Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis

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Abstract

The impact of DNA extraction protocol on soil DNA yield and bacterial community composition was evaluated. Three different procedures to physically disrupt cells were compared: sonication, grinding–freezing–thawing, and bead beating. The three protocols were applied to three different topsoils. For all soils, we found that each DNA extraction method resulted in unique community patterns as measured by denaturing gradient gel electrophoresis. This indicates the importance of the DNA extraction protocol on data for evaluating soil bacterial diversity. Consistently, the bead-beating procedure gave rise to the highest number of DNA bands, indicating the highest number of bacterial species. Supplementing the bead-beating procedure with additional cell-rupture steps generally did not change the bacterial community profile. The same consistency was not observed when evaluating the efficiency of the different methods on soil DNA yield. This parameter depended on soil type. The DNA size was of highest molecular weight with the sonication and grinding–freezing–thawing procedures (approx. 20 kb). In contrast, the inclusion of bead beating resulted in more sheared DNA (approx. 6–20 kb), and the longer the bead-beating time, the higher the fraction of low-molecular weight DNA. Clearly, the choice of DNA extraction protocol depends on soil type. We found, however, that for the analysis of indigenous soil bacterial communities the bead-beating procedure was appropriate because it is fast, reproducible, and gives very pure DNA of relatively high molecular weight. And very importantly, with this protocol the highest soil bacterial diversity was obtained. We believe that the choice of DNA extraction protocol will influence not only the determined phylogenetic diversity of indigenous microbial communities, but also the obtained functional diversity. This means that the detected presence of a functional gene—and thus the indication of enzyme activity—may depend on the nature of the applied DNA extraction procedure.

Keywords: DNA extraction; Bacterial community composition; Microbial diversity; Functional gene; DGGE

1. Introduction

DNA-based methods are routinely used in studies of microbial diversity and community composition. The quality and the quantity of the environmentally extracted nucleic acids obviously are very important to the answers that can be obtained by the performed experiments. Thus, the application of a proper DNA extraction protocol is crucial. There are two main approaches for the isolation of microbial DNA from soil: (i) the cell extraction method, and (ii) the direct lysis method (Saano et al., 1995). In both approaches, a number of different methods to extract DNA can be used including cycles of freezing and thawing, sonication, boiling, liquid nitrogen, bead beating, osmotic stress, SDS, lysozyme and others (Sørensen et al., 2002). The cell extraction method relies on the separation of soil microorganisms prior to cell lysis and recovery of DNA. It has been shown that the cell extraction method is biased as, e.g. methane-oxidising (Primé et al., 1996) and ammonia-oxidising (Aakra et al., 2000) bacteria are more difficult to dislodge from soil particles compared to the majority of soil bacteria. Today, primarily the direct lysis method is used.
Here, micro organisms are lysed directly in the soil after which DNA is extracted. Protocols developed for this approach are all virtually derived from Ogram et al. (1987), but several suggestions for modifications have been published during the last decade (Moreé et al., 1994; Borneman et al., 1996; Zhou et al., 1996; Frostegård et al., 1999; Krsek and Wellington, 1999; Miller et al., 1999; Martin-Laurent et al., 2001; Webster et al., 2003). Following cell lysis and nucleic acid extraction, DNA is purified. Soil contains a number of compounds that inhibit or decrease the sensitivity of following PCR and hybridisation analysis, e.g. humic acids, phenolic compounds and heavy metals (Sørensen et al., 2002). Many different procedures for the purification of DNA has been applied including CsCl–EtBr gradient centrifugation, hydroxyapatite columns, polyvinylpolypyrrolidone, silica matrix, magnetic capture hybridisation PCR (Sørensen et al., 2002).

Several studies have been performed to evaluate the impact of DNA extraction protocols on soil DNA yield (Moreé et al., 1994; Zhou et al., 1996; Frostegård et al., 1999; Krsek and Wellington, 1999; Miller et al., 1999; Webster et al., 2003), however, the impact on the detected soil microbial diversity is only sparsely investigated (Kozdroj and van Elsas, 2000; Martin-Laurent et al., 2001; Westergaard et al., 2001). The objective of this study was to evaluate the effect of different DNA extraction protocols on the detected soil bacterial community composition as measured by 16S rDNA denaturing gradient gel electrophoresis (DGGE). Furthermore, the effect on soil DNA yield and fragment size was evaluated.

2. Materials and methods

2.1. Soils

A total of six Danish soils were analysed in this study. The four topsoils from Roskilde, Jyndevad, Sorø, and Fladerne Bæk were collected from the top 5–25 cm. The two subsurface soils from Vejen aquifer were collected with a stainless steel piston sampler from the saturated zone, 5–6.25 m below the surface (Tuxen et al., 2002). All soils were homogenised and stored at −20 °C prior to DNA analysis. For soil characteristics, see Table 1.

### Table 1: Soil characteristics

<table>
<thead>
<tr>
<th>Soil</th>
<th>Soil horizon</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
<th>d50 (mm)</th>
<th>pH</th>
<th>TOC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roskilde</td>
<td>A</td>
<td>11</td>
<td>31</td>
<td>56</td>
<td>ND</td>
<td>6.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Jyndevad</td>
<td>A</td>
<td>4</td>
<td>3</td>
<td>91</td>
<td>ND</td>
<td>6.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Sorø</td>
<td>A</td>
<td>24</td>
<td>22</td>
<td>54</td>
<td>ND</td>
<td>4.1</td>
<td>4.5</td>
</tr>
<tr>
<td>Fladerne Bæk</td>
<td>A</td>
<td>3</td>
<td>3</td>
<td>90</td>
<td>ND</td>
<td>5.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Vejen-B2</td>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.58</td>
<td>5.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Vejen-NX2</td>
<td>C</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.50</td>
<td>5.5</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Clay, <0.002 mm; silt, 0.02–0.002 mm; sand, >0.02 mm. d50, the particle diameter size that 50% of the soil material passed through. TOC, total organic carbon; ND, not determined.

a Data from Jacobsen and Pedersen (1992).

b Data from Schoening (personal communication).

c Data from Vinther et al. (2001).

d Data from Tuxen et al. (2002).

2.2. DNA extraction methods

Six different methods were used for the extraction of DNA from soil. With method 1, a combination of sonication, lysozyme, SDS and heat was used to extract DNA from 100 mg of soil. The protocol described by Porteous et al. (1994) was followed, except that sonication was performed for 10 s on ice. Soil DNA was purified by low melting point agarose (Sea Plaque GTG agarose) gel electrophoresis (0.7%, 1 h at 75 V). DNA bands were cut from the gel and stored at −20 °C in microcentrifuge tubes. Prior to PCR amplification the gel blocks were melted for 5 min at 68 °C. Three volumes of MiliQ water were added and samples were re-incubated at 68 °C for 20 min (Porteous et al., 1994).

With method 2, a combination of freezing–thawing, grinding, SDS and heat was used to lyse soil microorganisms. Liquid nitrogen was added to 1.0 g of soil in a mortar. After evaporation of nitrogen, the soil was ground. Hereafter, the protocol described by Zhou et al. (1996) was followed. Soil DNA was purified as described for method 1. With method 3, the commercial FastDNA Spin Kit for Soil (BIO101, Vista, CA) was applied. This DNA extraction procedure includes a bead-beating step. DNA was extracted from 500 mg of soil and hereafter purified by the use of a GeneClean procedure (BIO101). The methods 4, 5, and 6 were all based on the BIO101 kit, however, with the supplement of additional steps to extract soil DNA. With method 4, the soil was treated with 7.5 mg lysozyme g⁻¹ soil for 15 min at 37 °C prior to bead beating.

With method 5, the soil was subjected to two cycles of freezing (1 h at −80 °C) and thawing (30 min at 37 °C) after the bead-beating step. With method 6, the soil was subjected to both lysozyme as well as freeze–thaw treatments.
2.3. PCR amplification of 16S rDNA

The DNA primers 338f and 518r (Muyzer et al., 1993) were used to amplify bacterial 16S rDNA segments. A 40-bp GC-clamp was added to primer 338f in order to increase the separation of DNA bands in DGGE analysis (Muyzer et al., 1993). Two different PCR mixtures were used. Mixture 1 was used to amplify DNA from Fladerne Bæk and Vejen soils, Jyndevad, and Sorø soils, while mixture 2 was used to amplify DNA from Fladerne Bæk and Vejen soils. PCR mixture 1 consisted of 1× PCR Master (Roche Molecular Biochemicals, Boehringer Mannheim, Germany), 0.1 μM of each primer (DNA Technology, Science Park Aarhus, Aarhus, Denmark), 5 μl of DNA extracts, and Milli-Q water to a total reaction volume of 50 μl. PCR was performed using the program: 1 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C, and 1 min at 72 °C; 5 min at 72 °C. PCR mixture 2 consisted of 1× GeneAmp® PCR Buffer (Perkin–Elmer, Applied Biosystem, Foster City, CA), 100 μM of each dNTP (Perkin–Elmer), 0.5 μM of each primer (GibcoBRL Custom Primers, Life Technologies, Paisley, Scotland), 1.25 U AmpliTaq Gold™ DNA polymerase (Perkin–Elmer), 5 μl of DNA extracts, and Milli-Q water to a total volume of 50 μl. PCR was performed using the program: 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1 min at 72 °C; 6 min at 72 °C.

The intensities of the PCR products were measured by comparing to a standard curve derived from 100 to 600-bp bands of a 100-bp molecular size marker (Promega, Madison, WI). Quantitative measurements were performed on a Gel Doc 1000 system (Bio-Rad, BioRad Laboratories, Inc., Hercules, CA) using the image analysing software Quantity One 4.0 for Macintosh (Bio-Rad).

2.4. Denaturing gradient gel electrophoresis

DGGE was performed with the Dcode™ Universal Mutation Detection System (Bio-Rad). Equal amounts of PCR product were loaded onto 8% (wt/vol) polyacrylamide gels (40% acrylamide/bis solution, 37.5:1, Bio-Rad) in 1× TAE (40 mM Tris, 20 mM acetate, 1.0 mM Na2-EDTA). Polyacrylamide gels with a denaturant-gradient ranging from 40 to 65% (100% denaturant contains 7 M urea 40% (wt/vol) deionised formamide) were run at 75 V and 60 °C for 17 h. After electrophoresis, gels were stained with SYBR® Gold (1:10,000 dilution, Molecular Probes, Eugene, OR) for 1 h and briefly rinsed with Milli-Q water. Gels were analysed using Quantity One 4.0 (Bio-Rad). The number of DNA bands in each lane was recorded. The relative intensity of a specific DNA band was expressed as the ratio between the intensity of that band and the total intensity of DNA in the entire lane.

2.5. Quantification of soil DNA yield

The amount of DNA extracted from the four topsoils was quantified by agarose gel electrophoresis by comparing the band intensities to a standard curve derived from HindIII cut λ-phage DNA (Promega). Quantitative measurements were performed using the image analysing software Quantity One 4.0 for Macintosh (Bio-Rad) as described in Section 2.3. For the topsoils, DNA yield is indicated as ng or μg DNA g⁻¹ soil. This procedure could not be performed with the Vejen aquifer sediments as the concentration of DNA extracted from these was too small to be visualised by agarose gel electrophoresis (data not shown). Instead, the DNA yield obtained from the Vejen soils was estimated by most-probable-number (MPN) PCR analysis of 16S rDNA fragments (see Section 2.3) by analysing three replicates of each sample dilution (10⁰ to 10⁻⁶). Here, the DNA yield is indicated as numbers of 16S rDNA gene copies g⁻¹ soil. The detection limit was theoretically set to fifty 16S rDNA gene copies g⁻¹ soil, assuming that one 16S rDNA gene copy μl⁻¹ DNA extract would result in a visible PCR product.

2.6. Statistical analysis

All extractions and further analysis of soil DNA were performed in triplicate. The statistical significance of differences was determined by using a Student’s t test.

3. Results

3.1. Soil DNA yield and bacterial community composition obtained by the application of three different methods for DNA extraction

The impact of DNA extraction procedure on soil microbial community composition was investigated using three different protocols for physically disrupting bacterial cells: sonication (method 1), grinding–freezing–thawing (method 2), and bead beating (method 3). The three methods were tested on topsoils from Roskilde, Jyndevad and Sorø. With DGGE analysis we found that each DNA extraction method gave rise to different community profiles and numbers of DNA bands (Table 2). For all three soils, method 3 gave rise to a significantly higher number of bands compared to methods 1 and 2 (**P<0.01). For the Jyndevad soil the level of significance was lower when comparing with method 1 (*P<0.05). Except for the Sorø

Table 2

<table>
<thead>
<tr>
<th>Soil</th>
<th>DNA bands in DGGE gels</th>
<th>DNA extraction method 1</th>
<th>DNA extraction method 2</th>
<th>DNA extraction method 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roskilde</td>
<td>15.3±0.6</td>
<td>14.3±1.1</td>
<td>19.0±0.0</td>
<td></td>
</tr>
<tr>
<td>Jyndevad</td>
<td>14.7±4.9</td>
<td>14.0±0.0</td>
<td>21.0±0.0</td>
<td></td>
</tr>
<tr>
<td>Sorø</td>
<td>13.0±0.0</td>
<td>15.0±0.0</td>
<td>25.0±0.0</td>
<td></td>
</tr>
</tbody>
</table>

Data show the mean±standard deviation of three replicate measurements.
soil, there was no significant difference between the number of DNA bands obtained with methods 1 and 2. Looking more detailed at the community profiles we found additional differences between the three DNA extraction methods. With the Sorø soil (Fig. 1) seven DNA bands were common for all three methods. Besides, three, two, and 10 specific bands were found with method 1, 2, and 3, respectively. Also the relative representation of the 16S rDNA fragments differed between the methods. For example, with method 1, the first band in the DGGE gel (first arrow in Fig. 1) represented 6.6% of the total DNA band intensity, whereas with method 2 and 3 this band made up only 4.8 and 1.5%, respectively. In contrast, one of the lower bands (third arrow in Fig. 1) constituted 7.7% with method 3, as opposed to 0.4 and 1.6% with methods 1 and 2, respectively. With method 1, the most skewed representation was obtained as one of the DNA bands (second arrow in Fig. 1) made up 31.9%. This band constituted 2.6% with method 2, but was not represented with method 3. The community profiles for Roskilde and Jyndevad soils showed similar variations in DNA band representation (data not shown). The DGGE analyses of replicate soil samples generally resulted in highly similar community profiles and low standard deviations (Table 2, Fig. 1). Especially with method 3, identical profiles were obtained for all replicate measurements (Table 2, Fig. 2).

The DNA yield obtained with the three methods was evaluated for Roskilde and Sorø soils (Table 3). With the Roskilde soil, we found no significant difference between methods ($P > 0.05$). However, with the Sorø soil, significantly more DNA was extracted using method 1 compared to methods 2 and 3 (**$P < 0.01$). The efficiency of method 3 was significantly higher compared to method 2 (**$P < 0.01$). Apart from the yield, the size of extracted DNA differed between methods (data not shown). With methods 1 and 2, a well-defined band of approx. 20 kb was

![Fig. 1. DGGE gel illustrating the bacterial community profile of Sorø soil obtained with DNA extraction methods 1, 2, and 3 (see Section 2). Only two of the three identical replicates are shown. Arrows indicate DNA bands discussed in the text.](image)

![Fig. 2. DGGE gel illustrating the bacterial community profiles obtained with Roskilde (R), Jyndevad (J), and Sorø (S) soils using a bead-beating procedure (method 3) for extraction of soil DNA.](image)

![Table 3 DNA yield obtained from Roskilde and Sorø soils using three different DNA extraction methods](table)

<table>
<thead>
<tr>
<th>Soil</th>
<th>DNA yield ($\mu$g g$^{-1}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DNA extraction method 1</td>
</tr>
<tr>
<td>Roskilde</td>
<td>18.0 ± 0.59</td>
</tr>
<tr>
<td>Sorø</td>
<td>24.4 ± 0.18</td>
</tr>
</tbody>
</table>

Data show the mean $\pm$ standard deviation of three replicate measurements.
Data show the mean visible PCR product. 

Table 4

<table>
<thead>
<tr>
<th>Soil</th>
<th>DNA yield (ng g⁻¹ Fladerne Bæk or ×10⁵ 16S rDNA copies g⁻¹ Vejen soil)</th>
<th>DNA extraction method 3</th>
<th>DNA extraction method 4</th>
<th>DNA extraction method 5</th>
<th>DNA extraction method 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fladerne Bæk</td>
<td>483 ± 32</td>
<td>492 ± 55</td>
<td>502 ± 42</td>
<td>553 ± 49</td>
<td></td>
</tr>
<tr>
<td>Vejen-NX2a</td>
<td>84.0 ± 62.3</td>
<td>&lt; 4.0 ± 6.9</td>
<td>660 ± 494</td>
<td>80.3 ± 68.7</td>
<td></td>
</tr>
<tr>
<td>Vejen-B2a</td>
<td>63.1 ± 53.2</td>
<td>&lt; 0.4 ± 0.6</td>
<td>443 ± 185</td>
<td>1.2 ± 0.0</td>
<td></td>
</tr>
</tbody>
</table>

Data show the mean ± standard deviation of three replicate measurements. a The detection limit was theoretically set to fifty 16S rDNA gene copies g⁻¹ soil, assuming that one 16S rDNA gene copy µl⁻¹ DNA extract would result in a visible PCR product.

obtained. In contrast, the DNA obtained with method 3 was more sheared and primarily (> 90%) composed of fragments of 6–20 kb. Method 3 was the most reproducible with coefficients of variation of only 8.7% (Roskilde) and 2.2% (Sorø) compared to 32.8% (Roskilde) and 7.2% (Sorø) with method 1, and 14.3% (Roskilde) and 19.2% (Sorø) with method 2.

3.2. Extraction of DNA from soil bacterial communities applying a bead-beating procedure supplemented with additional cell-rupture treatments

The bead-beating method (method 3) was supplemented with lysozyme and freeze–thaw treatments (methods 4–6) in order to evaluate if this would have an impact on soil DNA yield and bacterial community composition. Experiments performed with Fladerne Bæk soil showed that the additional cell-rupture treatments did not have any significant effect on the DNA yield (P > 0.05) (Table 4). Furthermore, no effect was seen on the distribution of soil DNA size fractions (data not shown). With the Vejen soils, the inclusion of a lysozyme treatment generally decreased the bacterial DNA yield, although this effect was only significant for the B2 soil (*P < 0.05). Likewise, the addition of a freeze–thaw treatment generally increased the bacterial DNA yield, although this effect was only significant for the NX2 soil (*P < 0.05). The inclusion of freeze–thaw treatments did not change the bacterial community composition obtained with the Vejen soils (data not shown). In contrast, treating with lysozyme significantly reduced the number of DNA bands obtained in DGGE analysis (data not shown).

We also evaluated the effect of increasing or decreasing the bead-beating time compared to the 30 s described in the original protocol. With the Fladerne Bæk soil, a decreased bead-beating time resulted in a lowered DNA yield (*P < 0.05) (Table 5). In contrast, increasing to 120 s of bead beating gave rise to an increased soil DNA yield (*P < 0.05). No additional effect was found on the DNA yield when increasing the bead-beating time to 240 or 480 s (P > 0.05). Similar results were obtained with the Vejen-NX2 soil (Table 5). Here, increasing the bead beating to 240 s resulted in an increased soil bacterial DNA yield (*P < 0.05). However, a further increase up to 480 s did not have a significant effect (*P > 0.05). The effect of bead-beating time on DNA fragment size distribution was evaluated for the Fladerne Bæk soil (Fig. 3). We found that the fraction of high molecular weight DNA (9.4–23.1 kb) decreased from 36 to 10 and 6%, when increasing the bead-beating time to 240 and 480 s, respectively. In contrast, the fraction of low molecular weight DNA (2.3–5.3 kb) increased from 20 to 44 and 50%, respectively. It was not possible to do similar evaluations with the Vejen aquifer sediments as the concentration of soil DNA was too low to be visualised on agarose gel electrophoresis (data not shown). Changing the bead-beating time had only minor effects on the soil bacterial community composition as measured by DGGE analysis. With the Vejen-NX2 soil, one DNA band disappeared (first arrow in Fig. 4) when increasing the bead-beating time to 120 s or more, whereas another band appeared (second arrow in Fig. 4). Similar observations were recorded for the Fladerne Bæk soil (data not shown).

4. Discussion

We found that the efficiency of various DNA extraction protocols differed with soil type. This is similar to what has been observed by others (Zhou et al., 1996; Frostegård et al., 1999; Miller et al., 1999). In the present study, it seemed that the extraction of DNA from the sandy surface soils, Roskilde and Fladerne Bæk, was indifferent to the type of physical cell disruption. In contrast, with the more clayey Sorø soil the sonication procedure (method 1) was the most
efficient. Similar observations were found by Frostegård et al. (1999). Here, the DNA yield obtained from soils with a low sand content (<35%) and high silt/clay content (>65%) was significantly increased when a sonication step was included, while the sandy soils were more or less unaffected by the choice of protocol. These findings could be due to a more easily dislodging of bacterial cells from the larger soil particles. With the sandy Vejen-NX2 aquifer sediment, we found an increased DNA extraction efficiency when the bead-beating protocol was supplemented with a freeze–thaw treatment. This effect may be due to the low nutrient status generally found in subsurface aquifers, resulting in starved bacteria of small size (Bakken, 1997). Bacteria of small size may be more difficult to disrupt compared to larger cells.

Generally, bead beating have been found to increase the yield of soil DNA (Moreé et al., 1994; Krsek and Wellington, 1999; Miller et al., 1999). We found that a prolonged bead-beating time resulted in higher DNA yields. Care must be taken, though, with such treatments as the fraction of high molecular weight DNA decreases dramatically when the bead-beating time increase. This was clearly observed with soil from Fladerne Bæk, and bead beating for more than 1 min is not recommended. Extracted DNA should be of high molecular weight, especially for the proper analysis of long gene segments, but also because DNA of small fragment size (<1000 bp) may give rise to the formation of chimeric PCR products (Liesack et al., 1991). In the original protocol (BIO101), bead beating is performed for 30 s and the major part of the extracted soil DNA has a size of 5–20 kb. If long gene segments are of interest, bead beating could preferentially be replaced by a sonication or freeze–thaw procedure as these gave rise only to well-defined fragments of high molecular weight. Westergaard et al. (2001) similarly found that sonication yielded DNA of only high molecular weight (approx. 23 kb). It must be noted, however, that others have found that sonication shear the DNA (Frostegård et al., 1999; Krsek and Wellington, 1999). It seems that the effect of sonication on DNA extraction is very dependent on the type of instrument applied.

We found that the choice of DNA extraction protocol had a high influence on the bacterial community composition as measured by DGGE analysis. Not only the mere presence

Fig. 3. Effect of bead-beating time on the distribution of soil DNA size fraction obtained with topsoil from Fladerne Bæk.

Fig. 4. Effect of bead-beating time (15–480 s) on the bacterial community composition obtained with Vejen-NX2 aquifer sediment as measured by DGGE analysis. One of three replicate samples is shown.
and absence of DNA bands were affected but also their relative abundance. Similar observations were found by Kozdrój and van Elsas (2000) and Westergaard et al. (2001). By applying ribosomal intergenic spacer analysis (RISA), Martin-Laurent et al. (2001) also demonstrated that the choice of DNA extraction protocol determines what microbial community composition will be obtained. This means that when a soil is subjected to different DNA extraction protocols then non-similar data on the abundance and phylogenetic relation of the indigenous soil bacteria will be obtained. Most commonly, the impact of PCR bias on data of microbial diversity is emphasised (von Wintzingerode et al., 1997). The importance of the DNA extraction protocol has not been equally notified, but should gain more attention. In the present study, the bead-beating procedure significantly increased the number of DNA bands obtained in DGGE analysis. Supplementing with additional cell-rupture treatments generally did not give rise to changed community profiles. However, with low-biomass soils such additions may be considered (Webster et al., 2003).

Clearly, the choice of DNA extraction protocol depends on soil type. We find, however, that of the methods tested the BIO101 kit is very appropriate for the analysis of indigenous soil bacterial communities. The protocol is fast (approximately half an hour compared to 5 and 7 h, for methods 1 and 2, respectively), reproducible, and gives very pure DNA of relatively high molecular weight. Very importantly, the protocol seems optimal for retrieving information on the diversity of microbial communities. Webster et al. (2003) similarly found that the BIO101 protocol was superior to other DNA extraction procedures when evaluating the DNA yield from diverse low-biomass deep sub-seafloor sediments. They found, however, that the addition of poly-adenyllic acid (poly A) to the lysis mixture increased the DNA yield significantly. Furthermore, the BIO101 kit offers a simple standardized protocol, which makes results obtained in different laboratories more comparable.

We believe that the choice of DNA extraction protocol will influence not only the determined phylogenetic diversity of indigenous microbial communities, but also the obtained functional diversity. This means that the detected presence of a functional gene—and thus the indication of enzyme activity—may depend on the nature of the applied DNA extraction procedure.

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