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Abstract. Microorganisms live in all parts of the biosphere and are critical to nutrient recycling in ecosystems. In recent years, the development of methodologies for the analysis of microorganisms and microbial ecology, at the molecular level, has progressed phenomenally. This review introduces and compares the various molecular methods for studying microbial diversity in soil environments, and the advantages and disadvantages of current methods are proposed as well.

Keywords: Microbial diversity; SIP; T-RFLP; RAPD; DGGE; Soil

1 Introduction

Microorganisms are ubiquitous in the environment and fulfill a range of important ecological functions, particularly those associated with nutrients cycling processes and maintenance of ecosystem health in soil[1]. Soil contain an estimated 109 prokaryotes and more than 2000 genome types per gram of soil, with an average genome type representing less than 0.05% of the soil community[2-3].

Until a few decades ago, soil microorganisms could only be studied by microscopic observation or culture-dependent methods. In less than a decade, using nucleic acid probe technique for the detection of microorganisms had exploded. Nowadays, molecular ecology techniques based on sequence comparisons of nucleic acids can be used to provide molecular characterization while providing a classification scheme that predicts natural evolutionary relationships[4-5].

These laboratory-based works have been spectacularly successful in revealing details of soil microbial diversity. So we attempt to draw together some of these studies, with emphasis on the advantages and disadvantages of current molecular methods, for studying microbial diversity in soil environments.

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2 Stable Isotope Probing

Stable isotope probing (SIP) has been coupled with nucleic acid methods to provide a culture-independent method of linking the identity of bacteria with their function in the environment[6-7]. Soil is either incubated after adding a ^{13}C-labeled substrate or a plant is labeled with ^{13}C-CO_2. Soil DNA or RNA is then extracted and centrifuged in a density gradient to separate ^{13}C-labeled nucleic acids from those containing. Then separated, labeled DNA can be amplified using PCR. Analysis of the PCR products, through cloning and sequencing for example, allows the microbes that have assimilated the labeled substrate to be identified[8-9]. SIP-based approaches hold great potential for linking microbial identity with function, but at present a high degree of labeling is necessary to be able to separate labeled from unlabeled marker molecules.

3 Terminal-Restriction Fragment Length Polymorphism

Terminal-restriction fragment length polymorphism (T-RFLP) has multiple advantages for its rapid gain in popularity: data are quantitative and comparable between laboratories, the final electrophoresis step can be performed with automated sequencing equipment at core sequencing facilities[10]. This method provides a picture of the community including incorporates diversity and phylogenetic details. The profiles can be generated by using the procedure from physical-capture[11], fluorescence scanner[12] or ^{32}P-labelled primers[13]. Because of its relative simplicity, T-RFLP has been applied to the analysis of soil microbial diversity, for instance, fungal ribosomal genes[14-15], bacterial 16S rRNA genes[16-17], and archaeal 16S rRNA genes[18]. In addition, T-RFLP has been used for the analysis of functional genes such as those encoding for nitrogen fixation and methane oxidation[19-20].

4 Fluorescence in Situ Hybridization

Fluorescence in situ hybridization (FISH) is a relatively new technique utilizing fluorescently labeled DNA probe to detect genes of microorganisms in soil samples. Apart from allowing direct visualization of bacteria in the environment, FISH also has the added advantage of being able to detect active cells by targeting rRNA. This method has been reliably used for identification and quantification of ammonia-oxidizing bacteria[21-22]. Several studies have used FISH coupled with microautoradiography (FISH-MAR), this combined approach allows in situ identification and provides information on substrate utilization in complex microbial communities[23-24].

5 Microarray

Microarray for microbial community analysis has been classified into three main categories depending on the combination of probe types and target molecules exploited: community genome arrays, rRNA-based oligonucleotide microarrays and functional gene arrays[25-26]. Tiquia constructed a 50-mer oligonucleotide microarray using 763 genes involved in nitrogen cycling[27]. The increased use of cultivation-independent metagenomic approaches employing large-insert cloning could lead to an important extension of the CGA approach for large genomic fragments from uncultivated microorganisms[28-29]. In the meantime, adapting enzymatic signal amplification approaches for microarray analysis[30-31], the use of high sensitivity microarray hybridization detection devices hold much promise for enhancing the sensitivity of direct detection of extracted rRNA[32-34].

6 Random Amplified Polymorphic DNA

Random amplified polymorphic DNA (RAPD) use DNA products by PCR which are based on random priming of the target DNA, these primers are usually 10 bases in length and are designed to a number of random target sites[35]. Huai studied the genetic variation and spatial distribution of the ectomycorrhizal fungus, the 33 sporophores studied belonged to distinct genotypes[36]. The advantage of this approach is that it requires very little sample material and obtains results rapidly. However, this method is less susceptible to base changes in the target DNA. Thus usually the PCR is performed at low stringency for the first few cycles. This technique allows the generation of product when mismatches between template and primers occur. Hence, similar patterns generated using this technique better reflect phylogeny of the phages. One disadvantage of this technology is the results may be difficult to repeat by other users[37-38].

7 DNA Fingerprinting

DNA fingerprinting is used to distinguish differences in the genetic makeup of microbial populations from different samples. The advantage of this technique is that it enable high sample throughput and can be used to target sequences that are phylogenetically or functionally significant. Nowadays, the most common used techniques are denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE). They have been used to profile fungal microbial communities from many diverse environments[39-40]. DGGE fingerprints of total DNA from rhizosphere revealed that there was a relationship between fungal community composition and rhizosphere development[41]. Fungal community diversity was studied by PCR-DGGE followed by sequence analyses of ITS fragments in soil samples[42]. Although DGGE is a promising technique, it can still underestimate fungal diversity. The number of bands depends on the resolution of the gels, this takes

time to optimize and is difficult to reproduce[43]. This has already been demonstrated in previous studies that phylogenetically distant taxa can have comigrating bands and that one band does not mean one unique phylotype[44-45].

A new DNA-based fingerprinting approach, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), can be used to separate PCR amplicon of the ITS regions[46]. But this technique does not lend itself to high sample throughput. However, its improved ability to discriminate between soil communities and retrieve sequence information make it a powerful technique for elucidating key differences in community structure.

8 Conclusions and Future Directions

Soil has been dubbed “The Final Frontier”. Current knowledge pertaining to the diversity and distribution of soil ecosystem is still rudimentary. In recent years, obviously improvement in traditional approaches combined with various molecular techniques, such as molecular and phylogenetic inventorying via clone libraries[47], quantitative real-time PCR[48], pyrosequencing[49], quantitative membrane hybridization[50] has provided new data on these aspects. Nonetheless, we feel that these sophisticated methods are highly relevant to the existing knowledge of the role of microorganism in ecosystem processes, but the application of the techniques to this end is far from complete.

From recent molecular ecological studies, we have seen that most carbon- and nitrogen- cycling gene sequences are divergent from those of the model organisms on which most of our existing appreciation of biogeochemical cycles are based. However, our understanding of ecologically relevant microorganism involved in these cycles is limited. Hence we are aware that further understanding of how they fit into the complexities of ecosystems will require both bottom-up and top-down approaches.

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