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Can denaturing gradient gel electrophoresis (DGGE) analysis of amplified 16s rDNA of soil bacterial populations be used in forensic investigations?

Anat Lerner [a], Yaron Shor [b], Asya Vinokurov [b], Yaacov Okon [a], Edouard Jurkevitch [a,*]

[a] *Department of Plant Pathology and Microbiology and The Otto Warburg Minerva Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot Campus, Israel*

[b] *The Division of Identification and Forensic Sciences, Toolmarks and Materials Laboratory, Israel Police National Headquarters, Jerusalem, Israel*

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Abstract

In criminal investigations, information on the origin of soils may be crucial for solving cases. The biological complexity of soil may potentially be used for sorting and differentiating between soil samples. Nucleic-acid based analyses of soil microbial populations are powerful tools, routinely used in studies of this habitat. Application of such approaches in forensics implies that a standardized DNA extraction method has to be applied to all samples. In this study, several DNA extraction protocols were compared. An improvement on the method proposed by Tsai and Olson (1991) was found to be most suited to extract DNA from various soil types, including from small samples. A blind test on soils from a crime, an alibi scene and unrelated locations was conducted to evaluate the potential of environmental PCR and denaturating gradient gel electrophoresis for use in forensic science. In most cases, soil patterns clustered according to soil type and location.

Keywords: DNA extraction; PCR; DGGE; Cluster analysis; Forensic science

1. Introduction

Criminal investigators often have to rely on tiny clues in their search for the truth. If these clues can provide clear evidence linking one or more individuals to a crime, they can turn to be essential proof, or supporting evidence in convicting or exonerating suspects. In several cases tiny amounts of soil can play an important role in the field of physical evidence (Marumo et al., 1995), hence the search for the origin of the soil is crucial for the solution of the case. Soils are particularly heterogeneous and complex habitats consisting of inorganic minerals, organic matter and living biota (O'Donnell and Gorres, 1999), supporting a tremendous microbial diversity that is not reflected in culture-based approaches (Ranjard et al., 2000; Kent and Triplett, 2002). Nucleic-acid based analyses of soils have, therefore, become standard and powerful tools in studies of this habitat (Felske et al., 1998; Kozdroj and van Elsas, 2000; Nannipieri et al., 2003). Bacteria are part of the soil microflora and they have the potential to reflect the history of a given environment (Ranjard et al., 2000). This potential can be useful for forensic purposes. Analysis of the living biota in soil based on nucleic acid-based methodologies may therefore provide the criminal investigator with yet another potent tool (Horswell et al., 2002).

However, successful application of molecular techniques relies on effective recovery of nucleic acids from the environment (Hurt et al., 2001). Moreover, for forensic analyses, there is a need for a simple procedure, which can provide sensitive detection from a wide variety of microorganisms and a wide variety of soils (Kuske et al., 1998). This procedure should be repeatable, be usable with small samples, and provide a large statistical confidence in its results.

Human DNA-based forensic data is now largely used in courts around the world and has played a major role in numerous publicized trials (Jost, 1999). In contrast, the use of soil bacterial DNA for forensic purposes is not a routine procedure (Horswell et al., 2002).

The objectives of this study were: (i) to test soil DNA extraction protocols on various soil types and their efficiency with small sized samples; (ii) to perform a feasibility study of the PCR-DGGE approach as a forensic tool for analyzing the microbial diversity existing in soils collected from crime scenes.

2. Materials and methods

2.1. Crime scene

A young woman was found stabbed to death on the banks of the Yarkon River in Tel-Aviv. No footprints, weapon or other

* Corresponding author.
E-mail address: jurkevi@agri.huji.ac.il (E. Jurkevitch).

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physical evidence was found in the crime scene. The main suspect, arrested a couple of days later, claimed to have been with the victim on a non-asphalted, nearby parking lot (the alibi area). The suspect washed his clothes and shoes after the murder and the only possible link to the crime scene was a small soil clot (0.2 g) found inside his shoe.

2.2. Soils

Soils samples were collected from different locations (Table 1). Soils used for the evaluation of DNA extraction protocols were sampled 5 cm below the surface, and kept on ice until processed. Samples originating from the crime scene were kept in plastic bags on the shelf and in the dark for 6 months until analysis. The soil clot from the suspect's shoe was not made available for analysis by the court because of the destructive nature of the analysis.

2.3. Soil wash

Soil samples in 1.8 ml of 0.05 M buffer phosphate and 0.5% cetyltrimethylammonium bromide (CTAB) were ground using a mortar and pestle. Samples were shaken for 3 h at 4 °C and 200 rev min^{-1}, and then centrifuged for 10 min at 4 °C and 700 rev min^{-1}. The supernatant was removed and DNA extraction was performed.

2.4. DNA extraction

Five direct methods were used for DNA extraction from bacterial communities: (a) Tsai and Olson (1991) (thereafter 'T') with slight modifications: After three freeze-thaw cycles, proteinase K was added to the solution to a final concentration of 50 μg ml^{-1} and the samples were incubated for 30 min at 37 °C; (b) Zhou et al. (1996) ('Z'); (c) Yeates et al. (1998) ('Y'); (d-e) methods based on the commercial kits FastDNA SPIN Kit for Soil (BIO 101, Qbiogene, Inc, Carlsbad, USA) ('F') and UltraClean™ Soil DNA kit (MO BIO Laboratories, USA) ('U'). The Y and F methods included a bead beating step. Samples weighing 0.2, 1, 10, 0.6, and 0.25 g were used with methods, T and Y, Z, F, and U, respectively. The weight of the soil samples was the optimum recommended for each protocol.

2.5. Purification of crude DNA extracts

The QIAquick Gel Extraction kit (QIAGEN GmbH, Hilden, Germany, and DNA Isolation kit (Biological Ind., Israel) were used to purify DNA.

2.6. PCR amplification

One to three microlitre of each DNA preparation from environmental sample were amplified in a PCR reaction mixture (50 μl) using an Eppendorf Mastercycler Gradient (Brinkmann Instruments, Inc., USA). Each PCR mixture contained 0.8 μM of each primer, 0.3 mM of each deoxynucleotide (dNTP), 5 μl of 10× buffer (Promega, Madison, USA), 0.03 unit μl^{-1} redTaq DNA polymerase (Sigma, Rehovot, Israel), 3.75 mM $MgCl_2$, 2 μl of 10 mg ml^{-1} BSA and double distilled, sterilized water to complete the mixture volume. The primers for PCR were specific for conserved bacterial 16S rDNA sequences (Heuer et al., 1997). PCR with primers Gm5f (5′-GC-clamp-CCT ACG GGA GGC AGC AG-3′) and 907r (5′-CCC CGT CAA TTC CTT TGA GTT T-3) amplified a bacterial 16S rDNA fragment from position 341–928 (*Escherichia coli* numbering). PCR amplification was performed for 35 cycles as follows: after initial denaturation of 1 min at 95 °C each cycle consisted of denaturation at 95 °C for 20 s, primer annealing at 57 °C for 25 s, and primer extension at 72 °C from 30 s. Cycling was followed by final primer extension at 72 °C from 1 min. PCR products were visualized by electrophoresis in 1% (w/v) agarose gels after 1 mg ml^{-1} EtBr staining (Sambrook et al., 1989).

2.7. Denaturing gradient gel electrophoresis

Strong PCR products of the expected size (550 bp) were subjected to DGGE analysis. DGGE was performed with an Ingeny phor U-2 system (Leiden, The Netherlands). Samples of 43 μl of PCR product were loaded onto 6% (w/v) polyacrylamide gels in 1.0 strength Tris-ethylene-diamineteraacetate

Table 1
Properties of the soils used in this study

Location	Texture	Sampling zone	Organic matter (%)	pH	Moisture content (%)
Kfar Menachem, Israel	Sandy loam (Chromoxererts brown alluvial)	Maize rhizosphere	0.9	7.23	5.5–6
Rehovot, Israel	Sandy (Haploxeralfs brown–red)	Maize rhizosphere	0.5	8.4	27.9
Hula, Israel	Peat (Lacustrine gley)	Surface soil	29.3	7.26	84.5
Coconut residues medium	Compost (15% polystyrene)		85	4.8–5.6	30–35
Crime scene	Sandy clay loam (Hamric alluvial soils and gley)	Surface soil	N.D.	N.D.	N.D.
Alibi scene	Sandy clay loam (Calcareous sandstone)	Surface soil	N.D.	N.D.	N.D.
Suspect's home	Sandy loam	Surface soil	N.D.	N.D.	N.D.

N.D.: not determined.