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DNA Recovery from Soils of Diverse Composition

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A simple, rapid method for bacterial lysis and direct extraction of DNA from soils with minimal shearing was developed to address the risk of chimera formation from small template DNA during subsequent PCR. The method was based on lysis with a high-salt extraction buffer (1.5 M NaCl) and extended heating (2 to 3 h) of the soil suspension in the presence of sodium dodecyl sulfate (SDS), hexadecyltrimethylammonium bromide, and proteinase K. The extraction method required 6 h and was tested on eight soils differing in organic carbon, clay content, and pH, including ones from which DNA extraction is difficult. The DNA fragment size in crude extracts from all soils was >23 kb. Preliminary trials indicated that DNA recovery from two soils seeded with gram-negative bacteria was 92 to 99%. When the method was tested on all eight unseeded soils, microscopic examination of indigenous bacteria in soil pellets before and after extraction showed variable cell lysis efficiency (26 to 92%). Crude DNA yields from the eight soils ranged from 2.5 to 26.9 μg of DNA g^{-1}, and these were positively correlated with the organic carbon content in the soil ($r = 0.73$). DNA yields from gram-positive bacteria from pure cultures were two to six times higher when the high-salt–SDS–heat method was combined with mortar-and-pestle grinding and freeze-thawing, and most DNA recovered was of high molecular weight. Four methods for purifying crude DNA were also evaluated for percent recovery, fragment size, speed, enzyme restriction, PCR amplification, and DNA-DNA hybridization. In general, all methods produced DNA pure enough for PCR amplification. Since soil type and microbial community characteristics will influence DNA recovery, this study provides guidance for choosing appropriate extraction and purification methods on the basis of experimental goals.

Isolation of bacterial nucleic acids from natural environments has become a useful tool to detect bacteria that cannot be cultured (11, 27), to determine the fates of selected bacteria or recombinant genes under natural conditions (10, 19), and to reveal genotypic diversity and its change in microbial ecosystems (22). Many workers have attempted to increase DNA yields from soils by using severe physical treatments such as mechanical bead beating and sonication to lyse indigenous microbial cells. Such treatments can shear DNA to sizes of 5 to 10 kb or less (11, 14), and in at least one study, the average fragment size was 100 to 500 bp (17). Such DNA may not be suitable for community analysis based on *Taq* DNA PCR, because of the risk of forming chimeric products with smaller template DNA (12). Because microbial cells may remain tightly bound to soil colloids, soils high in clay or organic matter pose particular challenges to obtaining high yields of high-molecular-weight DNA. Most DNA extraction methods have been tested on a limited number of soil types, so that their general applicability is unknown for comparative ecological studies.

Extraction of DNA from soils always results in coextraction of humic substances which interfere with DNA detection and measurement. This contamination can inhibit *Taq* DNA polymerase in PCR (18, 25), interfere with restriction enzyme digestion (15), and reduce transformation efficiency (21) and DNA hybridization specificity (19). Since humic substances are difficult to remove, DNA purification is a critical step following direct extraction to obtain DNA of sufficient purity.

Objectives of this study were to evaluate and improve DNA extraction and purification methods for speed and simplicity, DNA yields, DNA fragment size, and applicability to a broader variety of soils. We tested these methods on eight physically and chemically distinct soils, including soils from which DNA is difficult to extract and purify. We emphasized PCR amplification in evaluating DNA purity because *Taq* polymerase is sensitive to humic contamination and because PCR amplification is a major use of extracted soil DNA.

MATERIALS AND METHODS

Soils. Eight soils were used to evaluate the efficiency of DNA extraction and purification procedures. Six of these soils had been selected from a global soil collection (9) to represent a range of soil properties (Table 1). The other two soils, Native Kellogg (NK) and Cultivated Kellogg (CK), were obtained from the National Science Foundation Long-Term Ecological Research site at Kellogg Biological Station near Kalamazoo, Mich. NK and CK soils were from the same soil series (Kalamazoo sandy loam, typic hapludalf), but CK soil has been cultivated for the last 40 years while NK soil has been undisturbed. All soils came from regions having predominantly luvisolic soils, as described under the Food and Agriculture Organization Soil Classification System (7). The six soils from the global collection had been sampled between 5 and 30 cm in depth in 1993. NK and CK soils were sampled between 0 and 15 cm in 1993 and 1994. All soils were kept on ice or stored at 4°C until they were tested in the laboratory.

Soil moisture contents were determined by drying at 110°C for 48 h. Particle size analyses were performed by a modified hydrometer method, in which the clay content was determined after 8 h (4). Carbon and nitrogen contents were determined on oven-dried, ground samples in a Carlo Erba NA 1500 series 2 nitrogen/carbon analyzer (Fisons Instruments, Beverly, Mass.). Soil pH was determined in a slurry (5 parts distilled water, 1 part soil). Soil color was evaluated by visual examination in outdoor sunlight with Munsell color plates.

Bacterial strains and soil inoculation. *Pseudomonas* sp. strain B13 (5) was used as the seed organism. Cells were grown to late exponential phase on M9 medium supplemented with trace minerals and 5 mM 3-chlorobenzoate and resuspended in 2 ml of extraction buffer (see below) before being inoculated into the soils. This cell suspension was mixed with sterilized soils, which were obtained by autoclaving twice at 121°C for 60 min. Seeded soils were kept at room temperature for 30 min prior to DNA extraction.

Effect of CTAB and PVPP on humic contamination of crude extracts. Hexadecylmethylammonium bromide (CTAB) and polyvinylpolypyrrolidone (PVPP) have been used in previous studies to complex and remove contaminants from

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DNA (1, 10). Seeded NK soils were used to evaluate the effect of CTAB and PVPP in the extraction buffer on humic contamination in crude extracts. Soil (5 g) was mixed with extraction buffer (see below) containing (i) no CTAB, no PVPP; (ii) 1% CTAB, no PVPP; or (iii) no CTAB, 2 g of PVPP. Soil suspensions were then processed by the extraction method described below. Spectrophotometric A_{260}/A_{280} and A_{260}/A_{230} ratios were determined to evaluate levels of protein and humic acid impurities, respectively, in the crude extracts (14, 20).

SDS-based DNA extraction method. Since CTAB performed better in reducing humic contamination, it was used in the buffer for sodium dodecyl sulfate (SDS)-based DNA extraction. Soil samples of 5 g were mixed with 13.5 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 100 μl of proteinase K (10 mg/ml) in Oakridge tubes by horizontal shaking at 225 rpm for 30 min at 37°C. After the shaking treatment, 1.5 ml of 20% SDS was added, and the samples were incubated in a 65°C water bath for 2 h with gentle end-over-end inversions every 15 to 20 min. The supernatants were collected after centrifugation at 6,000 × *g* for 10 min at room temperature and transferred into 50-ml centrifuge tubes. The soil pellets were extracted two more times by adding 4.5 ml of the extraction buffer and 0.5 ml of 20% SDS, vortexing for 10 s, incubating at 65°C for 10 min, and centrifuging as before. Supernatants from the three cycles of extractions were combined and mixed with an equal volume of chloroform-isoamyl alcohol (24:1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 16,000 × *g* for 20 min at room temperature, washed with cold 70% ethanol, and resuspended in sterile deionized water, to give a final volume of 500 μl.

Cell lysis and direct microscopic counts. Cell lysis efficiency was estimated for six of the eight soils by direct microscopic counts of soil smears (3) obtained before and after the DNA extraction treatment. Before DNA extraction, 5 to 15 g of soil was blended in a Waring blender for 1 min in 150 to 190 ml of sterile, filtered (0.2 μm), deionized water. The coarse particles in the blended slurry were allowed to settle for 1 min, and then a 10-ml subsample was removed from the upper portion of the slurry and transferred to a 15-ml sterile tube. The subsample was vortexed for 10 s, and 4- or 6-μl aliquots were removed for smears. Each aliquot was spread evenly in a 7-mm circle on a coated slide (Cel-Line Associates, Newfield, N.J.) and quickly dried at 40°C. After DNA extraction, smears were prepared from the remaining soil pellet and the pooled supernatants from each sample. A subsample of soil pellet was removed to determine the moisture content, and the remaining pellet was blended with appropriate amounts of water to prepare smears. Before DNA was precipitated and quantified from each supernatant, a 100-μl subsample of the supernatant was diluted 1:20 in water to make smears.

Dried smears were flooded with 10 μl of a fluorescent staining solution containing 2 mg of DTAF [5-(4,6-dichlorotriazin-2-yl)amino fluorescein; Sigma Chemical Co., St. Louis, Mo.)] per 10 ml of buffer (0.85% NaCl, 50 mM Na_2HPO_4 [pH 9.0]) (16). Flooded slides were held for 30 min in a covered container to prevent drying. The slides were rinsed by immersion in fresh buffer three times for 20 min each, rinsed with water after the final immersion, and air dried. Slides were stored in the dark at 4°C for no longer than 48 h before microscopic analysis.

The slides were examined with a 63× objective on a Leitz Orthoplan 2 epifluorescence microscope, a Lep HBO 50 mercury lamp, and a Leitz I3 filter block (BP 450-490 excitation filter, RKP 510 beam splitter, and LP 515 suppression filter). A charge-coupled device camera (Princeton Instruments, Trenton, N.J.) was used to obtain digitized images of smears (26) by photographing fields selected at random along two central transects (8). Images were transferred to a Power Macintosh 7100/66 via an ST135 detector/controller and GPIB interface card (National Instruments, Austin, Tex.) for display by IP Lab Spectrum image analysis software (Signal Analytics Corp., Vienna, Va.). Bacterial cells were counted by visual examination of images on the computer monitor. All counts were obtained by one investigator.

DNA extraction from gram-positive bacteria. The gram-positive bacteria used for comparing cell lysis methods were grown on tryptic soy agar at 37°C overnight. Three methods were used to evaluate DNA extraction from these bacteria in terms of DNA yield and fragment size: (i) grinding, freezing-thawing, and SDS; (ii) freezing-thawing and SDS; and (iii) SDS. Cell pellets from pure cultures (15 ml; 10^{10} cells ml^{-1}) were ground with a mortar and pestle in the presence of sterile sand and liquid nitrogen before addition of extraction buffer and SDS. The preparations were then frozen at −70°C and thawed by microwave heating until they boiled briefly, a total of three times; then the DNA was extracted by following a protocol similar to the one described above. The DNA yield was determined by spectrophotometry.

Purification of crude DNA extracts. Four methods for purifying small portions of crude extracts were evaluated. One-tenth to one-fifth of the crude DNA extract from 5 g of soil was processed in four ways, and the final volumes of the eluates were adjusted to their original volumes. The four methods were as follows: (i) single minicolumn (extract was passed through one Wizard minicolumn containing 1 ml of Wizard PCR Preps purification resin [Promega, Madison, Wis.]); (ii) double minicolumn (eluate from the first minicolumn was purified further with fresh resin and passage through another minicolumn); (iii) gel plus minicolumn (extract was purified by agarose gel electrophoresis followed by passage of the excised and melted gel band through a Wizard minicolumn); and

TABLE 1. Sampling locations and properties of soils used in DNA extraction procedures

Soil sampling location	Soil texture	Climate/native vegetation	FAO[a] soil grouping	Amt (%) of:			Moisture content %	Moist color (hue, value/chroma)	pH	% C	% N
				Sand	Silt	Clay					
Waitville, Saskatchewan (WV)	Loam	Temperate/boreal forest	Albic luvisol	45	39	16	28	7.5 YR 2/0	6.1	5.85	0.51
Bittern, Saskatchewan (BT)	Sandy loam	Temperate/boreal forest	Albic luvisol	59	37	7	14	10 YR 4/3	5.7	0.86	0.05
Lake Beloye, Russia (RU)	Sandy loam	Temperate/boreal forest	Albic luvisol	54	41	5	29	10 YR 3/1	4.8	4.73	0.12
Native Kellogg, Mich. (NK)	Sandy loam	Temperate/deciduous hardwood	Orthic luvisol	52	43	5	14	10 YR 3/1	5.4	1.50	0.12
Cultivated Kellogg, Mich. (CK)	Sandy loam	Temperate/deciduous hardwood	Orthic luvisol	70	23	7	12	10 YR 3/2	6.7	0.95	0.08
Lagos Penuelas, Chile (LP)	Sandy loam	Mediterranean/sclerophyllous	Chromic luvisol	67	21	12	10	10 YR 3/2	6.1	0.97	0.09
Venice Hills, Calif. (VH)	Loam	Mediterranean/sclerophyllous	Chromic luvisol	42	41	17	11	7.5 YR 4/3	6.4	0.85	0.08
Merredin, Australia (ME)	Sandy clay loam	Mediterranean/sclerophyllous	Xanthic ferralsol	55	14	31	9	5 YR 4/3	9.1	0.59	0.04

[a] FAO, Food and Agriculture Organization.

TABLE 2. Comparison of DNA yield and purity of the crude DNA from seeded NK soil subjected to different treatments[a]

Treatment	DNA yields (μg/g [dry wt] of soil)[b]	A_{260}/A_{280} ratio[b]	A_{260}/A_{230} ratio[b]
No PVPP, no CTAB	17.1 ± 0.9	1.17 ± 0.02	0.72 ± 0.03
CTAB, no PVPP	17.5 ± 1.2	1.35 ± 0.04	0.91 ± 0.03
PVPP, no CTAB	10.9 ± 1.5	1.23 ± 0.05	0.88 ± 0.03
Pure culture		1.89	1.57

[a] NK soil was sampled in 1993 and stored at 4°C for 6 months.
[b] DNA yields (mean values, $n = 3$, ± 1 standard deviation) were determined by fluorometry. The ratios were calculated from spectrophotometric measurements.

(iv) gel plus centrifugal concentrator. (The crude extract was subjected to gel electrophoresis, and the DNA band was excised, melted, and treated with Gelase [Epicentre Technologies, Madison, Wis.] by following the rapid protocol of the manufacturer. Nucleic acids were then washed and concentrated in a Centricon-50 [Amicon Corp., Beverly, Mass.].)

Since the minicolumn capacity was limited to 1 ml of resin, only a fraction (1/5 to 1/10) of the crude extract from 5 g of soil could be purified at a time. A larger-scale gel-plus-column purification procedure was used to purify the entire crude extracts from 5-g samples of the eight test soils. The procedure employed a 20-ml-capacity column with a different resin (Wizard Minipreps Plasmid Purification resin [Promega]) because the resin used in the minicolumns contained a 230-nm-absorbing substance that interfered with spectrophotometric measurement of DNA. The DNA was eluted from the resin twice with 500 μl of hot (70°C) Tris-EDTA buffer to facilitate release of high-molecular-weight DNA.

DNA quantification. After small-scale purifications, DNA was quantified by fluorometry with a TK 100 fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.), by following the extended assay protocol provided by the manufacturer. The fluorometer was calibrated with herring sperm DNA (Boehringer Mannheim, Indianapolis, Ind.). The DNA yields were estimated on the basis of at least three replicate determinations.

DNA was quantified after large-scale purification by determining fluorescence intensities of extracts in agarose gel bands in scanned Polaroid photographs. Crude and final DNA extracts were subjected to electrophoresis in Tris-acetate-EDTA (TAE) buffer containing 0.5 μg of ethidium bromide per ml in 0.7% agarose gels containing DNA standards of 5 to 60 ng of lambda phage DNA (Boehringer Mannheim). Gel photographs were scanned with a Hewlett-Packard ScanJet IIc scanner, generating digitized images that were analyzed with IP Lab Spectrum software. A standard curve of DNA concentration (10 to 50 ng of DNA) versus integrated pixel intensity ($r^2 = 0.98$) was prepared for each gel and used to calculate the final DNA concentrations in the DNA extracts.

PCR, restriction enzyme digestion, and Southern blotting. Primers and PCR conditions for amplifying 16S rRNA and *clcD* genes were described previously (28, 29). Restriction enzyme digests were performed with approximately 0.2 μg of DNA and 4 U of an endonuclease (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, or *Xho*I) in 20 μl of the appropriate buffer as provided by the manufacturer. After incubation for 12 to 16 h, the DNA fragments were resolved in a 1% agarose gel. To evaluate DNA hybridization, 0.5 μg of the purified DNA from the seeded soils was digested with 10 U of *Bam*HI, separated in an 0.8% agarose gel, and transferred to a GeneScreen Plus membrane (Dupont, Boston, Mass.). Prehybridization, hybridization, and washings were carried out as described previously (29). A PCR-amplified 615-bp fragment of the *clcD* gene was used as the probe.

RESULTS

Soil properties. The physical and chemical properties of the eight soils used in the DNA extraction study were quite different (Table 1). Soils were classed as loams, sandy loams, or sandy clay loams, with clay contents ranging from 5 to 31%. The WV soil had the highest organic C content, which was reflected in its dark color (chroma = 0), and the highest N content. The ME soil had the lowest organic C and N contents, the reddest color, and the lowest moisture content. The pH of the soils ranged from 4.8 to 9.1.

Effect of CTAB or PVPP on DNA extraction from NK soil. When seeded NK soil was treated with different extraction buffers, no difference in DNA yield was observed among treatments with or without CTAB. Significant differences in DNA yield did occur among treatments with or without PVPP (Table 2). The crude DNA solutions from PVPP or CTAB treatments were lighter in color and had higher A_{260}/A_{230} and A_{260}/A_{280} ratios than did the solutions from treatments without PVPP or CTAB (Table 2). Both CTAB and PVPP can effectively remove humic materials, but unlike PVPP, CTAB resulted in no DNA loss. CTAB and PVPP did not completely remove humic compounds, since the A_{260}/A_{230} and A_{260}/A_{280} ratios for crude DNA from soils were significantly lower than the ratios for DNA solutions from pure cultures (Table 2).

DNA extraction efficiency was determined by comparing the total crude DNA obtained from a known cell density of sterile seeded soil samples with the DNA extracted from the pure culture. Crude DNA recoveries from seeded NK and CK soils were 92 to 99%.

No significant differences in DNA yields were observed when the crude DNA was allowed to precipitate in isopropanol for 1 h or overnight, either at room temperature or at −20°C. Repeated extractions of the soil pellets were beneficial, since small amounts of DNA were still recovered after the second and even the third wash, depending on the soil (data not shown). In the optimized method used subsequently, soil pellets were extracted three times. Most of the soil DNA fragments were larger than 23 kb and similar in size to DNA isolated from pure cultures (Fig. 1). These results suggest that the extraction protocol did not cause severe shearing of DNA.

Evaluation of DNA extraction and cell lysis on more challenging soils. Crude DNA was extracted from eight unseeded soils by the SDS-based method with CTAB, and mean yields ranged from 2.5 to 26.9 μg of DNA per g (dry weight) of soil (Table 3). The WV soil had the highest DNA yield, and the ME soil had the lowest yield. Significant correlation was observed between crude DNA yield and soil organic C content ($r = 0.73$, $P = 0.01$).

Lysis efficiencies of the DNA extraction procedure for six soils were estimated by microscopic examination of soil smears before extraction and of soil pellets and pooled supernatants after extraction. No cells were found in any of the 1:20 dilutions of the pooled supernatants, but cells were found in the residual pellets (Table 4). Postextraction counts of WV, RU, LP, and BT soils were significantly lower than preextraction counts, indicating high lysis efficiencies (67 to 92%). Two soils, VH and ME, appeared to show poor lysis by this method. Significant correlation was observed between cell lysis effi-

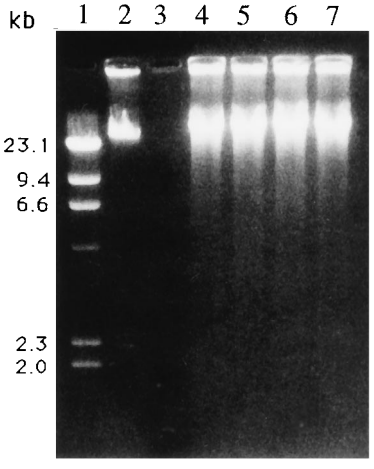

FIG. 1. Agarose gel electrophoresis of total DNA extracted from NK soil by different treatments. Lanes: 1, *Hin*dIII-cut bacteriophage lambda molecular size marker (1 μg); 2, pure-culture DNA from *Pseudomonas* sp. strain B13; 3, sterile soil; 4, sterile soil plus B13; 5, nonsterile soil DNA extracted without PVPP and CTAB; 6, nonsterile soil DNA extracted with PVPP; 7, nonsterile soil DNA extracted with CTAB.

TABLE 3. Crude DNA yields from eight soils and percentages of crude DNA recovered in the large-scale purification method involving gel electrophoresis and passage through one Megacolumn with 10 ml of Wizard Minipreps plasmid purification resin

Soil	Crude DNA yield[a] (μg/g [dry wt] of soil)	Final DNA yield[a] (μg/g [dry wt] of soil)	Crude yield CV (%)[b]	Final yield CV (%)[b]	% of crude DNA recovered
WV	26.9 ± 6.5	20.1 ± 5.0	24	25	75
BT	12.5 ± 5.1	3.4 ± 0.9	41	28	27
RU	13.7 ± 2.3	8.4 ± 2.8	17	34	61
NK	21.6 ± 5.1	12.0 ± 1.3	24	11	56
CK	4.9 ± 1.1	3.9 ± 1.2	22	31	80
LP	5.7 ± 1.1	3.1 ± 1.4	20	44	54
VH	3.0 ± 1.1	2.3 ± 0.5	35	22	77
ME	2.5 ± 0.6	2.0 ± 0.6	22	31	80

[a] DNA yields were estimated by gel staining. Yields are mean values (± 1 standard deviation) with $n = 5$, except for NK and CK soils, for which $n = 6$.
[b] CV, coefficient of variation.

ciency and clay content of the soils ($r = -0.67$; $P = 0.01$). Lysis was also evaluated by comparing the maximum and minimum expected DNA yields based on literature estimates of cellular DNA content for soil bacteria (2, 23). DNA yields for soils WV, RU, NK, CK, and ME were within the ranges of expected DNA yields, but observed values for BT, LP, and VH soils were below the minimum expected DNA yields (Table 4). By this method of evaluation, the ME soil gave the expected amount of DNA.

Comparison of different methods for lysing gram-positive bacteria. Since the SDS-based method may not have lysed some gram-positive bacteria, we evaluated two more physically severe cell lysis methods on pellets from pure cultures of gram-positive bacteria. The DNA yield was two to six times higher for most of the bacteria examined by the grinding-freezing-thawing-SDS method than by the freezing-thawing-SDS and SDS methods (Table 5). While a very small portion of the DNA was sheared by the grinding-freezing-thawing-SDS method, most of the DNA had a high molecular weight and a similar size to the DNA from the freezing-thawing-SDS and SDS methods.

Comparison of DNA purification methods with crude DNA from NK soil. To evaluate DNA purity for enzyme digestion, PCR amplification, and DNA hybridization, four purification methods were compared by using portions of the crude extracts from seeded and unseeded NK soil. Because of the small capacity of the minicolumn used in these methods, only 1/10 of the crude DNA extract from 5 g of soil was purified at a time. All four purification methods resulted in complete removal of the brown color from crude DNA solutions. DNA recovery varied with different purification methods. Higher recovery was obtained with gel-plus-concentrator purification than with column methods (Table 6). However, recovery by gel electrophoresis was more variable and depended on the size distribution of DNA fragments in crude extracts. In addition, loss of DNA was greater for the first minicolumn purification (~20%) than for the second minicolumn purification (~5 to 6%) (Table 6). This suggests that humic materials in crude extracts might interfere with DNA binding to the resin.

Restriction endonuclease digestion was possible only with purified DNA. While all enzymes cut the DNA purified by the gel-plus-minicolumn method, DNA resulting from single-minicolumn purification was only partially digested by most of the enzymes (Table 6). DNA quality could be improved by a second minicolumn purification, since the eluted DNA was digested by most of the enzymes. While all of the purified DNA samples were completely digested by *Bam*HI, most methods resulted in DNA that was only partially digested by *Hin*dIII (Table 6).

Amplification of the 16S rRNA genes was successful when DNAs purified by all tested methods were used as templates (Fig. 2). No PCR products were observed with DNA from

TABLE 4. Direct counts and DNA yields for individual samples of eight soils

Soil	Preextraction count/g (dry wt) of soil[a]	Postextraction count/g (dry wt) of soil	% Lysis efficiency[b]	Expected DNA yield[c] (μg/g [dry wt] of soil)	Crude DNA yield[d] (μg/g [dry wt] of soil)
WV	$(9.7 \pm 0.6) \times 10^9$	$(2.0 \pm 0.2)^e \times 10^9$	79	15–50	33.5
BT	$(7.3 \pm 0.4) \times 10^9$	$(5.7 \pm 2.1)^e \times 10^8$	92	11–39	8.7
RU	$(6.9 \pm 1.1) \times 10^9$	$(1.4 \pm 0.1)^e \times 10^9$	80	9–40	15.5
NK	$(5.1 \pm 1.0) \times 10^9$	ND[f]	ND	7–31	19.6
CK	$(2.9 \pm 0.7) \times 10^9$	ND	ND	4–18	4.9
LP	$(4.6 \pm 0.5) \times 10^9$	$(1.5 \pm 0.2)^e \times 10^9$	67	7–26	4.6
VH	$(3.5 \pm 0.3) \times 10^9$	$(2.6 \pm 0.4) \times 10^9$	26	5–19	2.0
ME	$(1.3 \pm 0.1) \times 10^9$	$(4.2 \pm 0.6) \times 10^9$	—[g]	2–7	2.3

[a] Mean count ± standard deviation (two smears per sample).
[b] Determined from one sample of each soil and calculated from mean counts: [100 − (postextraction count/preextraction count)] × 100.
[c] Range of expected DNA yield obtained by multiplying lower and upper limits of preextraction counts (mean counts ± 1 standard deviation) by low and high literature values reported for cellular DNA content of soil bacteria (1.6 fg cell^{-1}, as reported by Bakken and Olsen [2], and 5 fg cell^{-1}, as suggested by Torsvik and Goksoyr [23]).
[d] DNA yield from the supernatant associated with pellet used for the postextraction direct count. The DNA yield was estimated by gel staining.
[e] Significantly different from the preextraction count at the 5% level.
[f] ND, not determined.
[g] Not determined, since postextraction counts were higher than preextraction counts. Higher counts in ME soil following DNA extraction could have been due to cell masking by soil particles in preextraction ME smears, because they contained more soil (1.1 mg cm^{-2}) than did preextraction smears from other soils (0.14 to 0.7 mg cm^{-2}). Bloem et al. (3) have recently recommended a maximum soil density for direct counts on loam soil of 0.8 mg cm^{-2} to minimize cell masking by soil particles.

TABLE 5. Comparison of DNA yield from gram-positive bacteria[a] by different lysis methods

Treatment	DNA yield (μg/μl) from:							
	A. globiformis	*B. subtilis*	*C. renale*	*M. luteus*	*R. erythropolis*	Ea39	Ben-28	*S. lividans*
Grinding-freezing-thawing-SDS	0.47	0.33	0.40	0.22	0.23	0.19	0.26	0.37
Freezing-thawing-SDS	0.13	0.19	0.46	0.12	ND[b]	ND	ND	0.08
SDS	0.11	0.16	0.43	0.12	0.10	0.07	0.05	0.06

[a] The gram-positive bacteria are *Arthrobacter globiformis*, *Bacillus subtilis*, *Corynebacterium renale*, *Micrococcus luteus*, *Rhodococcus erythropolis*, *Rhodococcus* sp. strain Ea39, *Rhodococcus* sp. strain Ben-28, and *Streptomyces lividans*. The DNA yields were estimated by spectrophotometry.
[b] ND, not determined.

crude extracts. No amplification products were detected when target DNA sequences were not present in the reaction mixtures. A second set of primers (for the *clcD* genes) was used to further test for PCR amplification in parallel seeded soils. The DNA templates purified by all methods produced the expected product (data not shown).

DNA extracted from seeded NK soil was used to determine whether the DNA purified by each of the four methods was pure enough for Southern hybridization. Crude and purified DNA extracts and DNA obtained from pure cultures of the same strain were hybridized with the *clcD* gene probe. Signal intensities were very similar between pure-culture DNA extracts and purified soil DNA from each of the four methods (Fig. 3). Weak hybridization was observed for the crude DNA. The DNA purified by all tested methods was pure enough for Southern hybridization.

Evaluation of purification methods on crude DNA extracts from more challenging soils. Small-scale purification methods were also evaluated with crude DNA extracts from the six global soils. Only the double-minicolumn and gel-plus-minicolumn methods resulted in complete removal of the dark color from all six crude DNA solutions. The gel-plus-concentrator method did not completely remove the dark color from the WV and RU extracts, probably because of the high organic C contents of these soils. Crude extracts from WV, RU, and NK soils all contained greater amounts of high-molecular-weight humic acids, as observed during electrophoretic separation of DNA from humic contaminants. After electrophoresis, excised DNA bands could have contained such contaminants, which cannot be washed through concentrator filters with lower-molecular-weight cutoffs.

PCR amplification of the 16S rRNA genes was successful with DNA purified by the gel-plus-minicolumn and gel-plus-concentrator methods. DNA purified by the double-minicolumn method was amplified in only four of the six soils (WV and RU soils produced no PCR amplification), indicating that this method yielded DNA that was less pure. The DNA fragments from all soils were larger than 20 kb.

A larger-scale gel-plus-column method was also evaluated on these soils. The A_{260}/A_{280} and A_{260}/A_{230} ratios of DNA purified by the large-scale method were 1.6 to 1.8 and ≥1.9, respectively, indicating that the DNA was of good quality. For most of the extracts, 0.1-μl aliquots resulted in better PCR amplification than did 1-μl aliquots (Fig. 4). The WV soil extract still appeared to contain substances that interfered with the reaction, because no amplification was observed with 1-μl aliquots. DNA fragments purified by the large-scale procedure were all larger than 20 kb (data not shown). However, the large-scale purification method gave poorer DNA recoveries (27 to 80%; Table 3) than the small-scale methods did. Recoveries were improved slightly (e.g., from 53 to 75% for NK soils and from 68 to 85% for CK soils) when DNA Cleanup resin or Maxipreps Plasmid Purification resin (Promega Corp.) was used instead of the Minipreps resin. The resin choice may have

TABLE 6. DNA recovery for different purification methods and enzyme digestion of the DNA samples purified from unseeded NK soil

Purification method	Recovery[a] (%)	Digestion[b] by:					
		*Bam*HI	*Dra*I	*Eco*RI	*Eco*RV	*Hin*dIII	*Xho*I
Crude extracts	100	−	−	−	−	−	−
Column	80.6 ± 2.1	+	±	±	±	±	±
Column + column	74.5 ± 3.8	+	+	+	+	±	+
Gel + centricon	91.4 ± 7.5	+	+	±	+	±	+
Gel + column	84.1 ± 7.1	+	+	+	+	+	+

[a] Percentage of DNA recovered as measured by UV absorption compared with DNA in crude extract ± 1 standard deviation.
[b] +, complete digestion; ±, incomplete digestion; −, no digestion.

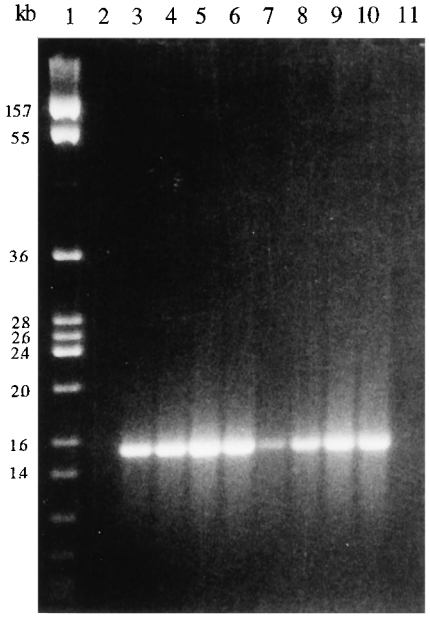

FIG. 2. Agarose gel electrophoresis of 16S rDNA amplification products from DNA of unseeded NK soil samples. Lanes: 1, *Hin*dIII-, *Eco*RI-, and *Bam*HI-cut bacteriophage lambda molecular size marker (1 μg); 2, crude DNA extracts; 3 and 4, undiluted (40.5-ng) and 10^{-1}-diluted (4.1-ng) DNA extracts purified by gel plus centrifugal concentrator; 5 and 6, undiluted (35-ng) and 10^{-1}-diluted (3.5-ng) DNA extracts purified by gel plus minicolumn; 7 and 8, undiluted (30-ng) and 10^{-1}-diluted (3.0-ng) DNA extracts purified by single minicolumn; 9 and 10, undiluted (28-ng) and 10^{-1}- diluted (2.8-ng) DNA extracts purified by two successive minicolumns; 11, reaction mixture only, without DNA template.

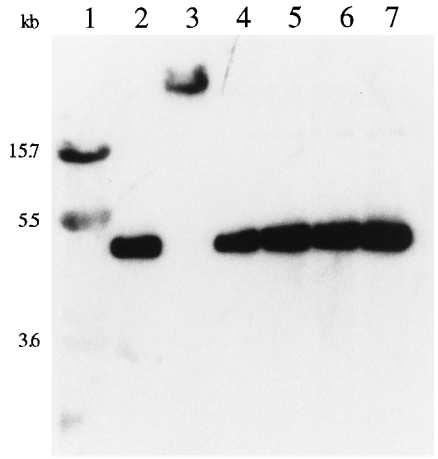

FIG. 3. Autoradiogram of hybridization signals with the *clcD* gene after Southern transfer of DNA from seeded sterile NK soil. Lanes: 1, *Hin*dIII-cut bacteriophage lambda molecular size marker (1 μg); 2, pure-culture DNA; 3, crude DNA extracts; 4, DNA extracts purified by gel electrophoresis plus centrifugal concentrator; 5, DNA extracts purified by gel electrophoresis plus column; 6, DNA extracts purified by single column; 7, DNA extracts purified by double column.

to be optimized when recovery of large amounts of DNA is important.

DISCUSSION

We divided the problem of DNA recovery from soil into two component methods, i.e., (i) cell lysis and extraction of crude DNA and (ii) purification of crude DNA, since there are advantages in combining different lysis and purification methods for different cases. The component methods were first developed on a standard soil (NK) and then tested on a set of more challenging soils. Three evaluation approaches were used to determine whether the SDS-based extraction method recovered most of the bacterial DNA: (i) DNA recovery efficiency from seeded bacteria in a standard soil; (ii) lysis efficiency of indigenous bacteria in more challenging soils; and (iii) comparison of crude DNA yields with expected DNA yields.

The SDS-based extraction method resulted in 92 to 99% recovery of the DNA from bacteria added to soil. These efficiencies were comparable to or higher than those obtained by other laboratories (6, 21). This first approach to evaluating DNA recovery, however, can overestimate extraction efficiency, because indigenous bacteria may be more difficult to lyse than seeded bacteria. When we determined net losses in indigenous cell counts after extraction, lysis efficiencies varied from 26 to 92% among five test soils. The variation in cell lysis apparently reflects differences in soil characteristics and bacterial community composition (i.e., soils exhibiting low cell lysis may have contained higher proportions of gram-positive cells). Crude DNA yields from the SDS-based extraction method agreed reasonably well with expected yields based on direct microscopic counts. We compared each experimental yield with a range of expected yields, rather than a single value, because of the uncertainty regarding the choice of an average cellular DNA content for soil bacteria.

SDS has been the most widely used cell lysis treatment for DNA extraction from pure cultures, soils, and sediments. Trevors et al. (24) found that the SDS-based cell lysis protocol provided the highest DNA yields in comparison with freezing-thawing and Sarkosyl-based lysis protocols. More et al. (13) showed that the percentage of indigenous cells remaining after SDS treatment of a sediment (13%) was lower than the percentage of cells left after 10 min of bead milling (26%). Our results indicated that SDS-based cell lysis, in combination with high-salt treatment and heating, was effective for most of the soils but appeared to be influenced by clay content and was not effective for at least some gram-positive bacteria. Thus, for soils exhibiting poor cell lysis or studies depending on extensive sampling of gram-positive DNA, other lysis treatments or combinations of treatments could be considered. Combining SDS with bead mill homogenization resulted in higher cell lysis efficiency for *Bacillus* endospores (13). However, bead mill homogenization and other physical methods such as sonication generally cause severe DNA shearing (11, 14). Our results showed that the combination of grinding, freezing-thawing, and SDS resulted in much higher DNA yields from most of the gram-positive bacteria but without severe shearing.

Although DNA purified by all methods could be amplified and hybridized, some variation in DNA purity was observed with respect to restriction enzyme digestion (Table 6). The gel-plus-minicolumn method appeared to result in the purest DNA, because the DNA was completely digested by all enzymes examined; the method also provided good recovery with the standard soil. However, the larger-scale gel-plus-column method gave variable DNA recovery from crude extracts from other soils. Single- or double-minicolumn purifications appeared to give DNA which was incompletely digested and less suitable for PCR amplification, as well as having lower recovery efficiency. Single- or double-minicolumn methods, however, were very rapid and less expensive.

The gel-plus-column method gave very pure DNA, while the gel-plus-concentrator method gave the highest recovery. The latter method, however, may not remove all humic contaminants from crude extracts of soils with low chromas (0 or 1), because these soils appear to contain higher proportions of high-molecular-weight humic acids. If gel-plus-concentrator

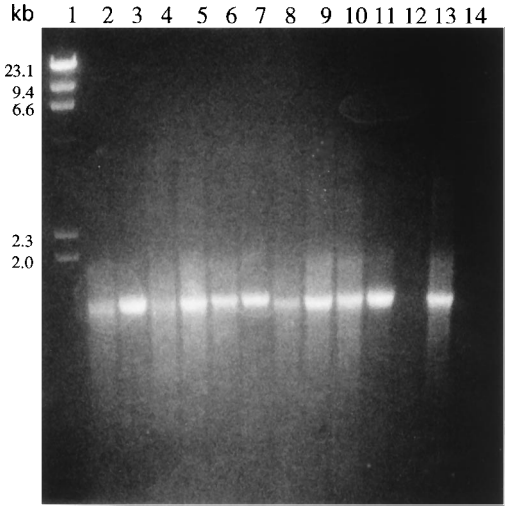

FIG. 4. Agarose gel electrophoresis of 16S rDNA amplification products of DNA samples from six soils purified by the large-scale procedure. Lanes: 1, *Hin*dIII, *Eco*RI, and *Bam*HI-cut bacteriophage lambda molecular size marker (1 μg); 2 and 3, undiluted (15-ng) and 10^{-1}-diluted (1.5-ng) BT extracts; 4 and 5, undiluted (20-ng) and 10^{-1}-diluted (2.0-ng) LP extracts; 6 and 7, undiluted (8.5-ng) and 10^{-1}-diluted (0.85-ng) ME extracts; 8 and 9, undiluted (36-ng) and 10^{-1}-diluted (3.6-ng) RU extracts; 10 and 11, undiluted (8-ng) and 10^{-1}-diluted (0.8-ng) VH extracts; 12 and 13, undiluted (40-ng) and 10^{-1}-diluted (4-ng) WV extracts; 14, reaction mixture only, without DNA template.

purification is used on these soils, concentrator units with the highest-molecular-weight cutoffs should be used, because some humic acids have molecular weights of 100,000 or greater. Larger-scale gel-plus-column purification can provide larger amounts of DNA but is more expensive, requiring additional agarose and DNA-binding resin.

In summary, the DNA extraction and purification methods evaluated here are simple, rapid, and efficient for most soils and purposes. DNA could be extracted from eight soil samples in 6 h by the SDS-based method. Because of the gentle nature of the extraction treatment, the DNA fragment size in crude extracts was >23 kb. DNA purification required 2 to 4 h for the single- or double-column method, 8 to 10 h for the gel-plus-column method, and 12 to 14 h for the gel-plus-concentrator method. If DNA purity is of the greatest concern, we recommend gel-plus-column methods. It is also important to recognize that no single method of cell lysis or purification will be appropriate for all soils and experimental goals. The basic methods suggested should be appropriate for the more common cases, but different combinations and modifications of lysis and purification protocols will probably be needed for some conditions.

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