BIOMEDICAL AND ENVIRONMENTAL SCIENCES **19**, 371-374 (2006)

Application of PCR-DGGE in Research of Bacterial Diversity in Drinking Water[1]

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Objective To analyze the structure of bacteria in drinking water by molecular biological techniques. **Methods** DNA of bacteria in drinking water was directly extracted without culture. 16S ribosomal DNA fragments, including V-6, -7, and -8 regions, were amplified with universal primers ($EUBf_{933}GC$ and $EUBr_{1387}$) and analyzed by DGGE. **Results** DGGE indicated that amplification products could be separated. The results showed that DGGE could be used in the separation of different microbial 16SrRNA genes extracted from drinking water. Though there were special bacteria in different water samples, the predominant bacteria were essentially the same. Three sequences of the reclaimed specific bands were obtained, and phylogenetic tree of these bands was made. **Conclusion** Bacterial diversity in drinking water is identified by molecular biological techniques.

Key words: Drinking water; Denaturing gradient gel electrophoresis (DGGE); 16S ribosome RNA; Microbial diversity

INTRODUCTION

The microbiological quality of drinking water is important since bacteria may survive, reproduce and disperse in water systems and become a source of microbial or pathogen contamination. Bacteria introduced into a water distribution system through raw water undergo changes in density and diversity due to the selective pressures of the system. Bacteria in water leaving the treatment plant, though probably inactivated by the disinfection process, may recover and grow in water distribution system and bulk water. It is necessary to provide sufficient information to control microbiological quality of drinking water. Identification of bacteria in drinking water is important in instances where specific waterborne bacteria may be detrimental and potentially harmful to the products or processes in which water is used. Such information about bacteria may also be very useful when the source of microbial contamination in a product or process is identified. Many researches showed that only 1% of bacteria in drinking water could be cultured in laboratory[1]. The real status of microbial community could not be reflected by traditional culture methods.

Recently, several molecular techniques have been developed in order to study natural samples[2-4]. These molecular techniques identify bacteria without isolation and reveal the enormous extent of microbial diversity. Specifically, denaturing gradient gel electrophoresis (DGGE) has emerged as a powerful tool.

16SrRNA gene is stable and widely existent, and contains more information. It is popularly accepted that the sequence of 16SrRNA gene to distinguish bacteria[5], and techniques based on 16SrRNA gene have already been widely used in the research of evolution, morphology and ecology of bacteria[6]. The key work in the research of bacteria by DGGE is amplifying the 16SrRNA gene of bacteria. After the specific PCR amplification of 16SrRNA gene and analysis by DGGE, the fragment of DNA can be separated. These techniques have been introduced into the field of molecular bacteria ecology, by which the genetic diversity of natural bacteria and the phylogenic relationship between the population members can be confirmed. There is no report about these techniques used in the research of bacteria diversity in drinking water.

In the present research, 9 sampling sites in the

[1]This research was supported by grants from Chinese National Science Foundation (50478086). The 10th Five-year Key Programs for Science and Technology Development of China (2002AA601120).

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0895-3988/2006
CN 11-2816/Q

water distribution network of a northern city residential area in China were chosen and were analyzed by DGGE. A pilot study was made to detect the bacterial diversity in the water samples. The result showed that the method of PCR-DGGE could be used in the research of bacterial diversity in drinking water. The information on bacteria in drinking water was obtained by sequencing and identification of the specific fragments. In a word, the work provides useful and credible information for the improvement of drinking water quality.

MATERIALS AND METHODS

Water Samples

The 9 sampling sites in the resident area included the entrance of the network (sites 1 and 9), the network (sites 3, 7, and 8), end points (sites 2, 4, and 5), and a tall building tank (site 6). The sampling program covered one month period from May to June, 2004. Samples were collected everyday in standard way. Water samples were carried back and monitored in the laboratory as soon as possible. Unless otherwise stated, all analyses were undertaken based on Standard Methods for the Examination of Water and Wastewater[7].

Instruments

Temperature, free- and total-chlorine were determined in the field using a HACH 46700-001, and turbidity was monitored by HACH 2100AN turbidimeter. PCRs was performed by the Mastercycler gradient, Eppendorf 5331 (Germany).

Culture Methods

Colony-forming bacteria were detected by the culture method, and drinking water samples were incubated at 20℃-25℃ for 7days on R2A medium (0.5 g of yeast extract, 0.5 g of peptones, 0.5 g of acid hydrolysate of casein, 0.5 g of dextrose, 0.5 g of soluble starch, 0.3 g of dipotassium phosphate, and 2.5 g of glucose per 1000 mL water).

DNA Extraction for 16S ribosomal DNA (rDNA) Analysis

DNA was extracted from bacterial cells in drinking water. Bacterial cells in drinking water were vacuum filtered with polycarbonate white filters (0.2 μm in pore size, Millipore, USA). These filters were placed into a sterilized tube with 500 μL sterile DNA-free water. The suspensions were mixed thoroughly, frozen in liquid nitrogen and thawed three times at room temperature in succession. The suspensions were used for PCR amplification[8].

Primers and PCR Amplification

Primers 16SrDNA fragments were amplified with primers GC-clamp-EUBf933 and EUBr1387 specific for universally conserved bacterial 16SrDNA sequences. The sequences of the two primers are EUBf933: 5′-GCACAAGCGGTGGAGCATGTGG -3′ and EUBr1387: 5′-GCCCGGGAACGTATTCA CCG-3′. For DGGE analysis of the PCR products, a 40 bp GC-rich sequence (GC-clamp) was attached to the 5′ end of primer EUBf933[9].

PCR mixture PCR mixture containing 2.5 U of Taq, 20 pmol of each primer, 5 μL of a 200 μmol/L of each deoxyribonucleoside triphosphate, 5 μL of 10×PCR buffer, was made up to 45 μL with DNA-free water. A DNA suspension was added in a 10 μL volume after irradiation of the PCR mixture with UV light.

Conditions of PCR Hot-start PCR was performed at 95℃ for 10 min. Touchdown PCR was performed as follows: the annealing temperature was initially set at 66℃ and then decreased 0.5℃ each cycle until it was 56℃. Twenty additional cycles were run at 56℃. Denaturation was carried out at 94℃ for 1 min, and primer extension was performed at 72℃ for 3 min. The final extension step was at 72℃ for 7 min. PCR products were detected by 1.5% agarose gel electrophoresis.

DGGE Analysis

PCR products were loaded onto a 6.5% (wt/vol) polyacrylamide gel in 1×TAE. The 6.5% (wt/vol) polyacrylamide gel (acrylamide/bisacrylamide ratio, 37.5:1) was made with denaturing gradients ranging from 40%-60% for 16SrDNA fragments. Denaturant (100%) contained 7 mol/L urea and 40% formamide. The electrophoresis was run at 60℃ for 10 min at 20 V, and subsequently for 7 h at 150 V. After electrophoresis, the gels were stained with silver stain.

RESULTS

PCR Products Separated on Agarose Gel

After the PCR amplification, fragments about 450 bp obtained from all drinking water samples were fragments of 16SrDNA V6-V8 sections. Fig. 1 shows the result of the 16SrDNA fragments amplified by PCR and separated on 1.5% agarose gel.

DGGE Analysis of Bacterial Community Structure in Drinking Water

Fig. 2 illustrates the result of DGGE analysis of 16SrDNA fragment. The products of bacterial DNA in drinking water amplified by PCR could be separated by DGGE.

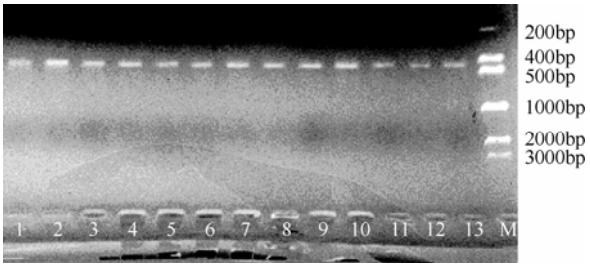

FIG. 1. 16SrDNA amplified by PCR separated on 1.5% agarose gel.

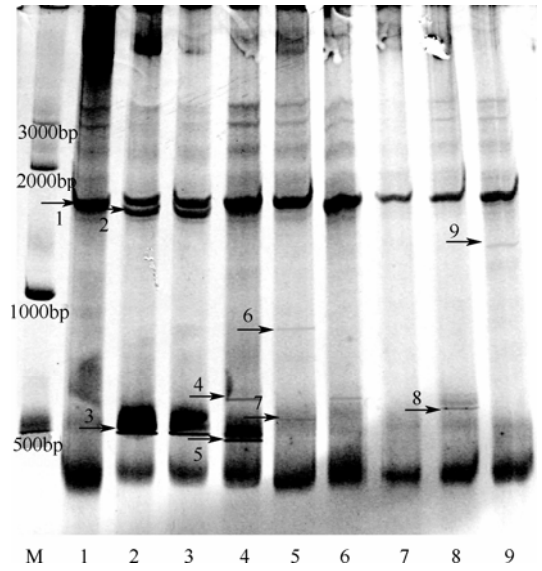

FIG. 2. DGGE analysis of 16SrDNA fragments obtained from drinking water samples.

It is shown that sites 1-9 all contained band 1, indicating that the predominant bacteria were the same in the water samples from the same area. At the same time, there were discrepancies between different site water samples from the same area. Each site had its special bands, for example, band 2 only existed in sites 2 and 3; band 3 exists in sites 2-4; band 4 exists in sites 4, 6, and 8; band 5 exists only in site 4; bands 6 and 7 exist in site 5; bands 8 and 9 exist in sites 8 and 9. This indicated that different waterpower conditions and distance from sampling site to the water plant were reasons why the water quality was different at different sites and resulted in different population of bacteria. The distribution of water sampling site has great influence on the structure of bacteria.

Sequencing and Identification

After DGGE, specific fragments of each water sample were reclaimed and sequenced. Comparison of 16SrDNA sequences with sequences available in GenBank revealed highly similar values for these reclaimed 16SrDNA fragments. The phylogenetic affiliation of the sequences was further analyzed with the sequences of bands 3, 4, 5, 8 (named as Strain WQ 3, Strain WQ 4, Strain WQ 5, and Strain WQ 8) by DNASTAR (Fig. 2). The phylogenetic tree shown in Fig. 3 indicates that there were different kinds of bacteria in drinking water, and some of them may be harmful to human beings.

DISCUSSION

Extracting total DNA from bacteria in drinking water is more rapid and accurate than traditional separation and identification methods, and can reflect the diversity of bacteria in drinking water more directly.

It is viable to research the structure of bacteria in drinking water by amplifying 16SrDNA of bacteria and separating the PCR products with DGGE. By reclaiming and sequencing of predominant or specific bands and identification by search for NCBI, more information about these bands could be obtained. With such information, species of the band and their phylogenic state could be known. Though the bands obtained by 16SrDNA PCR amplification are short and cannot be used to distinguish the exact taxonomic station, they are useful to understand the distribution of bacterial population. Longer 16SrDNA fragments can be obtained by improving the primers used in PCR and can be used to decide the taxonomic species effectively, and to study the structure of bacteria in drinking water. At the same time, conditions of un-culturable bacteria can be acquired, and the real environmental situation of drinking water can be reflected. Such information is important for water supply enterprises to ensure the quality of drinking water. In short, extracting total DNA from bacteria in drinking water is more rapid and accurate than traditional separation and identification methods, and can reflect the diversity of bacteria in drinking water more directly.

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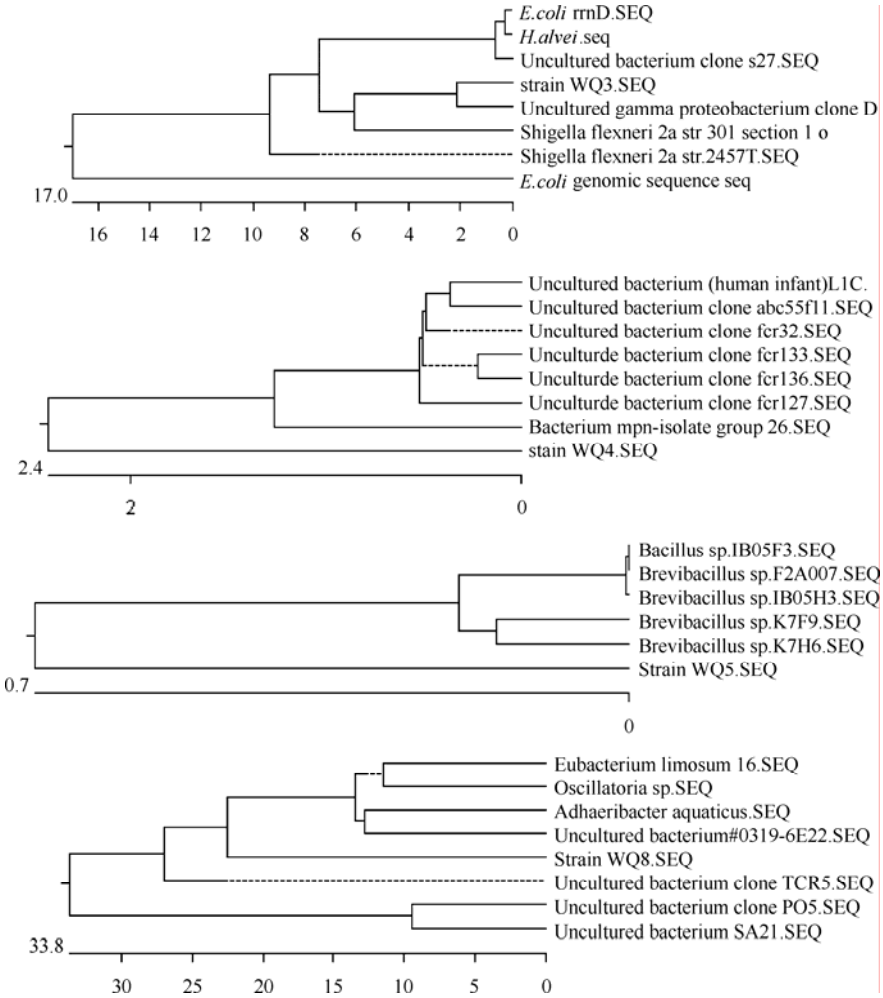

FIG. 3. Phylogenetic tree of 16SrDNA sequences from DGGE bands.

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(*Received September* 20, 2005 *Accepted May* 25, 2006)