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Choice of DNA extraction protocols from Gram negative and positive bacteria and directly from the soil

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DNA extraction is a fundamentally important step for the implementation of genotypic techniques in microbial identification, and the use of such techniques has become essential for the analysis of soil microbial diversity. Considering culture independent methodologies, it is still necessary to ensure that DNA is extracted in appropriate amounts and that extracted DNA is inhibitor-free. This study aimed at selecting a single protocol suitable for the extraction of total DNA from Gram positive and negative bacteria isolated from different sources, as well as a protocol for the direct extraction of DNA from soil. Four experimental protocols and a commercial kit were tested for the extraction of total DNA from isolated bacteria. Among the protocols, the detergent + salt + thermal incubation method (based on Harju et al., 2004) was considered the most promising because it produced satisfactory yields of DNA, with adequate quality for all isolates studied, especially *Staphylococcus aureus,* without the need to use enzymes and glass beads which can make the extraction process more expensive. Three experimental protocols and the commercial kit were tested for the direct extraction of DNA from soil. Regarding PCR amplification, the amount of total DNA extracted is less limiting than its quality. Thus, commercial kit PowerMax™ Soil DNA Isolation (MoBio) offered more promising results, because although this provided low yields of DNA, it was sufficient for polymerase chain reaction (PCR) amplification.

Key words: Genotypic characterization, bacterial diversity, polymerase chain reaction (PCR), agroforestry system, organic farming.

INTRODUCTION

The biodiversity of microbes within soil is significant for the maintenance of healthy soil because these microbes are involved in many vital functions like crucial cycles of C, N, P, formation of soil, toxin removal and so on.

Microbial characteristics of soils are being evaluated increasingly as sensitive indicators of soil health because of the clear relationships between microbial diversity, soil and plant quality, and ecosystem sustainability (Doran

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and Parkin, 1994). While the understanding of microbial properties such as biomass, activity and diversity are important to scientists in furthering knowledge of the factors contributing to soil health, results of such analyses may also be useful to extension personnel and farmers in devising practical measures of soil quality.

Traditional microbiological methods for diversity studies are based on the cultivation of microorganisms prior to phenotypic and genotypic identification. Isolation and purification of nucleic acids from bacteria requires its effective separation from other cellular constituents. Furthermore, it is essential to maintain the integrity of these molecules, which should remain unchanged throughout the extraction procedure, because the information contained in DNA depends on its sequence. Although the extraction method does not affect the sequence directly, some protocols result in breakage of DNA polymers, causing the loss of such information. Therefore, it is extremely important that the extracted nucleic acids remain as intact as possible.

Unlike Gram negative bacteria that are readily lysed by standard protocols, the Gram positive species are comparatively more resistant to cell lysis because of a greater concentration of peptide and cross-bond peptides in the cell wall. For example, *Staphylococcus* spp. has a thick cell wall of (20-80 nm in diameter) (Prescott et al., 1999). This hinders cell lysis, often requiring the addition of enzymes such as lysostaphin and acromopeptidase to the lysis buffer (Ezaki and Suzuki, 1982; Mason et al., 2001; Schindler and Schuhardt, 1964; Zschöck et al., 2000). This burdens the extraction process. On the other hand, Gram negative bacteria, such as *Escherichia coli,* with diameters of 1-3 nm and a thin layer of peptidoglycan (Prescott et al., 1999), may have the crosslinking cleaved by EDTA or lysozyme and are generally lyzed with boiling or SDS (Sambrook and Russel, 2001).

Characterization studies of microbial diversity in highly diversified environments, such as soil, have revealed the difficulties of application of specific DNA extraction protocols for each bacterial group. This makes it desirable to establish a single protocol for Gram positive and negative bacteria that enables the extraction of nucleic acids in quantity and quality required for subsequent application techniques, such as polymerase chain reaction (PCR) to identify them (Baratto and Megiolaro, 2012; Kramer and Coen, 2001; Sambrook and Russel, 2001; Weissensteiner et al., 2004).

According to the estimates, c. 99% of the microorganisms present in nature are not cultivable by standard techniques. Therefore, the genetic information and biotechnological potential of the majority of the organisms would be untapped by conventional approaches (Chernitsyna et al., 2008; Green and Keller, 2006). In order to circumvent limitations of the culture method, DNA-based approaches, have been adopted to explore the entire microbial community (Nordgard et al., 2005). Many of these techniques rely on PCR, such as nucleic acid hybridization, DNA cloning and sequencing, and denaturing gradient gel electrophoresis. However, the quantitative and qualitative efficiency of these techniques in obtaining DNA need to be guaranteed.

Soil is an extremely complex environment, with a multitude of colloids having electric charges capable of adsorbing DNA molecules. Thus, it is a limiting factor in obtaining DNA from this environment. Another critical issue is the presence of humic substances, which are acidic macromolecules that precipitate in the same pH range as DNA, and which can inhibit the activity of various enzymes, such as Taq DNA polymerase, and restriction enzymes used in various nucleic acid-based techniques (Tebbe and Vahjen, 1993; Torsvik, 1995).

Many protocols for the extraction of environmental DNA have been published, and some of them are commercialized as soil DNA extraction kits. The methods vary with respect to shearing, purity and quantity of the isolated DNA. However, the basic concept of cell lysis by enzymatic and hot detergent (SDS) treatment is still the core of many DNA extraction methods (Rondon et al., 2000). Besides, some protocols also apply mechanical forces generated by bead beating, freeze-thawing and sonication methods to disrupt the rigid cell structure (Kennedy and Marchesi, 2007; Sharma et al., 2007; Voget et al., 2003).

The soil extraction protocols are generally classified as direct and indirect DNA extraction procedures. Direct DNA isolation is based on cell lyses within the sample matrix and subsequent separation of DNA from the matrix and cell debris (Voget et al., 2003). While the indirect approach involves the extraction of cells from the environmental material prior to the lytic release of DNA (Kauffmann et al. 2004; Santosa 2001), direct DNA extraction protocol involves soft and harsh lysis methods.

Soft lysis method is based on the disruption of microorganism solely by enzymatic and chemical means, whereas harsh lysis approach involves the mechanical cell disruption by bead beating, sonication, freeze-thawing and grinding. Because the composition of different habitats varies with respect to their matrix, organic and inorganic compounds and biotic factors, standardization of total DNA extraction technique is desirable. Improved DNA extraction techniques could help to ensure analyses that adequately represent the entire community's genome without inhibitory substances.

Hence, the aim of this study was to evaluate the comparative effectiveness of different protocols available in the literature and commercial kits to select a single protocol for the extraction of DNA from Gram positive and negative bacteria isolated from different sources and a protocol for the direct extraction of DNA from soil from different regions and cropping systems in Bahia (Brazil).

Table 1. Morphotinctorial characteristics and sources of bacterial isolates used in this study.

Isolates	Species	Morphotinctorial characteristics	Source
30A	*Vibrio cholerae*	Gram-negative curved-rod shape	Mussels
40B	*Vibrio cholerae*	Gram-negative curved-rod shape	Oysters
BP11	*Pseudomonas* sp.	Gram-negative bacilli	Soils
BN5	*Bacillus* sp.	Gram-negative bacilli	Soils
BT108	*Bacillus* sp	Gram-negative bacilli	Soils
BP1	*Bacillus* sp.	Gram-negative bacilli	Soils
SA	*Staphylococcus aureus*	Gram-positive cocci	Milk
MecA	*Staphylococcus aureus*	Gram-positive cocci	Milk
BN1	*Staphylococcus epidermids*	Gram-positive cocci	Soils

MATERIALS AND METHODS

Protocols for bacteria DNA extraction

Five protocols for DNA total extraction (detergent + thermal incubation, glass beads, detergent + enzymatic + thermal incubation, detergent + Salt + thermal incubation method and commercial kit QuickExtract™ Bacterial DNA Extraction - Epicentre) from nine bacterial samples collected from different sources were evaluated (Table 1).

Bacteria were inoculated into 5 ml of Brain-Heart Infusion Broth (Merck) and incubated at room temperature on a shaker at 150 rpm for 24 h. The concentration of cells was adjust to OD_{600} = 1.0. Subsequently, 1.5 ml of the culture was transferred to microcentrifuge tubes and centrifuged at 12,396 ×*g* for 5 min. The supernatant was discarded. This step was repeated three times.

Cell lysis step (except for commercial kit) are shown in the Table 2, and deproteinization and precipitation steps were common to them. Deproteinization was performed using the same volume of phenol/chloroform : isoamyl alcohol [1-1 (24:1)] and the same volume of chloroform : isoamyl alcohol (24:1) and to the precipitation, two volumes of iced ethanol 100% were added, followed by incubation at -20°C for 2 h. The microtubes were centrifuged at 14,549 ×*g* for 30 min, and the sediments were washed with 500 µl of 70% ethanol, dried at room temperature, and resuspended in 30 µl of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)].

For the commercial kit QuickExtract™ Bacterial DNA Extraction Kit (Epicentre), the sediments were resuspended in 50 µl of Quick Extract plus 0.5 µl of Bacterial Extraction of Ready-Lyse Lysozyme Solution. After 2 h at room temperature, the microtubes were incubated at 80°C for 2 min. Subsequently, the samples were centrifuged at 12,396 ×*g* for 2 min, and the supernatant was transferred to a new microtube.

Protocols for soil DNA direct extraction

For the direct extraction of DNA from soil, four protocols (glass beads, enzymatic, glass beads + PEG8000 method and commercial kit PowerMax™ Soil DNA Isolation - MoBio) were tested using four soil samples from two farms in southern Bahia and two areas with different cocoa planting systems. Soil samples collected from each area were composed of 10 subsamples collected at a depth of 0-10 cm in July 2011.

Cell lysis step (except for commercial kit) are shown in the Table 2, and deproteinization and precipitation steps were common to them. One gram of soil was added to 1 ml of extraction buffer followed procedure described in Table 2. Deproteinization was performed using the same volume of phenol/chloroform : isoamyl alcohol [1-1 (24:1)] and the same volume of chloroform : isoamyl alcohol (24:1). For precipitation, the sample was added to a same volume of 100% iced isopropanol and incubated at -20°C for 2 h. Subsequently, the microtubes were centrifuged at 14,549 ×*g* for 30 min, and the sediments were washed with 500 µl of 70% ethanol, dried at room temperature, and resuspended in 30 µl of TE.

For the commercial kit PowerMax™ Soil DNA Isolation (MoBio Laboratories, Inc), two hundred and fifty milligrams of soil was added to the PowerBead tube, and DNA extraction and purification was performed according to the protocol provided by the manufacturer.

Yield and purity of the DNA

All samples were stored at -20°C, and the amount and quality of DNA obtained were measured using a spectrophotometer (NanoDrop ND-1000 spectrophotometer, Thermo Fisher Scientific). To estimate the purity of the extracted nucleic acids, the ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}) was used. The A_{260}/A_{280} values between 1.7 and 2.0 indicate DNA samples with good quality. Values below this range indicate contamination with proteins, and values above this range indicate the presence of RNA or polysaccharides.

To DNA extracted from soil, the ratio of absorbance at 260 to 230 nm (A_{260}/A_{230}) was evaluated. The A_{260}/A_{230} values can help evaluate the level of salt and organic compounds, like humic acids carryover in the purified DNA. The A_{260}/A_{230} ratio should be greater than 1.5, ideally close to 1.8 (Moore et al., 2004).

PCR of the 16S rDNA region

PCR was performed in a 20 µl volume containing 1 U of Taq DNA polymerase (Fermentas), 1x reaction buffer, 200 µM of each dNTP, 3.0 mM $MgCl_2$, 0.5 mM of primers 27f (Suzuki and Giovannoni, 1996) and 1512r (Kane et al., 1993) and 10 ng of DNA. Blank and positive controls were included alongside each set of PCR reactions. Amplifications were performed according to the following parameters: 5 min initial denaturation at 94°C, 30 cycles of 94°C for

Table 2. Cell lysis step employed in the DNA extraction protocols.

Protocols	Lysis step	Reference
	Bacteria DNA extraction	
Detergent + thermal incubation method	- 800 µl of saline solution-EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.0). SDS was added to a final concentration of 2%, followed by incubation at 60°C for 10 min.	Marmur (1961)
Glass beads method	- 400 µl of STE buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and centrifuged at 14,549 ×*g* for 5 min. The supernatant was discarded, and the pellets were resuspended in 200 µl of TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)]. To this mixture, 50 mg of glass beads (150–212 µm in diameter) and 100 µl of saturated phenol in Tris-HCl (pH 8.0) were added, and the mixture was vortexed for 60 s.	Cheng and Jiang (2006)
Detergent + enzymatic + salt + thermal incubation method	-570 µl of TE, SDS (final concentration, 0.5%), and proteinase K (final concentration, 100 mg mL^{-1}), and incubated at 37°C for 1 h. To this mixture, 100 ml of 0.8 M NaCl and 80 µl of CTAB/NaCl (10% CTAB in 0.7 M NaCl) were added, and the microtubes were incubated for 10 min at 65°C.	Wilson (1997)
Detergent + salt + thermal incubation method	- 600 µl of extraction buffer [200 mM Tris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 25 mM NaCl, 1% SDS] and incubated at 65°C for 30 min.	Harju et al. (2004)
	Direct extraction of total DNA from soil	
Glass beads method	- 1 ml of extraction buffer [50 mM Tris-HCl (pH 7.6), 50 mM EDTA, 50 mM NaCl, 5% SDS], 0.4 g of glass beads (150–212 µM in diameter), and 1 µl of 1 M ditiotreitol, and vortexed for 3 min. The samples were incubated at 65°C for 30 min and centrifuged at 14,549 ×*g* for 10 min. The supernatant was transferred to a new microtube.	Costa et al. (2004)
Enzymatic method	- 1 ml of extraction buffer (100 mM Tris-HCl, 100 mM EDTA, 100 mM NaH_2PO_4, 1.5 M NaCl, 1% CTAB, pH 8.0) and proteinase K (final concentration, 0.1 mg mL^{-1}). The samples were incubated by shaking at 250 rpm for 30 min. SDS was added to a final concentration of 3%, and the samples were incubated at 65°C for 2 h. The samples were incubated at 65°C for 30 min and centrifuged at 14,549 ×*g* for 10 min. The supernatant was transferred to a new microtube.	Hang et al. (2006)
Glass beads + PEG8000 method	- 1 ml of extraction buffer [0.1 M Tris-HCl (pH 8.0), 0.1 M EDTA, NaH_2PO_4, 1.5 M NaCl, 1% CTAB)] and 0.4 g of glass beads (150–212 µM in diameter). SDS was added to a final concentration of 5%. The samples were incubated at 65°C for 1 h and centrifuged at 14,549 ×*g* for 10 min. The supernatant was transferred to a new microtube, and the same volume of iced isopropanol was added, followed by incubation at room temperature for 15 min. The samples were centrifuged at 14,549 ×*g* for 10 min, and the pellets were resuspended in 80 µl of TE, 1 mL of NaCl, and PEG 8000 (final concentration, 1.6 M). These were again incubated for 2 h at room temperature and centrifuged at 14,549 ×*g* for 10 min. The pellets were resuspended in 400 µl TE and $NH_4C_2H_3O_2$ (final concentration, 2.5 M) and incubated on ice for 5 min. Subsequently, the samples were centrifuged at 14,549 ×*g* for 20 min, and the supernatant was transferred to a new microtube.	Yeates et al. (1998)

Table 3. Estimated amount (ng μl^{-1}) and purity (A_{260}/A_{280} ratio) of DNA extracted from gram-positive and gram-negative bacteria isolated from different sources.

Isolates[1] / Protocols	DNA yield (ng μl^{-1})									
	30A	**40B**	**BP11**	**BN5**	**BT108**	**BP1**	**SA**	**MecA**	**BN1**	**Average**
Detergent + thermal incubation method	1019 bc	1149^{c}	3517	506^{c}	263^{b}	283^{b}	199^{b}	190^{c}	184^{c}	812
Glass beads method	1924 a	800^{cd}	5270	1437^{ab}	125^{b}	101^{b}	135^{b}	226^{c}	1508^{a}	1281
Detergent + enzymatic + salt + thermal incubation method	1353 abc	3626^{a}	299	107^{c}	69^{b}	321^{b}	25^{b}	24^{c}	29^{c}	650
Detergent + salt + thermal incubation method	1614 ab	298^{d}	5039	813^{bc}	596^{b}	534^{b}	1339^{a}	1754^{a}	550^{b}	1393
KitCE*	839 c	2003^{b}	3170	1908^{a}	1618^{a}	1717^{a}	1041^{a}	575^{b}	465^{b}	1482
Protocols	A_{260}/A_{280} ratio									
Detergent + thermal incubation method	2.29	2.10	2.05	2.00	1.95	2.06	1.95	1.98	2.01	2.04
Glass beads method	2.04	2.01	1.99	2.03	2.02	2.00	2.04	2.03	2.06	2.02
Detergent + enzymatic + salt + thermal incubation method	2.01	2.00	1.86	1.73	2.09	1.13	1.78	1.67	1.69	1.79
Detergent + salt + thermal incubation method	2.00	1.93	1.71	2.10	2.05	2.06	1.92	1.87	2.02	1.97
KitCE	1.60	1.96	1.61	1.51	1.38	1.56	1.64	1.58	1.35	1.72

*KitCE: QuickExtract™ Bacterial DNA Extraction Kit (Epicentre). [1]Bacterial isolates: 30A = *Vibrio cholera*; 40B = *V. cholerae*; BP11 = *Pseudomonas* sp.; BN5 = *Bacillus* sp.; BT108 = *Bacillus* sp.; BP1 = *Bacillus* sp.; SA = *Staphylococcus aureus*; MecA = *S. aureus*; BN1= *S. epidermidis*. The results shown represent the average of triplicates. The averages followed by the same letter in each column do not differ by Tukey's test at 5% probability.

60 s, 58°C for 60 s, 72°C for 60 s, followed by a final elongation at 72°C for 10 min. The PCR products were separated by electrophoresis on a 1.5% agarose gel plus SYBR Safe DNA Gel Stain (Invitrogen), and the gel was visualized under UV light at 254 nm.

Statistical analysis

All the experiments were carried out in triplicates. Data were subjected to analysis of variance, and when significant, Tukey's test ($P < 0.05$) was used. The statistical program SAEG (SAEG 2009) was used for analysis.

RESULTS AND DISCUSSION

Choice of a single protocol for Gram positive and negative bacteria DNA extraction

Gram positive and negative bacteria isolated from soil samples, mussels, oysters and milk were subjected to five DNA extraction protocols, including the QuickExtract™ Bacterial DNA Extraction Kit (KitCE), with subsequent qualitative-quantitative assessment of the extracted DNA. Although total DNA was successfully extracted from all bacteria with all protocols used, differences were observed in the efficiency of extraction processes considering the different bacterial groups (Table 3). Among the Gram negative bacteria, the KitCE yielded the highest quantity of DNA, 1908, 1618 and 1717 ng μl^{-1} DNA for *Bacillus* isolates. For *Vibrio* sp., the best method for one isolate was detergent + enzymatic + thermal incubation, for another it did not have difference between evaluated methods. For *Pseudomonas* sp. isolates, glass beads and detergent + salt + thermal incubation methods yielded the highest quantity of DNA, 5270 and 5039 ng μl^{-1} DNA, respectively. Among the Gram positive bacteria, detergent + salt + thermal incubation method yielded the highest

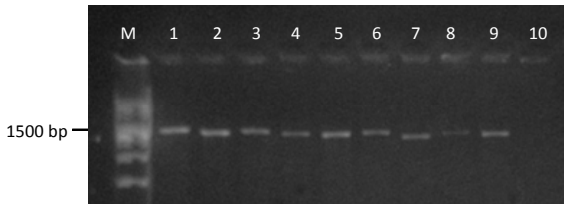

Figure 1. Electrophoresis of amplification products of the rDNA 16S region of the DNA extracted using the detergent + salt + thermal incubation method based on Harju et al. (2004) on a 1.5% agarose gel. M: Molecular weight marker 1 Kb (Amresco) 1: SA = *Staphylococcus aureus*; 2: MecA = *S. aureus*; 3: BT108 = *Bacillus* sp.; 4: BP1 = *Bacillus* sp.; 5: BN1 = *Staphylococcus epidermidis*; 6: 30A = *Vibrio cholerae*; 7: 40B = *Vibrio cholerae*; 8: BP11 = *Pseudomonas* sp.; 9: BN5 = *Bacillus* sp.; 10: Blank.

quantity of DNA for *Staphylococcus aureus* isolates, 1339 and 1754 ng μl^{-1} DNA, respectively. For *Staphylococcus epidermidis,* the method that yielded the highest quantity of DNA was the glass beads with 1508 ng μl^{-1} DNA. On average, the best results was with KitCE, detergent + salt + thermal incubation method and glass beads method that yielded 1482, 1393, and 1281 ng μl^{-1} DNA, respectively.

The ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}) was used as an estimate of nucleic acid purity. On average, detergent + salt + thermal incubation, detergent + enzymatic + salt + thermal incubation method and KitCE yielded better quality DNA (A_{260}/A_{280} between 1.7 and 2.0) with ratios of 1.97, 1.79, and 1.72, respectively (Table 3).

The detergent + salt + thermal incubation method based on Harju et al. (2004) was considered the most promising because it produced satisfactory yields of DNA, with adequate quality for all isolates studied, especially *S. aureus,* without the need to use enzymes and glass beads which can make the extraction process more expensive.

The quality of extracted DNA using the detergent + salt + thermal incubation method, based on Harju et al. (2004) was confirmed by PCR amplification using universal bacterial primers, and all samples were amplified generating a fragment of approximately 1500 bp (Figure 1).

Various protocols have been described for the extraction of DNA from specific groups of microorganisms; however, the efficiency of these protocols varies among different groups (Baratto and Megiolaro, 2012; Chapaval et al., 2008; Ligozzi and Fontana, 2003; Rivera et al., 2003; Wilson, 1997). This variation is mainly due to the inherent characteristics of the different bacterial groups and the structure of their cell walls, which reflects the efficiency of lysis. The presence of capsular polysaccharide makes it difficult to separate DNA, and the association of DNA with proteins influences its purification (Marmur, 1961; Navarre and Schneewind, 1999).

Choice of a protocol for direct extraction of DNA from soil

Direct DNA extraction from soil has three basic objectives: lysis of representative microorganisms within the sample, obtaining intact DNA with high molecular weight, and removal of inhibitors from the extracted DNA for subsequent molecular manipulations, such as for PCR amplification.

Four protocols, including the commercial kit PowerMax™ Soil DNA Isolation (KitCMB) were used for the direct extraction of DNA from different soil samples. The highest yields were observed for DNA samples extracted using enzymatic and glass beads method, on average, 258.04 and 233.39 µg g^{-1} of soil, respectively (Table 4). Samples extracted using glass beads + PEG8000 method and KitCMB exhibited the lowest yield of DNA of approximately 7.81 and 6.29 µg g^{-1} of soil, respectively (Table 4).

Although samples extracted using enzymatic and glass beads method gave higher yields of DNA, they resulted in the worst A_{260}/A_{280} ratio, on average, 1.38 and 1.32, respectively, suggesting contamination with proteins. The glass beads + PEG8000 method resulted in a low A260/A280 ratio too. A better A_{260}/A_{280} ratio, on average 1.87, was observed in the samples extracted using KitCMB (Table 4).

All samples showed low value of A_{260}/A_{230} ratio indicating high humic acid (Table 4). The ratio of absorbance at 260 and 230 nm (A_{260}/A_{280}) is used as a secondary measure of nucleic acid purity (Boehm et al., 2009; Lim et al., 2009; Ning et al., 2009; Wilfinger et al., 2006).

To confirm the quality of the extracted DNA samples and the impact of the presence of contaminants on the PCR samples, the samples were subjected to PCR amplification using universal primers for bacteria. None of the samples extracted using enzymatic and glass beads methods were amplified. Two samples extracted using glass beads + PEG8000 method and all samples extracted using KitCMB were amplified, generating a fragment of approximately 1500 bp (Figure 2).

The enzymatic and glass beads protocols produced the highest yields of DNA for all soils; however, these extracted samples were not sufficiently pure for PCR amplification using universal primers for bacteria. Despite the lower yield of DNA, KitCMB protocol gave the best quality of DNA, enabling its amplification by PCR.

In non-amplified samples, it was observed that despite producing amounts of DNA suitable for PCR amplification (several dilutions were tested), the purity of DNA was compromised, as evidenced by the A_{260}/A_{280} ratios. It is likely not all contaminants that adhered to the DNA have been removed, which may have led to the inhibition of amplification by Taq DNA polymerase (Roh et al. 2006).

Table 4. Estimated amount (µg µg^{-1}) and purity (A_{260}/A_{280} and A_{260}/A_{230} ratio) of total DNA extracted from soil samples collected from different cocoa planting systems

	DNA yield (µg g^{-1})				
Protocols	**[1]Conv.1**	**ASF**	**Conv.2**	**Org.**	Average
Glass beads method	384.38^{a}	193.47^{b}	243.65^{b}	112.05^{b}	233.39
Enzymatic method	99.17^{b}	268.74^{a}	287.74^{a}	376.52^{a}	258.04
Glass beads + PEG8000 method	29.94^{c}	0.54^{c}	0.31^{c}	0.46^{c}	7.81
KitCMB*	0.33^{d}	12.07^{c}	6.35^{c}	6.40^{c}	6.29
	A_{280}/A_{260} ratio				
Glass beads method	1.31	1.37	1.25	1.36	1.32
Enzymatic method	1.34	1.38	1.42	1.37	1.38
Glass beads + PEG8000 method	1.41	1.38	1.27	1.21	1.32
KitCMB	1.69	1.97	1.85	1.98	1.87
	A_{260}/A_{230} ratio				
Glass beads method	0.79	0.73	0.85	0.78	0.79
Enzymatic method	0.79	0.73	0.82	0.70	0.76
Glass beads + PEG8000 method	0.66	0.47	0.71	0.35	0.55
KitCMB	1.09	0.42	1.11	0.26	0.72

*KitCMB: - PowerMax™ Soil DNA Isolation Kit (MO BIO Laboratories, Inc). [1]Soils: Conv. 1. - Conventional system, Farm 1; ASF. - Agroforesty system, Farm 1; Conv.2 - Conventional system, Farm 2; Org. - Organic Farming, Farm 2. The results shown represent the average of triplicates. The averages followed by the same letter in each column do not differ by Tukey's test at 5% probability.

Figure 2. Electrophoresis of amplification products of the 16S rDNA region of the DNA extracted directly from soil on 1.5% agarose gel. Lanes 1-4: Glass beads method (Costa et al., 2004); Lanes 5-8: Enzymatic method (Hang et al., 2006); Lanes 9-12: Glass beads + PEG8000 method (Yeates et al., 1998); Lanes: 13-16: Commercial kit - PowerMax ™ Soil DNA Isolation (MO BIO Laboratories, Inc.); 17: Positive control; 18: Blank. Soils: 1, 5, 9 and 13: Conv. 1 - Conventional system, Farm 1; 2, 6, 10 and 14: Conv. 2 - Conventional system, Farm 2; 3, 7, 11 and 15: ASF. - Agroforesty system, farm 1; 4, 8, 12 and 16: Org - Organic Farming, Farm 2;. M: 100 bp DNA ladder (Fermentas).

Regarding PCR amplification, the amount of total DNA extracted is less limiting than its quality. Thus, KitCMB protocol offered more promising results, enabling PCR amplification. Soil contaminants, particularly humic substances, might preclude PCR amplification, interfere with DNA hybridization, and increase the background in microarray hybridization (Braida et al., 2003; Lemarchand et al., 2005; Niemi et al., 2001; Zhou et al., 1996).

Some of the most popular DNA purification methods involve removal of humic material through agarose gel electrophoresis, polyvinylpolypyrrolidone (PVPP), size exclusion chromatography or silica-based DNA binding (Berthelet et al., 1996; Miller et al., 1999; Miller, 2001). The commercial kits like KitCMB essentially rely on silica gel spin columns for purification of the DNA and it was effective in this case, providing the amplification of DNA of soil samples.

Conflict of Interests

The authors have not declared any conflict of interests.

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