is necessary for nucleic acid extraction. Altogether, make uncertain whether the collection of sequences obtained from an environment represents the natural assemblage accurately. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA genes is a molecular technique that is used to study the dynamic behavior of complex microbial assemblages (Muyzer, 1998).

In this report we would like to give an overview of our recent work on biodiversity study. By studying the microbial community in Pancuran 7 Hot Spring, a thermal pool in the Baturraden Park, Purwokerto, we would like to explore the community that detected by 16S rRNA analysis on culture-dependent and uncultured-independent procedure. The potential for DGGE to offer a more rapid and comprehensive means to simultaneously survey various bacterial populations prompted us to evaluate the technique in our system. DGGE banding patterns were evaluated, and bacterial populations were identified by sequencing individual bands.

MATERIALS AND METHODS

Sampling procedure

Pancuran 7 hot spring belongs to Baturraden field that located in Purwokerto, Central Java. This hot spring has temperature at 52^{o}C and pH 7. The microbial samples were collected on August 2004 from one of the hot stream. The microbial diversity studies were studied through two strategies: cultivation and filtration. The cultivation procedure was carried out using two kinds of minimal mediums which are BR-A and BR-B. The formula of BR-A medium contains 0,5% peptone, 0,25% yeast and 0,5% NaCl and BR-B medium composed of 0,5% triptone, 0,25% yeast and 0,5% NaCl, except the water which were used is the spring water. These medium were incubated at 60^{o}C without shaking for 24 hours. For the filtration procedure, the water sample was kept in a sterile plastic container (2L) and brought to the laboratory immediately within 2 hours. Afterwards, cells were harvested by filtration of 1 L volumes of spring water gently through 0,2 µm (nominal pore size) Millipore filters. Resulting filtrates containing bacterial communities were stored at -20°C until DNA was extracted.

Nucleic acid extraction

Each microbial sample which is from cultivation or filtration was pellet by centrifugation at 5000xg for 5 min. DNA was extracted using slight modifications of a method described by Klijn et al. (1991). The pellet were suspended in 200 µl of 10 mM Tris HCl buffer (pH 8.0) containing 8mg/ml of lysozyme and incubated at 37°C for 1 h, the cells were lysed by adding 200 µl lysis buffer containing 2% sodium dodecyl sulfate, 0,8 mg/ml proteinase K and 200 mM EDTA at pH 8,0. The lysis process was done by incubation at 50^{o}C for 30 min. The purification step was carried out by adding 200 µl of chloroform:isoamil-alcohol (24:1), vortex and centrifugation at 1300 g, 30 second. The upper solution was moved to clean tube. This step was repeated for 3 times. Subsequently, the DNA was precipitated by adding 60 µl of 3 M sodium acetate and 1 ml of 96% ethanol (stored at -20°C). After centrifugation, the DNA pellet was dissolved in 10 mM Tris HCl buffer (pH 8.0). The DNA pellet was washed with 70% ethanol and finally dissolved in 50 µl of TE buffer (10 mM Tris HCl [pH 8.0], 1 mM EDTA).

PCR

The amplification of partial 16S rRNA genes of the domain *Bacteria* were as previously described (Ferris, et al, 1996). One primer (P1) complements a region conserved among members of the domain *Bacteria* (*Escherichia coli* positions 1055 to 1070). The other primer (P2) is based on a universally conserved region (*E. coli* positions 1392 to 1406; and incorporates a 40-base GC clamp). The primer sequence:

P1: 5'-ATGGCTGTCGTCAGCT-3'

P2: 5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCACGGGC
GGTGTGTAC-3'.