The specificity of this primer is imparted by the underlined region. Theoretically, these primers should amplify a 323-bp fragment of the 16S rRNA genes for members of the *Bacteria*, including the highly variable V9 region. PCRs were performed by using *Taq* DNA polymerase according to the instructions provided by the manufacturer (Promega). PCR products were examined on ethidium bromide-stained agarose gels, and the reaction mixtures yielding products of the expected size were used for DGGE analysis. The temperature cycle for the PCR was 1 min of denaturation at 94^{o}C, 1 min of annealing (see below), and 3 min of primer extension at 72^{o}C. During an initial touchdown cycle, the annealing temperature was decreased from 53 to 43^{o}C in intervals of 18^{o}C per cycle; 20 additional annealing cycles were done at 43^{o}C. The final primer extension was for 10 min.

DGGE

All reagents and techniques were as previously described (Ferris, et al, 1996) except as noted. Acrylamide gels (10%) were prepared and run with 0.5x TAE buffer (13 TAE is 0.04 M Tris base, 0.02 M sodium acetate, and 1.0 mM EDTA; pH adjusted to 7.4). A gel electrophoresis unit (Ingeny Phor U) was used with glass plates (16 by 18 cm), 1.0-mm spacers, and 1-cm-wide loading wells. A 15 liter aquarium served as the lower buffer chamber. DGGE gels contained a varied of 30 to 80% gradient of urea and formamide (UF) solution increasing in the direction of electrophoresis. A 100% UF solution is composed of 40% (vol/vol) formamide plus 7.0 M urea (1, 20). DGGE was conducted at 60^{o}C, firstly at 20V for 10 min and then at a constant voltage of 200 V for 3 h. The results were stained using silver staining method (Bassam, B.J. et al., 1991).

Sequencing and Phylogenetic Analysis

Amplified bacterial 16S rRNA PCR products (400 bp) from bands excised from the DGGE gel were subsequently add with TE buffer and let overnight at refrigerator. Selected DGGE bands that excised from the gel, re-amplified sequenced as described in detail previously. All confirmed DGGE bands were subjected to double-stranded DNA sequence analysis and sequence comparison to determine the best match to known sequences using the BLAST program at National Centre of Biotechnological Infornation website (http://www.ncbi.nlm.nih.gov). The phylogenetic tree was constructed using MegAlign program at DNA Star software. The alignments were carried out using Clustal Method.

RESULTS AND DISCUSSION

Our perspective on microbial diversity has improved enormously over the past few decades. Since 1990 many new bacteria have been identified solely from their 16S rRNA gene sequences and many of these are known to be numerically dominant in nature. In 1990 about 10 divisions of the domain *Bacteria* were known. Now 40 have been described and this remarkable expansion in our knowledge of bacterial biodiversity has occurred entirely due to the recent explosive growth of molecular approaches. Furthermore, 13 of these divisions are currently known only from sequences and have no cultured representatives.

In this study, the microbial diversity of the 52^{o}C of Pancuran 7 hot spring (BR) was studied. The study of this hot spring was carried out using cultivation and direct analysis through filtration of the spring water. Figure 1 represents the hot spring that has 7 streams with brown-yellow sediment and the filtrate of Pancuran 7 water that pass through membrane filter.