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SSCP Profiles of 16S rRNA Gene of Thermophilic Bacterial Communities Inhabiting the Local Hot Stream

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ABSTRACT

Identification of the bacterial communities from one hot stream at Gedongsongo (GS3), Ungaran, Central Java; was carried out using Single Strand Conformation Polymorphism (SSCP) method. Two minimal media, GYa and GTa were used to grow semi anaerobic microbial communities. Cultures media were combined by filtration through 0.2-μm-pore-size filter for total hot stream community. PCR amplifications of partial 16S rRNA gene sequences were performed with chromosomal DNA extracted from cells of filtration and cultures using primers targeting the V4–V5 region of 16S rRNA genes. These primers amplified about 400-bp section of the 16S rRNA genes. The SSCP profiles showed that there were five bands from filtration sample, two bands from GTa culture and three bands from GYa culture. All of bands represent different phylotypes.

INTRODUCTION

Traditionally, the analysis microbial communities have been conducted using viable plate counts or most probable number techniques. However, due to the high degree of selectivity and bias inherent in culture methods, only ca. 5000 bacterial species have been described (Amann, et al. 1994). Direct visualization of stained bacteria suggests that the proportion of culturable cells is usually 65% of the total number of viable cells present in the soil sample (Bakken, 1995). Furthermore, this culturable subset is not representative and contains significantly less genetic information than the collective genomes (metagenome) of the total microbiota (Rondon, et al. 2000). 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, 1987). Such approach has been successfully applied for hot spring (Ferris, et al., 2003), compost (Ueda, et al., 2001), marine bacterio-plankton (Fuhrman, et al. 1993), soil (Nakatsu, et al. 2000) as well as hydrothermal environment (Moyer, et al. 1994).

PCR combined with single strand conformation polymorphism (SSCP) has also been applied to the analysis of microbial communities. This method was initially developed to detect mutations in human genes (Orita, et al, 1989). In non-denaturing conditions the tertiary folded structure of single-stranded DNA (ssDNA) is affected by intra-molecular interactions (Lee, et al, 1996). The electrophoretic mobility of the folded ssDNA is, therefore, affected by these sequence-dependent properties and the molecular mass of the molecule, allowing separation of PCR products of the same size but different sequence due to the differing mobility of their folded structure (Hayashi, 1991). Since no GC clamp, special equipment or gradient gels are required, SSCP is highly applicable for community analysis. SSCP has been used to monitor: pure cultures of microorganisms; rhizosphere microbial communities (Schwieger and Tebbe, 1998); differences between bacterial populations in an oligotrophic lake and a eutrophic pond (Lee, et al, 1996); succession of microbial communities during hot composting (Peters, et al. 2000); and community dynamics in an anaerobic bioreactor (Zumstein, et al. 2000).

Our objective in this study was to describe the diversity of bacterial community inhabiting one hot stream at Gedongsongo field of Ungaran volcano, central Java. The