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Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results?

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Abstract

Bacterial communities of four arable soils – pelosol, gley, para brown soil, and podsol brown soil – were analysed by fingerprinting of 16S rRNA gene fragments amplified from total DNA of four replicate samples for each soil type. Fingerprints were generated in parallel by denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), and single strand conformation polymorphism (SSCP) to test whether these commonly applied techniques are interchangeable. PCR amplicons could be separated with all three methods resulting in complex ribotype patterns. Although the fragments amplified comprised different variable regions and lengths, DGGE, T-RFLP and SSCP analyses led to similar findings: (a) a clustering of fingerprints which correlated with soil physico-chemical properties, (b) little variability between the four replicates of the same soil, (c) the patterns of the two brown soils were more similar to each other than to those of the other two soils, and (d) the fingerprints of the different soil types revealed significant differences in a permutation test, which was recently developed for this purpose.

Keywords: 16S rRNA gene; DGGE; Soil bacterial diversity; SSCP; Total community DNA; T-RFLP

1. Introduction

Microbe-driven functions are responsible for a wide range of nutrient cycling and geochemical processes in soil. Thus microbial diversity in soil is crucial for soil functioning and health (Tiedje et al., 1999), and so there is a need to understand at a community level the spatial and temporal variability of microbial community structure and functions, e.g., in response to agricultural practices, to pollutions or to the climate. To address these questions methods which allow a rapid, simultaneous and reproducible analysis of multiple samples are required (Muyzer and Smalla, 1998; Forney et al., 2004). Therefore, in the last decade molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE; Muyzer et al., 1993), terminal restriction fragment length polymorphism (T-RFLP; Liu et al., 1997) or single strand conformation polymorphism (SSCP; Schwieger and Tebbe, 1998) became important and frequently used tools in microbial ecology. These three techniques can be used to generate fingerprints not only of rRNA gene fragments but also of other functional genes PCR-amplified from total community DNA or cDNA (Prosser, 2002; Liesack and Dunfield, 2002). Fingerprinting techniques provided information on the diversity and dynamics of, e.g., ribotypes in an environmental sample in response to environmental triggers but with a resolution which is surely not satisfactory to describe the full microbial diversity in complex habitats such as soil (Dunbar et al., 2000, 2001; Gans et al., 2005). However, the strength of these

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fingerprinting techniques is that large numbers of samples can be analysed and compared, making them ideal tools for ecological studies. Responding organisms can be characterised by cloning and sequencing of differentiating bands or by probing. To improve the interpretation of data obtained by fingerprinting methods a polyphasic approach is often used which comprises the parallel analysis of marker genes amplified from clones, isolates and total community DNA by the same fingerprinting technique. The general principle of most molecular fingerprinting techniques is based on the electrophoretic separation of marker gene fragments PCR-amplified from nucleic acids directly extracted from soil samples, due to differences in their nucleotide sequence.

The choice to select DGGE, SSCP or T-RFLP will often be influenced by the expertise and equipment available in the laboratory. A really intriguing question is whether the analysis of 16S rRNA gene fragments amplified from soil DNA by DGGE, SSCP or T-RFLP would come up with comparable results for the same type of sample. To address this question, DNA extracted directly from four replicate soil samples originating from four long-term observation field sites was shared between several laboratories and used for PCR amplification of 16S rRNA gene fragments. Subsequently the PCR products were analysed by SSCP, T-RFLP or DGGE. All data were evaluated by cluster analysis (UPGMA based on Pearson's indices). Furthermore, a permutation test was applied to test for significant differences between the similarities of profiles within a treatment group and between treatment groups (Kropf et al., 2004).

2. Materials and methods

2.1. Sampling and soil sample characteristics

The bulk soil samples used in this study originated from four different sites in Lower Saxony (Germany) that belong to the long-term soil observation programme BDF (Bodendauerbeobachtungsflächen). Each site is characterised by a different soil type, and its soil physico-chemical and microbiological parameters which were determined by Höper and Kleefisch (2001), are summarised in Table 1. The different sites were planted with winter oil seed rape (BDF8), pea (BDF27), winter wheat (BDF31) and winter rye (BDF39). Four composite samples were taken per site at the beginning of the vegetation period. Each composite sample consisted of 16 bulk soil cores (10 cm of the upper horizon), taken from four areas of 250 m^2 per site. The four replicate samples from each field were transported on ice, dried to 50% of the maximal water capacity at room temperature, homogenised well by sieving (2 mm) and sub-samples were stored at −70 °C until total DNA extraction was performed.

2.2. Total DNA extraction from bulk soil

Total DNA was extracted from 0.30 g (dry weight) using the FastDNA® SPIN Kit for Soil (BIO101, Carlsbad, CA) in combination with the FastPrep® Cell Disruptor FP120 (Qbiogene, Heidelberg, Germany). Slight modifications were made to the manufacturer's protocol regarding the cell lysis: in order to achieve a harsh cell wall disruption the speed was 30 s at speed 6.5 instead of 5.5. Subsequently, the crude DNA extract was purified by using the GENECLEAN® SPIN® Kit (BIO101). The yield and fragmentation of the crude and purified DNA was checked by agarose gel electrophoresis (0.8% w/v agarose) and UV visualisation of the ethidium bromide stained gels.

2.3. DGGE analysis of PCR-amplified 16S rRNA gene fragments

A 433 bp fragment of the 16S rRNA gene between positions 968 and 1401 of the *Escherichia coli* 16S rRNA gene sequence was amplified as described by Heuer et al. (1997). Each 25 µl reaction contained Stoffel buffer, 3.75 mM $MgCl_2$, 4% (w/v) acetamide, 0.2 mM deoxynucleoside triphosphates (Roche Diagnostics, Mannheim, Germany), 0.2 µM of each primer (GC-F968-984, R1378-1401, synthesised by TIB-Mol Biol, Berlin, Germany) and 2.5 U Taq DNA polymerase Stoffel fragment (Applied Biosystems, Darmstadt, Germany), to which 1 µl template DNA (ca. 20 ng) was added. After 5 min of denaturation at 94 °C, 35 thermal cycles including 1 min at 94 °C, 1 min at 53 °C and 2 min at 72 °C were performed, followed by a final extension at 72 °C for 10 min. At least two independent PCRs were done per sample and analysed separately. Amplicons were checked on 1% agarose gels after ethidium bromide staining.

DGGE analysis was performed as described by Heuer et al. (2001) with a denaturing gradient of 26 to 58% denaturant but with an additional acrylamide gradient of 6 to 9% to enhance the bands' resolution and sharpness (Gomes et al., 2005). The DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, München, Germany) was used. Approximately equal DNA amounts of the PCR products were loaded on the DGGE either in blocks of samples from the same site, or randomly. The products were separated during the running in 1× TAE buffer (40 mM Tris–acetate, 1 mM EDTA, pH 8.0) for 6 h at a constant voltage of 220 V and temperature of 58 °C. The gels were silver stained, dried at 37 °C and scanned as shown by Heuer et al. (2001). At least two different DGGE runs were carried out for all samples and for both loading orders of the samples on gel, in order to estimate the reproducibility of the statistical analysis of the DGGE profiles generated with different loading schemes of samples.

Table 1
Characteristics of analysed BDF soils

Location	Soil type	Soil texture	Clay [%]	Silt [%]	Sand [%]	C_{org} [%]	N_{tot} [%]	pH
8 Hofschwichelt	Pelosol	Clayish loam	35.2	44.5	20.4	2.26	0.24	7.4
27 Barrien	Para brown soil	Sandy silt	6.0	51.4	42.7	1.39	0.1	6.0
31 Vinnhorst	Gley	Sandy loam	19.2	40.4	40.4	4.04	0.3	5.3
39 Handeloh	Podsol brown soil	Silty sand	3.7	11.1	85.2	1.74	0.17	5.2