Comparison of commercial DNA extraction kits for isolation and purification of bacterial and eukaryotic DNA from PAH-contaminated soils

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Abstract: Molecular characterization of the microbial populations of soils and sediments contaminated with polycyclic aromatic hydrocarbons (PAHs) is often a first step in assessing intrinsic biodegradation potential. However, soils are problematic for molecular analysis owing to the presence of organic matter, such as humic acids. Furthermore, the presence of contaminants, such as PAHs, can cause further challenges to DNA extraction, quantification, and amplification. The goal of our study was to compare the effectiveness of four commercial soil DNA extraction kits (UltraClean Soil DNA Isolation kit, PowerSoil DNA Isolation kit, PowerMax Soil DNA Isolation kit, and FastDNA SPIN kit) to extract pure, high-quality bacterial and eukaryotic DNA from PAH-contaminated soils. Six different contaminated soils were used to determine if there were any biases among the kits due to soil properties or level of contamination. Extracted DNA was used as a template for bacterial 16S rDNA and eukaryotic 18S rDNA amplifications, and PCR products were subsequently analyzed using denaturing gel gradient electrophoresis (DGGE). We found that the FastDNA SPIN kit provided significantly higher DNA yields for all soils; however, it also resulted in the highest levels of humic acid contamination. Soil texture and organic carbon content of the soil did not affect the DNA yield of any kit. Moreover, a liquid–liquid extraction of the DNA extracts found no residual PAHs, indicating that all kits were effective at removing contaminants in the extraction process. Although the PowerSoil DNA Isolation kit gave relatively low DNA yields, it provided the highest quality DNA based on successful amplification of both bacterial and eukaryotic DNA for all six soils. DGGE fingerprints among the kits were dramatically different for both bacterial and eukaryotic DNA. The PowerSoil DNA Isolation kit revealed multiple bands for each soil and provided the most consistent DGGE profiles among replicates for both bacterial and eukaryotic DNA.

Key words: DNA extraction, PAH biodegradation, PCR, DGGE.

Résumé : La caractérisation moléculaire des populations microbiennes de sols et de sédiments contaminés aux hydrocarbure aromatique polycycliques (HAP) constitue souvent la première étape de l'évaluation de leur potentiel de biodégradation intrinsèque. Cependant, les sols sont problématiques pour une analyse moléculaire à cause de la présence de matières organiques comme les acides humiques. De plus, la présence de contaminants comme les HAP peut constituer un défi supplémentaire lors de l'extraction, la quantification et l'amplification d'ADN. Le but de notre étude était de comparer l'efficacité de quatre trousses commerciales d'extraction d'ADN du sol (UltraClean Soil DNA Isolation kit, PowerSoil DNA Isolation kit, PowerMax Soil DNA Isolation kit et FastDNA SPIN kit) afin d'extraire de l'ADN bactérien et eucaryote pur et de haute qualité à partir de sols contaminés aux HAP. Six sols contaminés différents ont été utilisés pour déterminer s'il existait certains biais entre les trousses à cause des propriétés des sols ou des niveaux de contamination. L'ADN extrait a été utilisé comme matrice pour amplifier l'ADNr 16S bactérien et l'ADNr 18S eucaryote, et les produits de la PCR ont été ensuite analysés par électrophorèse sur gel en gradient dénaturant (DGGE). Nous avons trouvé que la trousse FastDNA SPIN générait des rendements en ADN significativement plus élevés pour tous les sols, mais il comportait aussi les niveaux les plus élevés de contamination par les acides humiques. La texture du sol et le contenu en carbone du sol n'affectaient pas les rendements en ADN pour aucune des trousses. De plus, une extraction liquide–liquide des extraits d'ADN ne montrait aucun HAP résiduel, indiquant que toutes les trousses étaient efficaces pour enlever les contaminants lors du processus d'extraction. Même si la trousse PowerSoil DNA Isolation donnait des rendements relativement faibles, elle produisait l'ADN de la meilleure qualité d'après les résultats de l'amplification des ADN bactériens et eucaryotes des six sols. Les empreintes en DGGE différaient de façon importante selon les trousses, tant pour l'ADN bactérien qu'eucaryote. La trousse PowerSoil DNA Isolation a révélé de multiples bandes dans chaque sol et générait les profils en DGGE les plus constants parmi les répliquats, tant pour l'ADN bactérien qu'eucaryote.

Mots-clés : extraction d'ADN, biodégradation des HAP, PCR, DGGE.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment through petroleum refining and transport, the use of coal tar and creosote, and the incomplete combustion of organic matter (Cerniglia 1984; Freeman and Cattell 1990; Lijinsky 1991; Lim et al. 1999). PAHs have gained widespread attention because some of them are carcinogenic, teratogenic, and mutagenic (Harvey 1996; Xue and Warshawsky 2005). Due to their hydrophobic structure, PAHs have low water solubility and are readily absorbed onto soils and sediments where they persist until they are degraded (Cerniglia 1992). Microbial degradation of PAHs has been well documented under in situ and laboratory conditions and is thought to be an important process in remediating contaminated sediments and soils (Cerniglia 1984, 1992; Peng et al. 2008). Molecular characterization of the natural microbial population in PAH-contaminated soils and sediments is often a first step in assessing the potential for intrinsic biodegradation. The amplification of ribosomal RNA genes using domain-specific primers, followed by analysis of the ribotypes present via cloning, fragment analysis, or denaturing gel electrophoresis methods is routine for community characterization. Regardless of downstream applications, high-quality, purified DNA is an essential requirement for the successful DNA amplifications that underlie all subsequent procedures. Over the years, several different DNA extraction and purification methods have been developed specifically for soils, and a variety of commercial extraction kits are available that provide consistent solutions for the central problems — cell lysis and humic acid removal (Tsai and Olson 1992; Young et al. 1993; Harry et al. 1999; Varanini and Pinton 2001). Commercial DNA extraction kits are widely available and have become favorable because they are often cheaper and faster than traditional extraction methods. Many popular commercial DNA extraction kits lyse microbes in the soil by a combination of heat, detergent, and mechanical force against specialized beads (Roose-Amsaleg et al. 2001). While the effectiveness of these kits has been examined in various soils, the efficiency and ability of various commercial kits to extract high-quality DNA from contaminated soils has yet to be investigated. Furthermore, the observed microbial community structure and diversity has shown to be impacted by the mechanism used to isolate and purify DNA (Krsek and Wellington 1999; Martin-Laurent et al. 2001; Maarit Niemi et al. 2001). This can be a significant issue, especially for contaminated soils where inaccurate community analysis can lead to potentially erroneous estimations regarding the biodegradation capability of the natural microbial population.

The goal of our study was to compare the effectiveness of commercial soil DNA extraction kits to extract pure, high-quality bacterial and eukaryotic DNA from PAH-contaminated soils. The importance of eukaryotic species, especially fungi, in the degradation of PAHs has been demonstrated in recent years. Fungi have greater degradation potential than bacterial species because they can reach PAHs immobilized in micropores because of their multicellular mycelium (Cerniglia 1997; Bennett et al. 2002). Thus, any kit used to obtain microbial community DNA must also successfully extract eukaryotic DNA to accurately assess the degradation potential of the natural microbial community. Six different contaminated soils were used to determine if there were any biases among the kits due to soil properties or level of contamination. DNA yield was measured after completion, and the extracted DNA was used as a template for bacterial 16S rDNA and eukaryotic 18S rDNA amplification. PCR products were analyzed using denaturing gradient gel electrophoresis (DGGE) to determine the effect of the extraction kit on microbial diversity.

Materials and methods

Soil samples

Samples were collected from the upper 0.5–0.6 m of surface soil at an industrial site in southern Ontario where soils were contaminated with varying levels of PAHs. Soils were transported to McMaster University upon collection and stored at –20 °C prior to DNA analysis. PAH concentrations were assessed using the EPA method 8270. Grain size was determined using the Beckman Coulter LS 230 laser diffraction particle size analyzer (Brea, California), and soil textures were assessed using the standard textural triangle. Finally, organic carbon content was analyzed using a continuous flow system consisting of a Costech 4010 elemental combustion system (Milan, Italy) with peak intensities being measured using a Delta Plus XP isotope ratio mass spectrometer (Thermo Finnigan, Herts, UK) (Table 1).

DNA isolation

The soil nucleic acids were extracted using the UltraClean Soil DNA Isolation kit, PowerSoil DNA Isolation kit, PowerMax Soil DNA Isolation kit (all from MoBio Laboratories Inc., Carlsbad, California), and FastDNA SPIN kit (MP Biomedicals, Solon, Ohio), according to the manufacturer's protocol. Triplicate DNA extractions of each soil using each kit were completed, and the maximum amount of soil as suggested by the manufacturer was used for all kits. In the case of the PowerMax kit, the eluted DNA was concentrated to 200 μL, according to the manufacturer's protocol. A total of 5 μL of each DNA extract was run on 1% agarose gel with a 1 kb DNA ladder (GeneRuler). Gels were stained with ethidium bromide and photographed with the G-Box gel documentation system (Syngene, Cambridge, UK). However, since many of DNA extracts were below the detection limit (1 ng/μL), the final DNA yield was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, Delaware), and the purity of DNA was determined by the value of OD_{260}/OD_{280} and the value of OD_{260}/OD_{230}.

PCR

Amplification of bacteria 16S rDNA within the V3 region was done using the eubacterial-specific universal primers 341F-GC (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′, which includes a 40 bp GC clamp on its 5′ end; Invitrogen Canada) and 534R (5′-ATTACCGCGGCTGCTGG-3′; Invitrogen Canada) (Muyzer et al. 1993). The universal eukaryotic primers forward 1427–1453 (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCTCTGTGATGCCCTTAGATGTTCTGGG-3′, which includes a 40 bp GC clamp on its 5′ end) and reverse 1616–1637 (5′-GCGGTGTGTACAAAGGGCAGGG-3′) were used to amplify eukaryotic 18S

Table 1. Analysis of soil samples used for DNA extraction.

Soil sample	% Clay	% Silt	% Sand	Soil texture	Total [PAH] (μg/g soil)	Organic carbon (%)
1	23	77	0	Silt loam	<0.25	0.5
2	27	73	0	Silt loam	161.00	0.4
3	22	78	0	Silt loam	1299.00	3.0
4	4	25	70	Sandy loam	1079.00	2.4
5	26	74	0	Silt loam	3552.00	0.4
6	2	13	84	Loamy sand	4802.00	2.0

rDNA within the V4 region (van Hannen et al. 1998). PCR reactions were 50 μL in total and contained approximately 50 ng of template DNA, 1 μmol/L (each) forward and reverse primers, and 2.5 U of HotStarTaq DNA polymerase (Qiagen, Valencia, California). PCR cycling was done using a PTC-100 thermal cycler (MJ Research Inc., Waltham, Massachusetts). Bacterial 16S rDNA fragments were amplified using the following conditions: initial enzyme activation of 95 °C for 5 min; 34 cycles of denaturation at 94 °C for 1 min, denaturation at 55 °C for 1 min, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. Eukaryotic 18S rDNA fragments were amplified using the following conditions: 95 °C for 5 min; 30 cycles at 94 °C for 30 s, 52 °C for 1 min, and 68 °C for 1 min; followed by a final extension at 68 °C for 10 min. PCR products were run on a 1.2% agarose gel (*m*/*v*) stained with ethidium bromide prior to DGGE analysis to confirm successful amplification. PCR amplification was subsequently replicated to assess reliability and consistency of the extracted DNA.

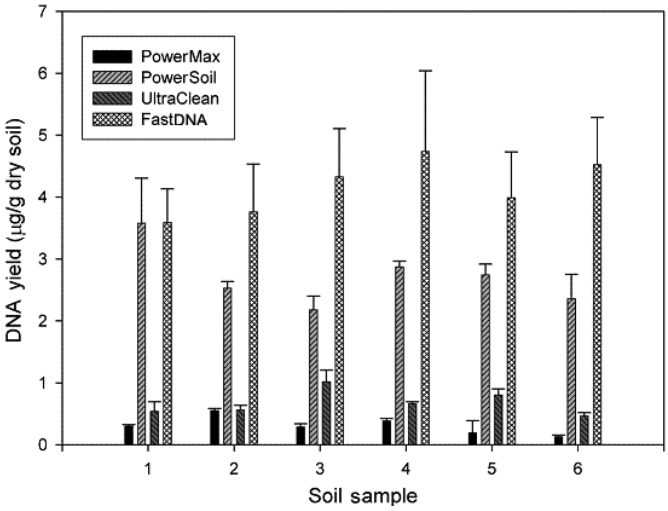
DGGE

Bacterial and eukaryotic PCR amplicons were applied onto 8% polyacrylamide gels with a denaturing gradient of 40%–70% (bacterial) or 30%–55% (eukaryotic) (100% denaturant contains 7 mol/L urea and 40% (*v*/*v*) formamide). Ten microlitres of a sample was mixed with 5 μL of loading dye and loaded onto wells. Electrophoresis was performed in 0.5× Tris–acetate–EDTA buffer at 70 V at 60 °C for 16 h using a DGGE-2401 apparatus (C.B.S. Scientific, DelMar, California). Gels were stained with ethidium bromide and visualized with the G-Box gel documentation system (Syngene, Cambridge, UK). The DGGE profiles were normalized and compared using GelCompar II version 6.5 (Applied Maths, Belgium).

Presence and effect of PAHs on extracted DNA

To determine if there were any residual PAHs in the extracted DNA using the commercial kits, a liquid–liquid extraction was performed on the remaining DNA extracts after amplification. Nanopure water was added to the DNA extract to bring the volume to 500 μL. An equal volume of dichloromethane was added to the extract, and the resulting solution was vortexed for several minutes. The organic phase was extracted using a Pasteur pipette and concentrated to 20 μL using N_2 gas. The concentrated organic phase was then run on an Agilent 6890 gas chromatograph coupled to a 5973 quadruple mass spectrometer to identify PAHs.

Fig. 1. Final yield of extracted DNA from commercial DNA kits, as determined by UV absorbance.



Results and discussion

DNA yield and quality

The DNA yields, as determined by UV absorbance, were consistently low for the UltraClean and PowerMax kits but were consistently high for the FastDNA SPIN kit, which provided significantly higher DNA yields for all soils based upon an ANOVA analysis of yield measurements, $p < 0.05$, (Fig. 1). The PowerSoil kit gave intermediate DNA yields for most soils and was equivalent to the FastDNA SPIN kit for soil 1. Across the soils, DNA extraction yield was not significantly affected by the soil texture or the organic carbon content of the soil (Pearson's *r* values were not significant).

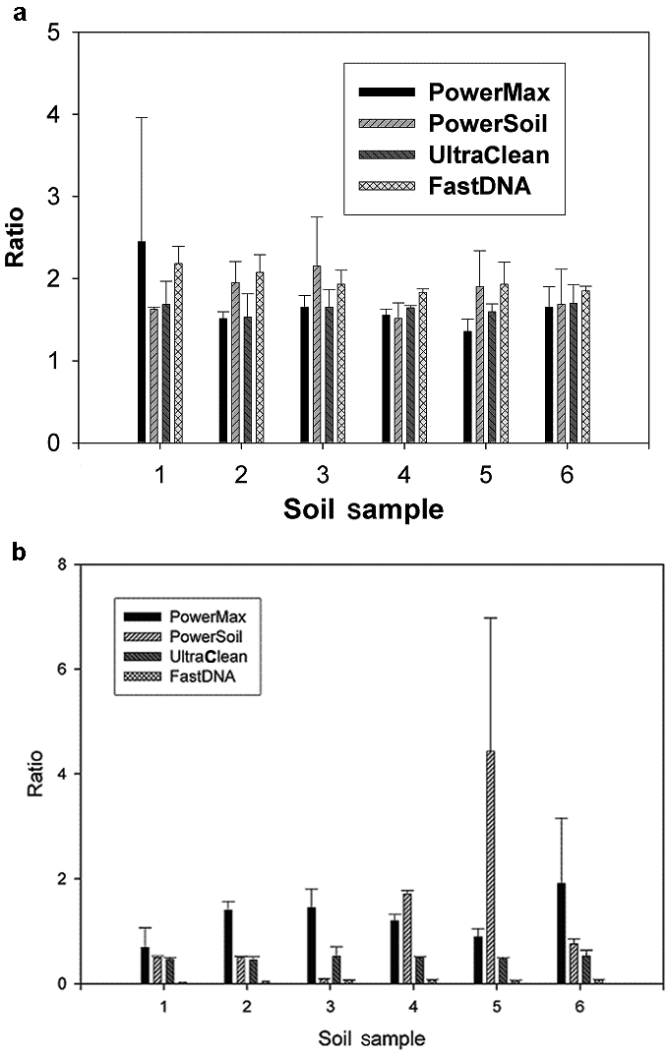
One of the most important requirements for all kits is high-quality DNA free of contaminants, which allows for successful amplification of the extracted DNA. The level of contamination can be determined by examining absorbance ratios, since DNA has an absorption peak at 260 nm. DNA purity can be assessed for contamination from residual proteins using a ratio of A_{260}/A_{280}, where ratios lower than 1.7 reflect protein contamination and ratios greater than 1.7 reflect pure DNA. Similarly, purity from humic compounds can be determined using a ratio of A_{260}/A_{230}, where ratios <2 reveal humic acid contamination and ratios >2 are characteristic of pure DNA. All four kits were roughly equivalent with respect to A_{260}/A_{280} ratios (Fig. 2*a*).

Table 2. DNA yields of six soil samples, obtained from four commercial DNA extraction kits.

Soil sample	PowerMax Soil DNA Isolation kit	PowerSoil DNA Isolation kit	UltraClean Soil DNA Isolation kit	FastDNA SPIN kit
1	0.21±0.02 μg/g	4.80±0.50 μg/g	<1 ng/μL	1.52±0.30 μg/g
2	0.18±0.02 μg/g	7.20±0.70 μg/g	<1 ng/μL	3.20±0.40 μg/g
3	<1 ng/μL	<1 ng/μL	<1 ng/μL	<1 ng/μL
4	0.06±0.01 μg/g	4.24±0.40 μg/g	<1 ng/μL	2.40±0.20 μg/g
5	<1 ng/μL	3.60±0.40 μg/g	<1 ng/μL	1.20±0.10 μg/g
6	0.12±0.01 μg/g	<1 ng/μL	<1 ng/μL	6.00±0.40 μg/g

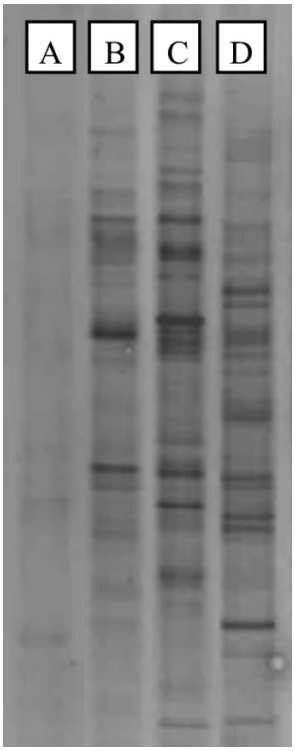
Note: DNA yields were determined using agarose electrophoresis assay. Values are the means ± standard deviations.

Fig. 2. Efficiency of commercial kits at removing (*a*) residual proteins using an absorbance ratio of A_{260}/A_{280} (ratios <1.7 reflect protein contamination, >1.7 pure DNA) and (*b*) humic acids using an absorbance ratio of A_{260}/A_{230} (ratios <2 reveal humic acid contamination, >2 pure DNA).



However, A_{260}/A_{230} ratios greatly varied among kits (Fig. 2*b*). Although none of the extracts were visibly coloured, the FastDNA SPIN kit gave the lowest A_{260}/A_{230} ratios and, therefore, had the most residual humic acid contamination. DNA yields estimated by band intensities on agarose gels support that the FastDNA SPIN kit yields were overestimates caused by high levels of UV absorbant compounds (Table 2). The UltraClean kit gave the lowest DNA yields, having both low absorbance and relatively low A_{260}/A_{230} ratios, resulting in amounts not detectable in 5 μL of DNA extract on agarose gel. The highest A_{260}/A_{230} ratios and, therefore, the cleanest DNA, was provided by the PowerMax or PowerSoil kits, depending on the soil. The higher yield given by the PowerSoil kit versus the PowerMax kit makes the former a better choice by providing the greatest amount of high-quality DNA.

Fig. 3. Denaturing gradient gel electrophoresis DGGE profiles of 16S rDNA of the same soil sample (soil 2) extracted using four different commercial DNA isolation kits: UltraClean (lane A), FastDNA SPIN (lane B), PowerMax (lane C), PowerSoil (lane D).



Low A_{260}/A_{230} ratios can be caused by humic acids but also by other aromatic compounds such as residual PAHs. This was illustrated by adding several different concentrations of naphthalene (ranging from 6 to 102 ppm) to DNA oligomer mixtures (consisting of 17–57 bp primers) of known concentrations and reassessing DNA concentration. The addition of low concentrations of naphthalene, up to 13 ppm, did not give significantly higher absorbance read-

Table 3. 16S PCR amplification results of six soil samples, based on quality of DNA extracted from four commercial extraction kits.

Soil sample	PowerMax Soil DNA Isolation kit	PowerSoil DNA Isolation kit	UltraClean Soil DNA Isolation kit	FastDNA SPIN kit
1	++/++	++/+	–/++	–/+
2	++/++	++/++	+/++	++/+
3	++/++	++/++	–/–	++/–
4	++/–	++/++	++/–	++/–
5	+/+	++/++	–/–	++/++
6	–/+	++/++	++/+	++/+

Note: ++ indicates successful amplification, + indicates partial amplification, and – indicates no amplification. Amplification results are for sample/replicate.

Table 4. 18S PCR amplification results of six soil samples, based on quality of DNA extracted from four commercial extraction kits.

Soil sample	PowerMax Soil DNA Isolation kit	PowerSoil DNA Isolation kit	UltraClean Soil DNA Isolation kit	FastDNA SPIN kit
1	++/–	++/++	+/+	++/++
2	++/++	++/++	+/+	++/++
3	++/++	++/++	–/+	++/++
4	–/++	++/++	+/–	+/++
5	–/+	++/++	+/+	++/++
6	++/++	++/++	+/++	++/++

Note: ++ indicates successful amplification, + indicates partial amplification, and – indicates no amplification. Amplification results are for sample/replicate.

Table 5. Percent similarity of replicate samples based on normalized denaturing gradient gel electrophoresis profiles.

Soil sample	PowerMax Soil DNA Isolation kit	PowerSoil DNA Isolation kit	UltraClean Soil DNA Isolation kit	FastDNA SPIN kit
1	50.0%	75.0%	55.0%	0.0%
2	50.0%	65.0%	50.0%	70.0%
3	80.0%	70.0%	0.0%	0.0%
4	40.0%	72.5%	0.0%	0.0%
5	30.0%	80.0%	0.0%	80.0%
6	40.0%	20.0%	35.0%	55.0%

Note: A value of 0 indicates that replication of the DNA was unsuccessful resulting in no replicate for comparison.

ings; however, mixtures containing amounts above 51 ppm were detectable on the Nanodrop instrument. To determine whether PAH contaminants were interfering with either extraction efficiency or assessment of DNA yields, a liquid–liquid extraction of the DNA extracts was performed. The resulting extract was run on a gas chromatography – mass spectrometer with a detection limit of 1 ppm for PAHs; there were no PAHs found in any of the DNA extracts tested. Therefore, DNA yields and concentrations presented are not biased by any PAHs found in the soils because PAHs found below 1 ppm would not have any significant effect on absorbance readings.

PCR amplification

The PowerSoil kit provided the highest quality DNA based on successful amplification of both bacterial and eukaryotic DNA for all six soils (Tables 3 and 4). The FastDNA SPIN kit extracted high-quality DNA, as demonstrated through successful eukaryotic amplifications of all six soils; however, amplification of prokaryotic DNA was not successful. DNA extracted from both the UltraClean and PowerMax kits was unreliable for PCR and lead to some successful PCR reactions depending on the soil. Specifically, the PowerMax kit produced DNA from which amplification was possible for soils 1, 2, and 3; however, amplification was problematic for soils 4, 5, and 6. It was much less successful at providing high-quality DNA from soils with higher levels of contamination, such as soils 5 and 6.

Phylotype diversity

Products from successful PCR reactions were analyzed via DGGE to assess the impact of the extraction kit on the rDNA fingerprints, i.e., the perceived phylogenetic diversity of the samples. Replicability varied between kits (Table 5), and they gave dramatically different fingerprints, ranging from 2% to 10% similarity between kits for the 16S profiles and from 10% to 25% for the 18S profiles. This result indicates that the kits differ with respect to degree of cell lysis, and

so, observed phylogenetic diversity depends greatly on the extraction kit being used (Fig. 3).

For bacterial diversity, the PowerSoil, PowerMax, and FastDNA SPIN kits displayed the greatest number of bands. However, the PowerSoil kit was the most consistent and revealed the greatest number of bands for all six soils, whereas the PowerMax kit and the FastDNA SPIN kit varied and would often reveal fewer bands than the PowerSoil kit, depending on the soil. The UltraClean kit displayed very few or almost no bands for all soils, which may reflect the poor quality of the extracted DNA.

Apparent eukaryotic diversity also varied greatly between kits. Similar to bacterial diversity, the UltraClean kit revealed very few or almost no eukaryotic bands for all six soils. The FastDNA SPIN kit revealed the greatest number of bands for all soils; however, this was not consistent, and replicates would often reveal significantly fewer bands, indicating that this kit is not the most reliable for estimating eukaryotic diversity. On the other hand, the PowerSoil kit revealed multiple bands for each soil and was very consistent between replicates.

In conclusion, the results clearly demonstrate that commercial DNA extraction kits can be used on a wide variety of soils, including heavily contaminated soils, and residual PAHs do not coextact with the DNA. In our hands, the PowerSoil kit was the most effective and reliable kit for contaminated soils because it provided the highest quality DNA that was consistently amplifiable using both eukaryotic and prokaryotic primers.

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