Microbial Diversity in Ground and Surface Water Analyzed by Denaturing Gradient Gel Electrophoresis Using the DCode™ System

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Introduction

Bacterial diversity in environmental samples is usually determined by a characterization of isolated strains. A problem for the analysis and characterization of microbial communities is the inability to culture most of the bacteria species present in the sample. Therefore, isolated bacteria may account for only a minor portion of the total bacterial diversity originally present in the sample. This problem is particularly severe in oligotrophic habitats like ground water, where approximately only 0.1–1% of the bacterial species are culturable (Amann et al. 1995). A new approach in microbial ecology is based on the analysis of bacterial genetic information without cultivation. This culture-independent approach has greatly enhanced the ability to assess bacterial diversity in ecosystems such as ground and surface water environments. After isolation of total bacterial DNA, variable regions of the 16S rRNA gene are amplified by the polymerase chain reaction (PCR). The similar-sized PCR products are separated by subsequent denaturing gradient gel electrophoresis (DGGE), and the resulting diversity pattern are analyzed and compared (Kuhlmann et al. 1997, Eschweiler et al. 1998).

Methods

DNA from surface and ground water was isolated and purified as described earlier. Two universal bacterial 16S rDNA primers were used to amplify a 527 bp fragment from total genomic DNA. PCR was performed in a total volume of 100 µl containing 1x PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin), 200 µM each dNTP, 0.5 µM each primer, 0.3 mg/ml bovine serum albumin (BSA), and 2.5 U AmpliTaq DNA polymerase (PerkinElmer, Inc.), 10 µl DNA solution. The temperature cycle for the PCR was 60 sec of denaturation at 94°C, 60 sec of annealing (see below), and 90 sec of primer extension at 72°C. During an initial touchdown cycle, the annealing temperature was lowered from 65°C to 55°C in intervals of 1°C per cycle. The additional annealing cycles were done at 55°C. Ten PCR cycles were performed for the touchdown procedure and then 20 additional cycles at the actual annealing temperature of 55°C. This touchdown procedure reduces the formation of spurious by-products during the amplification process (Don et al. 1991, Muyzer et al. 1993). DGGE was performed with the DCode universal mutation detection system (Bio-Rad Laboratories, Inc.). The polyacrylamide gels (7.5%) contained a denaturing gradient from 40% to 70% (100% denaturant: 7 M urea and 40% formamide) and were run in 0.5x TAE buffer (40 mM Tris base, pH 7.4, 20 mM sodium acetate, 1 mM EDTA). Gels were poured with a stacker on top (7.5% polyacrylamide, no denaturant). The optimum denaturant gradient was determined by performing perpendicular DGGE according to the DCode instruction manual. Electrophoresis was performed at constant voltage (70 V) and temperature (57°C) for 16 hr. After electrophoresis, the gels were stained with silver nitrate (Heukeshoven and Dernick 1985), dried, and photographed.

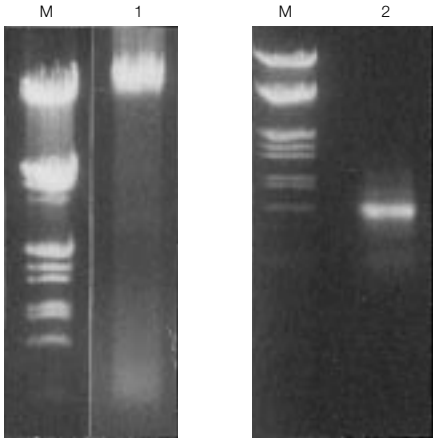

Fig. 1. Agarose gel electrophoresis from isolated and PCR-amplified DNA from surface water. Lane 1, isolated DNA from river Ruhr; lane 2, PCR-amplified DNA. M, size standard (λ DNA x HindIII x EcoRI).

Results and Discussion

Isolated and PCR-amplified DNA from surface water is shown in Figure 1. Separation of 16S rDNA amplified fragments by DGGE revealed the great diversity in ground and surface water samples. Complex banding patterns could be observed in samples with different hydrochemical conditions. These patterns were characteristic for each sample and showed the differences and common features in species composition.

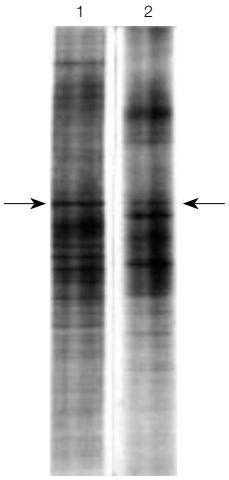

Fig. 2. DGGE patterns from ground water with different hydrochemical characteristics. Lane 1, anoxic ground water; lane 2, oxic ground water. Arrows indicate example of bands found in both samples.

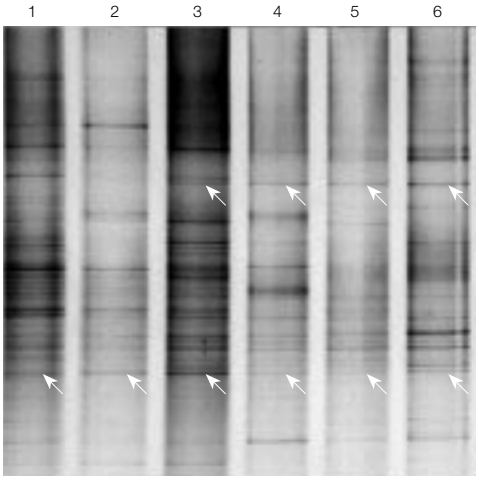

Fig. 3. DGGE patterns from surface water. Lanes 1–6, surface water (river Ruhr) from March through August, respectively. Arrows indicate species found over a limited period of time (top) and species found in all samples (bottom).

The DGGE patterns from ground water with different hydrochemical characteristics are shown in Figure 2. Patterns from anoxic ground water are different from those obtained with oxic ground water. Some bands were found in both samples, indicating bacterial populations that could adapt to both habitats. In order to demonstrate temporal changes in microbial populations, DGGE patterns from surface water (river Ruhr) were recorded over a period of six months. Figure 3 shows changing patterns where certain species of bacteria are found over a limited period of time, while others are found in all samples.

The complex DGGE patterns are further analyzed and compared by digitized gel imaging using the Model GS-700 imaging densitometer and Multi-Analyst™ software (Bio-Rad). DGGE patterns from surface and ground water reveal a specific and unique bacterial population depending on the hydrochemical properties. In addition, DGGE patterns are used to demonstrate temporal and spatial variations in species composition. Sudden changes in DGGE patterns might indicate the introduction of harmful compounds (for example, pesticides and heavy metals) to surface and ground water. Analysis by DGGE is also suitable for a subsequent species identification by sequencing individual bands (Ferris et al. 1996).

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