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Bacterial Community Analysis of Gedongsongo Hot Spring: Denaturing Gradient Gel Electrophoresis

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Abstract

The bacterial communities from one of hot spring at Gedongsongo (WGS2) Ambarawa, Central Java, Indonesia; was investigated by molecular analysis based on the 16S rRNA gene. Two minimal media, MM_1 and MM_2 were used for growth of aerobic microbial communities. Cultures media were combined by filtration through 0.2-μm-pore-size filter for total genomic DNA extraction. The DNA that was extracted both from cells of filtration and cultures have been well characterized as microbial chromosomal DNA and used as PCR template. Partial 16S rRNA gene sequences were PCR amplified using one primer set. One primer complements a region conserved among members of the domain Bacteria (Escherichia coli *positions 1055 to 1070. The other primer is based on a universally conserved region* (E. coli *positions 1392 to 1406 and incorporates a 40-base GC clamp. These primers amplified a 323-bp section of the 16S rRNA genes. The amplicons were separated by denaturing gradient gel electrophoresis (DGGE) for community analysis. The DGGE profiles showed that there were three distinct bands, but only two of them that represent the predominant bacteria.*

1. Introdcution

Biotechnologists have been searching for microorganisms that can function in extreme environments to enable the creation of new processes and industries. A number of thermophiles and hyperthermophiles have been isolated from samples of hot sediments, mud, rocks, soils and waters. The classical approach to determine the microbial diversity in a natural or artificial ecosystem starts with culturing of the microorganisms in a sample. Culture based approaches to isolate microorganisms from any natural environment do not provide comprehensive information on the composition of microbial communities. This technique also failed to determine the majority of microorganisms in nature typically are not cultivated by using standard techniques (Amann, R.F. *et al.*1995). It has been already realized that only a minor fraction of the microorganisms (1-5%) is amenable to standard culturing techniques (Fuhrman et al., 1993; Liesack, 1992; Schmidt, 1991). Due to this difference between cultivable and in situ diversity, it is often difficult to assess the significance of cultured members in resident microbial communities. In order to overcome the limitations associated with cultural approaches a molecular alternative has been developed. The development of techniques for the analysis of 16S rRNA sequences in natural samples has greatly enhanced our ability to detect and identify bacteria in nature (Pace et al., 1986). This involves DNA extraction of community DNA directly from water, soil or sediments followed by PCR amplification and then sequencing of 16S rRNA genes, which are known to be one of the established phylogenetic markers (Woese, C.R. 1987). Such approach has been successfully applied for hot spring (Ferris, M.J. et al., 2003), compost (Ueda, K. et al., 2001),marine