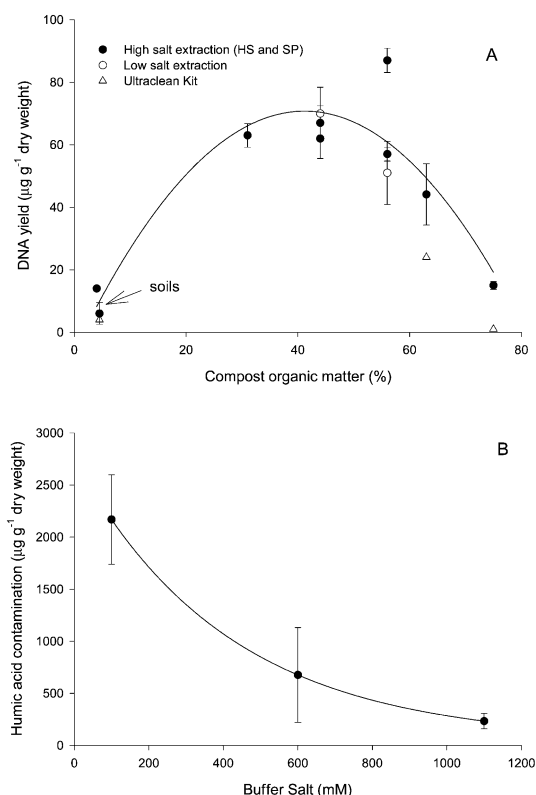


Fig. 1. DNA yield and purity. Panel A: Relationship between DNA yield and organic matter content using different DNA extraction protocols. A binomial model was fit to DNA yields (\pm S.D.) obtained from samples extracted in high-salt buffers (protocol HS and SP; Table 1). Composts were washed with phosphate-EDTA before extraction. For comparison, composts F and G were also extracted using low-salt buffer as per Malik et al. (1994). Composts D, E, and grassland soils were also extracted using the UltraClean™ Kit. Panel B: Effect of buffer salt on humic acid content of DNA. Compost E was extracted using Protocol SP. Humic acid content (\pm S.D.) was measured in duplicate extractions and the A_{230} of crude DNA was compared to a standard (see Methods).

significant ($P=0.001$) inverse correlation between humate contamination in the crude DNA extracts and A_{260}/A_{280} ratios after using Sepharose 4B column purification. For example, DNA extracted using protocol GP and purified by Sepharose 4B column chromatography or affinity chromatography using HTP, yielded DNA that had A_{260}/A_{280} ratios of 1.5–1.8, consistent with a relatively pure DNA. By comparison, DNA extracted using protocols HS and FT and purified by HTP columns had A_{260}/A_{280} ratios of 1.2–1.9.

Presence of high levels of humate contamination in crude DNA appeared to decrease its PCR amplifiability even after subsequent purification by gel electrophoresis or Sepharose 4B column chromatography. PCR amplifiability of the purified DNA was tested using eubacterial 16S rDNA primers in the presence of BSA as BSA addition was shown to improved PCR amplification of extracted DNA (Abu Al-Soud and Rådström, 2000). Our results also showed improved PCR amplifiability of compost DNA in the presence of BSA (data not shown). DNA isolated using protocols GP or HS and purified by gel electrophoresis gave consistently satisfactory PCR amplification. Harry et al. (1999) also reported gel electrophoresis as the most consistent method of separating DNA from humic acids. However, we found that DNA samples extracted using protocol FT did not consistently PCR amplify even after purification by gel electrophoresis. Also, DNA samples purified using HTP columns did not consistently PCR amplify even when A_{260}/A_{280} ratios reached 1.8 (consistent with relatively pure DNA). In contrast, DNA samples purified with Sepharose 4B columns gave consistently satisfactory PCR amplification, when A_{260}/A_{280} ratios exceeded 1.5. Thus, starting with a relatively pure crude DNA, Sepharose 4B column purification gave consistently PCR-amplifiable DNA from compost. Our results are consistent with a report by Miller (2001) who indicated that use of Sepharose 2B columns enhances separation of DNA and humics.

Table 3
Effect of PEG vs. isopropanol precipitation in DNA extraction

Compost	Precipitation method			
	Isopropanol ^a		PEG ^b	
	Yield ^c	Humic ^d	Yield ^c	Humic ^d
F	56 \pm 14	7.7 \pm 2.8	59 \pm 8	2.1 \pm 0.7
G	62 \pm 15	6.3 \pm 0.6	62 \pm 4	1.3 \pm 0.3

^a Compost was extracted as per protocol HS.

^b Compost was extracted as per protocol HS but polyethylene glycol (PEG) was substituted for isopropanol in the terminal concentration step.

^c Mean (\pm S.D.) DNA yield (μ g DNA g⁻¹ dry weight) of triplicate samples was measured in crude DNA preparations with Picogreen™ as per manufacturer's instructions (Molecular Probes).

^d Mean (\pm S.D.) humic acid content (mg humic acid g⁻¹ dry weight) of triplicate crude DNA preparations was measured as A_{230} .

Using the same compost sample, protocols GP and HS yielded DNA that gave similar TRFLP profiles; however, protocol FT, which did not yield consistently PCR-amplifiable DNA, gave unsatisfactory TRFLP profiles in that the relative number and magnitude of the TRFLP peaks was quite low (Fig. 2). Unique TRFLP peaks were found for different compost types. For example, in compost A, 5 peaks were found in all TRFLP profiles of DNA isolated using GP or HS protocol and these were not seen in DNA isolated from compost B using the same protocols. Conversely,

11 peaks were found consistently in DNA from compost B which were not seen in DNA from compost A. PCA of similarity indices generated based on the presence and absence of peaks showed that TRFLP patterns separated composts A and B but did not differentiate protocols GP and HS (Fig. 3A). Several peaks differed in intensity between composts. For example, all profiles showed a 240-bp fragment. However, this peak constituted 36% of the cumulative peak height in compost A and only 4% of that in compost B. Similar variation in ribotype intensity

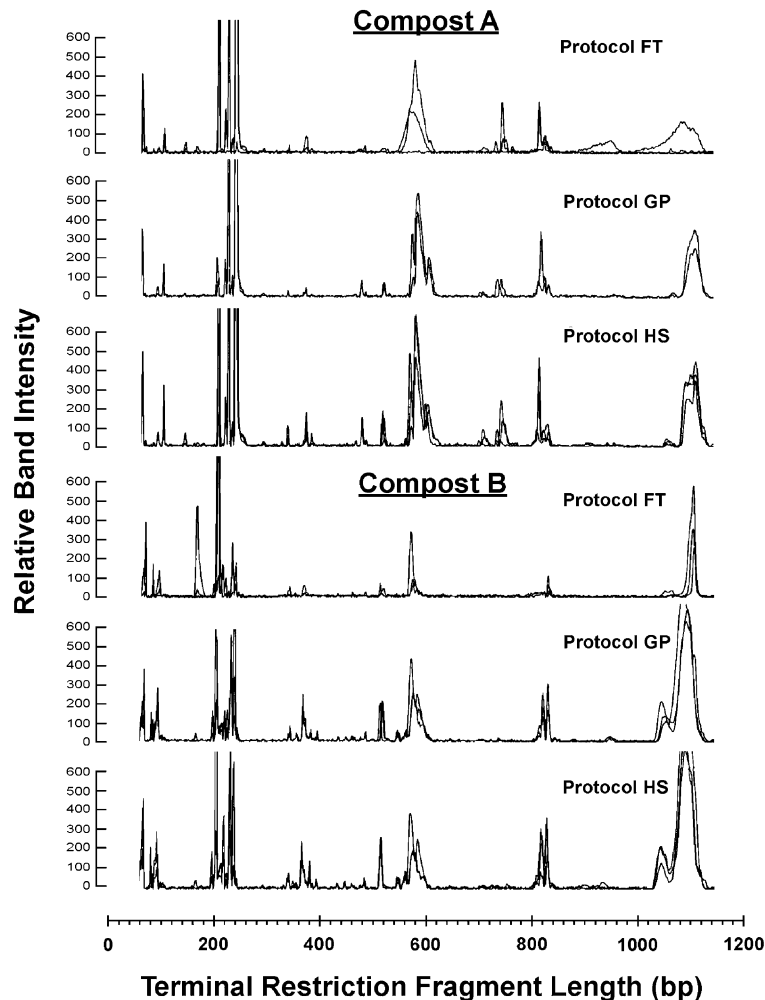


Fig. 2. Terminal restriction fragment length polymorphisms of PCR-amplified 16S rDNA using DNA extracted from composts A and B. DNA was extracted using protocols HS, GP and FT, gel purified, and PCR amplified as described in text. PCR products were digested with *Hha*I. Triplicate profiles are overlaid.

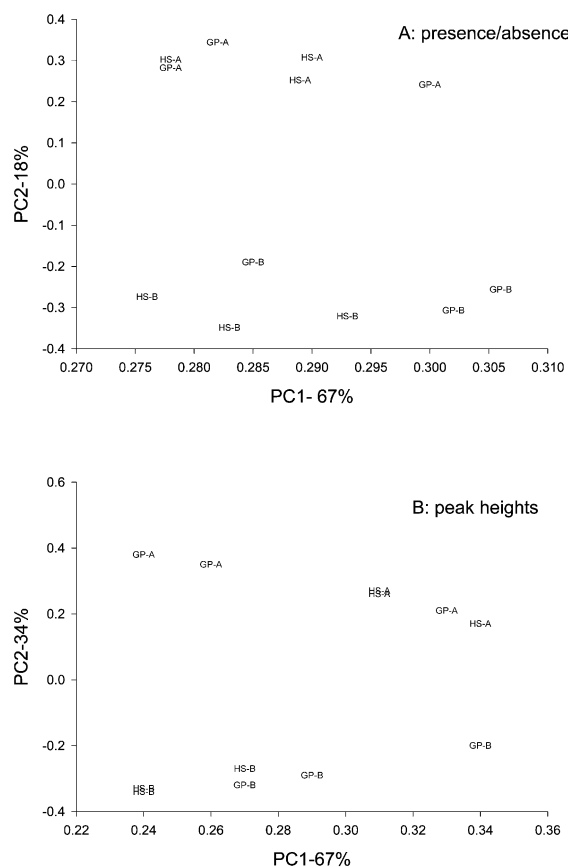


Fig. 3. Principal components analysis (PCA) of TRFLPs. Prefix refers to extraction method: GP (Porteous et al., 1997); or HS (Zhou et al., 1996). Suffix refers to compost A or B. Variability explained by each principal component (PC) is indicated. Panel A: Variation in number of peaks shared. PCA of variability reduced from similarity indices calculated by the presence/absence of TRFLP peaks (see Methods). Panel B: Variation in peak heights. PCA of variability reduced from correlation coefficients calculated from the heights of reproducible peaks (see Methods).

between PCR-generated microbial profiles has been attributed to different DNA extraction protocols (Martin-Laurent et al., 2001; Stach et al., 2001) and the amount of pure culture added (Clement and Kitts, 2000). This variation in peak heights could obfuscate some TRFLP peaks (Dunbar et al., 2001). To assess variation in peak heights due to chemical–enzymatic extraction protocols, we applied PCA to the heights of peaks shared between different TRFLP profiles. Based on variation in heights, PCA separated composts A and B but not protocols GP and HS (Fig. 3B).

The number of TRFLP peaks detected differed between composts, but not between protocols GP and HS. Thus, lower DNA yield observed with protocol GP versus HS did not appear to introduce a bias towards lower community diversity (Table 4). As mentioned above, DNA prepared with protocol FT gave few TRFLP peaks and these peaks were not reproducible. For example, DNA extracted from compost B using the FT protocol gave only 5 peaks common to all the three profiles (Fig. 2). From the same compost, DNA obtained using HS and GP protocols gave 28 and 29 reproducible peaks, respectively. Thus, purity of DNA template appears to affect molecular community analyses as has been shown previously for DGGE analysis (Frostegård et al., 1999; Krsek and Wellington, 1999). The quality of the DNA template appears to affect TRFLP estimates of diversity as does the quantity of template as previously reported by Osborn et al. (2000). There was no significant difference in Shannon–Weaver diversity indices for identical compost samples extracted using protocols GP and HS (Table 4); however, Shannon–Weaver diversity indices were significantly ($P < 0.001$) higher for compost B than compost A. This higher index may reflect inclusion of soil in compost B, which could provide a more diverse inoculum, and is consistent with the similarity in TRFLP profiles obtained with protocols GP and HS (Fig. 3B).

Table 4
Diversity estimates^a from TRFLPs of 16S rDNA

Compost	Protocol			
	HS ^b		GP ^c	
	TRFs ^d	H ^e	TRFs ^d	H ^e
A	22 ± 4	2.4 ± 0.3	22 ± 2	2.1 ± 0.1
B	33 ± 8	2.9 ± 0.3	33 ± 8	3.1 ± 0.3

^a Mean (± S.D.) of triplicate samples.

^b Compost was extracted in high-salt buffer and was isopropanol precipitated as per Zhou et al. (1996).

^c Compost was extracted using GP protocol as per Porteous et al. (1997).

^d Number of restriction fragments detected.

^e Shannon–Weaver indices of diversity (H) were calculated by adapting the index formula so that $H = C/N [\log_{10} N - \sum (n_i \log_{10} n_i) / i = 1 \text{ to } N]$, where $C = 2.3$, N equals the number of TRFLP peaks and n_i equals the magnitude of the i th peak (see Atlas and Bartha, 1993).

In conclusion, as compared to soils, composts generally yield greater amounts of DNA but with higher levels of humate contamination. Hot-detergent lysis in either relatively high-ionic strength (>500 mM) buffer or in guanidine thiocyanate followed by PEG precipitation appears to be suitable for obtaining relatively clean high-molecular weight DNA. Additional purification by column chromatography or gel electrophoresis is needed to obtain consistently PCR-amplifiable DNA suitable for molecular genetic analysis of complex microbial communities. PCA of TRFLP profiles appears to distinguish compost types. Lower DNA yield obtained using the GP protocol versus HS protocol does not appear to introduce a bias towards lower diversity as determined by TRFLP profiles; however, lower DNA purity as observed with the FT protocol appears to introduce a bias towards lower diversity and results in low reproducibility of TRFLP profiles.

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