Use of Terminal Restriction Length Polymorphism (T-RFLP) Analysis to Evaluate Uncultivable Microbial Community Structure of Soil

Puneet Singh Chauhan, Charlotte C. Shagol, Woojong Yim, Sherlyn C. Tipayno, Chang-Gi Kim, and Tongmin Sa*

Department of Agricultural Chemistry, Chungbuk National University, Cheongju, Chungbuk, 361-763, South Korea, *Bio-Evaluation Center, KRIBB, Cheongwon 363-883, South Korea

Various environmental ecosystems are valuable sources for microbial ecology studies, and their analyses using recently developed molecular ecological approaches have drawn significant attention within the scientific community. Changes in the microbial community structures due to various anthropogenic activities can be evaluated by various culture-independent methods e.g. ARISA, DGGE, SSCP, T-RFLP, clone library, pyrosequencing, etc. Direct amplification of total community DNA and amplification of most conserved region (16S rRNA) are common initial steps, followed by either fingerprinting or sequencing analysis. Fingerprinting methods are relatively quicker than sequencing analysis in evaluating the changes in the microbial community. Being an efficient, sensitive and time- and cost effective method, T-RFLP is regularly used by many researchers to access the microbial diversity. Among various fingerprinting methods T-RFLP became an important tool in studying the microbial community structure because of its sensitivity and reproducibility. In this present review, we will discuss the important developments in T-RFLP methodology to distinguish the total microbial diversity and community composition in the various ecosystems.

Key words: Microbial diversity, Community structure, T-RFLP, 16S rRNA

Introduction

Microbial communities are present in diverse environments and these play an important role in their respective environments. The study of their structure and dynamics is an important, yet intimidating task due to the large number of inhabitant bacterial population in a sample (e.g. approximately 10^8-10^10 g^-1 in soil). Micro-organisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation, decomposition of organic matter, toxin removal, and the cycling of carbon, nitrogen, phosphorus, and sulphur (Garbeva et al., 2004). In association with larger organisms these microbial communities play decidedly important role in maintaining the health of soil and host organism. The management or treatment of soil affects microbial community structures has long been recognized (Islam et al., 2011). The physicochemical properties of soil, soil particle size distribution, the presence and age of specific plant species, and crop rotations are key determinative factors. Application of pesticides, amendment with chemical fertilizers, compost or manure, and the introduction of plant growth promoting living microbial inoculants or genetically modified microorganisms have all been shown to affect soil microbial community structures (Nautiyal et al., 2010).

Due to the vast importance of bacterial communities it is imperative to understand how they interact with their environment and the changes that take place. Soil microbial communities are often difficult to fully characterize, mainly because of their immense phenotypic and genotypic diversity and heterogeneity. While all of the cultivation based methods are excellent in being able to yield actual microorganisms which can be biochemically characterized, the cultivation methods miss an estimated 85%-99.9% of the microorganisms in an environmental sample with many microorganisms being unculturable (Amann et al., 1995; Hill et al.,
Various cultivation independent methods are used to determine the diversity of microbial communities [(DGGE/ TGGE (Muyzer et al., 1993), SSCP (Lee et al., 1996), ARDRA/RFLP (Liu et al., 1997), ARISA (Fisher and Triplett, 1999)]. All these molecular methods are now widely used to detect microbe specific genes because they are efficient, time- and cost effective and sensitive compared to conventional cultivation dependent methods. Among these molecular methods, fingerprinting techniques based on 16S rRNA genes have been successfully used in numerous studies to explore microbial diversity of predominant populations in various habitats and offer the advantage that they are more amenable to high throughput and more comprehensive than cultivation-dependent methods (Rappe and Giovannoni, 2003; Torsvik et al., 2002; Weng et al., 2006). This review will provide the information on the important developments in T-RFLP methodology to discriminate between uncultivable microbial populations in the various ecosystems.

**Direct Cultivation Techniques**

Identifying the makeup of the microbial communities provides insights into how the microbes interact and what potential metabolic processes are occurring within these communities. There are a plethora of methods available for elucidating the microbial populations in these communities. These methods may be cultivation based, wherein samples are diluted and individual microorganisms are grown in some form of growth media or these may be cultivation independent methods, whereby DNA is extracted from the sample and the microorganisms are identified by molecular markers. Standard culture techniques have a limited ability to adequately describe microbial communities. Culture techniques are typically time consuming, and cumbersome, requiring a battery of individual biochemical and nutritional tests to characterize each isolate, which may require excessive incubation time for adequate growth. Most soil microorganisms are not easily grown in the laboratory, if they can be grown at all. Consequently, culture techniques grossly underestimate diversity, only describing approximately 0.3% of a community (Amann et al., 1995). For cultivation methods the media that is used can either be in solid or liquid form, and either a rich media or a defined minimal media. The cultivation conditions can also be varied depending on the environmental requirements to replicate as closely as possible the original environment from where the microorganisms were isolated (Dedysh et al., 2006; Elshahed et al., 2004; Hopkins et al., 2001; Ladapo and Barlaz, 1997; Nottingham and Hungate, 1968; Spieck et al., 2006; Wise et al., 1999). While microorganisms have been traditionally isolated and characterized in this manner, there are some drawbacks to these plating and cultivation methods. Many traditional cultivation methods are biased in the microorganisms they select for and some of these viable but non-culturable microorganisms could be cultivated under laboratory conditions by changing the culture media to more accurately reflect their environment. Zengler et al. (2002) used a gel micro-droplet technology to create a high through put cultivation system to allow a greater number of microorganisms to be isolated and characterized from the environment.

**Culture-Independent Techniques**

Since the cultivation methods require that the microorganisms be culturable in growth media, and these methods miss such a large proportion of the microbial communities, cultivation independent methods have been developed to bypass the need for growing the microorganisms in the lab. These methods rely on the use of total community DNA isolated from the environmental sample, followed by amplification with specific genes of interest targeted by polymerase chain reaction. These amplified gene products are then analyzed through various fingerprinting and sequencing methods and identified (Table 1). These identified gene products can then be used to shed some light on the source organisms. Collection of these gene products from the environmental sample, a profile of the microbial community can be developed indicating what microorganisms are present, which can be used to develop phylogenetic relationships between the sequences. In order to analyze the phylogenetic relationship, gene products need to be chosen that are common among the members of the microbial community in question. Ribosomal genes are the preferred gene of choice for microbial community analysis. The ribosomal DNA genes code for ribosomal RNA (rRNA) which provides the structural backbone for both the 30S and the 50S