Research Progress of Molecular Biology Technology in Soil Microbial Ecology

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Abstract

Microorganisms play a very important role in the ecosystem and the diversity of its structure and function reflects the basic conditions of the ecosystem. The traditional methods of microbial culture and identification are very one-sided, does not fully represent the true condition of microorganisms in an ecosystem. The molecular biological techniques developed in recent decades have broken through the limitations of traditional methods and played a great role in the development of microbiology and Ecology, in this paper, the research status of several common molecular biological methods in microbial field is introduced.

Keywords

Microbiology; ecosystem; Molecular biology; Research status.

1. Introduction

Soil microorganism is a general term for all the microscopic organisms in soil that are invisible to the naked eye, strictly including bacteria, archaea, fungi, viruses, protozoa and microscopic algae. Individuals are tiny, usually measured in micrometers or nanometers, and there are usually hundreds of millions to ten billion of them in a gram of soil, varying in variety and number according to the soil forming environment and its depth. They can oxidize, nitrify, ammonify, fix nitrogen and sulfurize in the soil, and promote the decomposition of soil organic matter and the transformation of nutrients[1].

In the past, the traditional microcounting method, which was developed in 1990 by Martilainen et AL, involved directly observing and counting a small number of soil samples under a microscope by adding water to make a suspension and placing it on a specific type of glass slide with a defined volume, the number, volume and specific gravity of microorganisms in the observed area are usually calculated by 1.18 GAM Or 259 / and 47% of the carbon content of the dry matter are usually used for the dry matter content of the microorganism, which is further converted into the carbon content of the microorganism in the soil per gram. This method is cheap and fast, and it can provide information of living and different living species, especially in water environment. Some special target species with certain function have been separated by this method, and many valuable microbial species have been obtained. This method is simple to operate, but because this method is artificially limited, some culture conditions, can not fully simulate the natural conditions of microbial growth, often resulting in selective enrichment of some microorganisms, and can not obtain, other microorganisms, resulting in the vast majority of microorganisms in nature still can not be known through traditional microbial culture methods. Therefore, the application of traditional research methods to reflect the microbial information is less, a large number of valuable microbial information can not be obtained. Therefore, the traditional culture method can only be used as a supplementary means, often in conjunction with other advanced methods, so as to reflect more objective and comprehensive information about the environment, sample microbial community composition and structure[2].

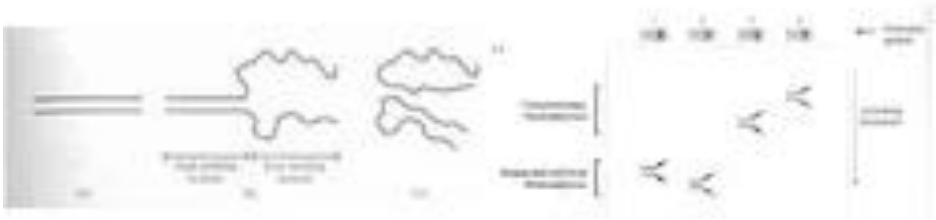
2. Several common methods of molecular biology

These methods include denaturing Gradient Gel electrophoresis and denaturing gradient Gel electrophoresis, terminal restriction fragment length polymorphism analysis, cloning library, random amplified DNA polymorphism analysis, Single-strand conformation polymorphism analysis, Fluorescence in situ hybridization（FISH）, gene chip（Microarry）, stable isotope probe（SIP）, metagenomics, real time fluorescent quantitative PCR, transcriptional genomics, etc.It provides an important means to reveal the microbial population structure and genetic diversity in soil.

2.1 Denaturing Gradient Gel Electrophoresis

Denaturing Gradient Gel Electrophoresis (DGGE) was first proposed by Fischer et AL in 1979 to detect DNA mutations. DGGE has higher resolution than Agarose, electrophoresis and polyacrylamide Gel Electrophoresis, and is a common single-base mutation screening method[3-4]. In 1993, Muzyer began using the technique to study the genetic diversity of microbes, at present, the technique has been widely used in soil, sludge, water, food, human intestinal flora and other samples of microbial diversity analysis, microbial identification and variation, and population succession, etc[5-7]. It has some advantages in revealing the genetic diversity and population difference of natural microbial communities.

In general polyacrylamide Gel Electrophoresis, the migration behavior of double-stranded DNA molecules depends on their molecular size and charge. DNA segments of different lengths can be distinguished, but DNA segments of the same length move through the gel the same way, so they can not be distinguished. DGGE / TGGE technology, based on the common polyacrylamide Gels, adds a gradient of denaturants (urea and formamide) to differentiate DNA fragments of the same length but with different sequences[9-11]. A particular DNA fragment has its own sequence composition, which determines the melting domain, MD, and melting behavior. A DNA fragment with hundreds of base pairs typically has several unlinked regions, each of which has a continuous base pair composition. When the denaturant concentration gradually increases to the lowest concentration of the unlinked region, the continuous base pair in the region will be unlinked. When the concentration increases to the concentration of other unchained regions, the unchained regions will occur in turn[11-14]. Until the denaturant concentration reaches the highest concentration of the unlinked region, the highest unlinked region also occurs, thus double-stranded DNA complete unlinked.



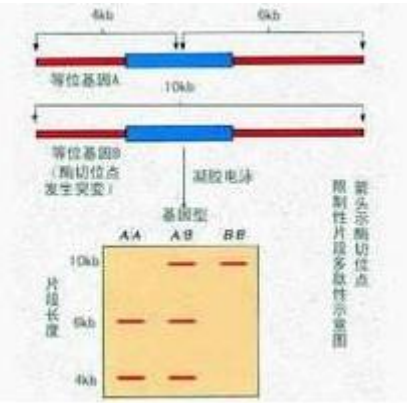
Because the sequence composition of different double-stranded DNA fragments is different, the concentration of the unlinked region and the unlinked region are different. When they were applied to Dgge, the denaturant concentration at the beginning was too small to unchain the lowest unchain region of the double-stranded DNA fragment, and the Migration Behavior of the DNA fragment was the same as that in the polyacrylamide Gel[15-22]. However, when the denaturant concentration of the DNA segment is just enough to release the least unlinked region of the double-stranded DNA segment, the unlinked region of the double-stranded DNA segment will release immediately. The rate of migration of fragments of partially broken-down DNA in the GEL is dramatically reduced. As a result, DNA fragments of the same length but with different sequences will reach the lowest concentration at different locations in the GEL, so they will be partially broken down at different locations in the

Gel and the migration rate will be greatly reduced, to be distinguished from each other in the glue. However, once the denaturant concentration reaches the highest temperature at which the DNA fragments can be completely unlinked, they become single-stranded DNA molecules, and they can then continue to migrate in the GEL. Thus, if the sequence differences between the different DNA segments occur in the highest unlinked region, the segments can not be distinguished. Adding a piece of DNA rich in GC at one end (a GC clip, typically 30-50 base pairs) solves the problem. The highest unchain region of DNA fragments containing GC clips is located in the sequence of GC clips. The unchain concentration of GC clips is high, which can prevent DNA fragments from completely unchain in DGGE Gel. When a GC clip is added, almost every base sequence difference in the DNA fragment can be distinguished[23-25].

DGGE / TGGE has been widely used to analyze the biodiversity of bacteria, cyanobacteria, archaea, eukaryotes, eukaryotes and viruses in natural environment. This technique can provide information on dominant species in a community and analyze multiple samples at the same time. It has the characteristics of repeatability and simple operation, and is suitable for investigating the spatio-temporal change of the population, the community composition can be identified by sequence analysis of the bands or hybridization with specific probes. DGGE and TGGE can separate DNA fragments with the same length but different base by increasing the linear concentration gradient and linear temperature gradient of chemical denaturant respectively[25]. The double strands of DNA are separated at a temperature that depends on the hydrogen bond content of the complementary strands (higher melting temperatures in regions rich in GC) and the gravitational pull of neighboring bases[26].

2.2 Terminal Restriction Fragment Length Polymorphism

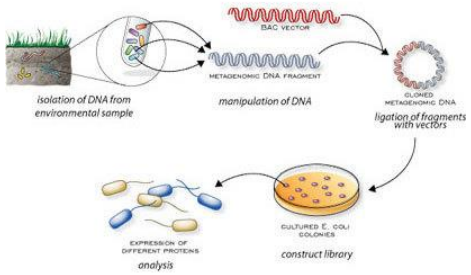
Restriction fragment length polymorphism (RFLP) involves the design of appropriate primers to allow the amplified fragment to contain one or more polymorphic Restriction enzyme sequences, which are then used to cut the PCR product, according to Amp-FLP versus VNTR[27-29]. The repeat sequences such as STR are highly polymorphic due to the different number of repeat units. Therefore, the amplified fragments were amplified by PCR with specific primers on both sides of the repeat sequence. The amplified fragments were highly polymorphic and could be separated by PAGE. The RFLP technique works by measuring the size of specific DNA fragments that form when DNA is digested by Restriction enzyme enzymes. Therefore, RFLP can be induced by any mutation which can cause the variation of the restriction site, such as the new generation of the point mutation and the removal of the restriction site, and the re-organization of a piece of DNA, such as the change of the length between the restriction sites caused by insertion and deletion[30-33].



2.3 Metagenomic Approaches

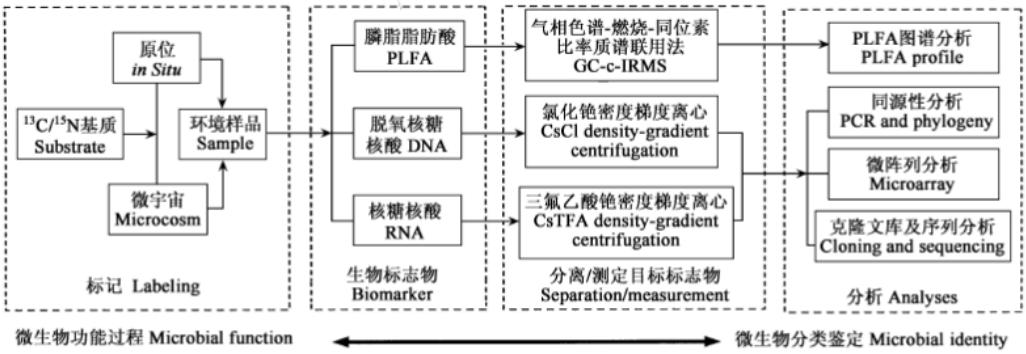
In 1998, Handelman and others first proposed that metagenomics, also called metagenomics, environmental genomics or community genomics, refers to the summation of different microbial genomes in a community.Metagenomics technology is a kind of microbial research method which does not need microbial culture and can be used to analyze and study microbial samples directly from environment. The process involves extracting DNA from the sample, sequencing it, and then analyzing the results using computer software. This approach solves at least two very important

problems[34]. First, it allows us to analyze and study 99 percent of the microbes in nature — those that still don't make it to the lab. Second, it's possible to study microbes in the whole environment[35]. Because the DNA comes from different species, finding the genome sequence of each species from metagenomic data is a very complex task. Until recently, scientists have been able to sequence the genomes of microbes from environmental samples that are rich in genotypes but relatively simple[36].



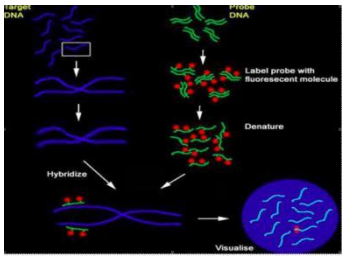
2.4 Stable Isotope Probing

Stable isotope is a powerful tool to study the impacts of global climate change and forest management on soil carbon, nitrogen and nitrogen dynamics, and is an important tool to deepen the understanding of carbon, nitrogen and nitrogen dynamics in terrestrial ecosystems. An important tool for understanding n-cycle process. The application of stable isotope in soil biology has led to significant advances in the understanding of soil microbial processes that regulate the C and n cycles in terrestrial ecosystems. With the development of stable isotope technology, and the combination of molecular biology technology, a new stable isotope detection technology has been formed, which can well relate the microbial community to its function. Recent studies have identified-groups of microorganisms that are actively involved in specific metabolic processes. The stable isotope is a collection of technologies, it includes the selection of stable isotope, the selection of suitable biomarkers, the labeling of environmental samples, the extraction, separation and purification of labeled biomarkers, etc[37-41]. The basic principle and technical route are: Exposing Environmental Samples of the in situ or microcosmic Mirocomsm to a matrix enriched with stable isotope, some of the microorganisms present in these samples were able to metabolize and satisfy their own growth needs using stable isotope as carbon or nitrogen sources in the Matrix, and stable isotope in the matrix were absorbed and assimilated into the microorganisms, it is involved in the synthesis of certain substances such as nucleic acid, DNA, RNA and Phospholipid-derived fatty acids. By extracting, isolating, purifying and analyzing stable isotope biomarkers in these microorganisms, in order to relate the composition of the microbe to its function. In this way, microorganisms in the environment can be combined with their functions without conventional cultivation, in order to deepen the understanding of the functions of microorganisms in different environments and the specific Biogeochemistry processes in which they participate. In this paper, the application, advantages, disadvantages and development trends of stable isotope detection technology and its application in the field of environmental Microbial ecology are described.



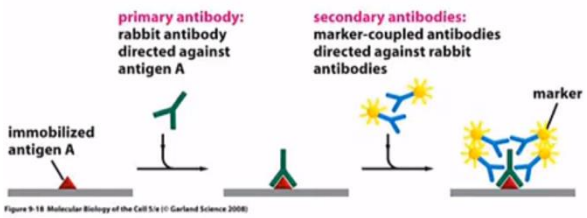
2.5 Fluorescence In Situ Hybridization

Fluorescence in situ hybridization hybridizes a specific fluorescent labeled nucleic acid probe with the corresponding target DNA or RNA molecule in the cell, and then uses Fluorescence microscope or confocal laser scanners to observe the fluorescence signal, the positions of DNA or RNA molecules in chromosomes or other Organelles were determined, and the morphology and distribution of stained cells or Organelles could be observed after hybridization with specific probes. This technique can be used to study the characteristics of microbial community structure and to track the dynamic changes of microbial population in real time. For example, FISH technology has been used to monitor the dynamic changes of microbial communities in Rivers, and some new microbial information and species that are difficult to cultivate under artificial conditions have been obtained[42-43]. Clsm-fish was used to study the distribution of acetic acid oxidizing bacteria, desulfurizing bacteria, methanogenic bacteria and sulfate reducing bacteria in the biofilm of anaerobic reactor. Of course, there are some problems with FISH technology, such as false positive or false negative, many microorganisms have their own fluorescence, such as mold, yeast, Pseudomonas, Legionella, cyanobacteria and archaea, etc. , this can lead to false positives; the accuracy and reliability of FISH are dependent on the specificity of the Oligonucleotide probe, so the design and evaluation of the probe is important if the design lacks specificity, can also lead to false positives. Similarly, false negatives can sometimes occur, mainly because the structure of the Cell Wall affects the penetration of the probe, which may lead to a reduction in the intensity of the hybridization signal, especially the Gram-positive bacteria, in order to increase the penetration of the probe, special immobilization and pretreatment are required, and Gram-negative is relatively permeable, allowing even polynucleotide probes to penetrate well into cells. In addition, the three-dimensional structure of rRNA and its content in cells also affect the accuracy of probe hybridization.



2.6 Quantitative Real-time PCR

Quantitative Real-time PCR is a method of measuring the total amount of products after each polymerase chain reaction cycle using fluorescent chemicals in DNA amplification reactions. Method of Quantitative analysis specific DNA sequences in a sample to be tested by internal or external reference methods. Real-time PCR is the real-time detection of PCR process by fluorescence signal during PCR amplification. Because of the linear relationship between the CT value of the template and the initial copy number of the template during the exponential period of PCR amplification, it becomes the quantitative basis.



Quantitative Real-time PCR is the most sensitive and accurate method to determine the number of DNA or cDNA copies in samples. If used for RNA detection, this is known as Real-time rt-PCR, or Real-time PCR, which is the amplification of DNA or RNA by polymerase chain reaction and Real-time monitoring of DNA, the amplification products were measured during the exponential growth phase of amplification, because there was a correlation between the exponential growth phase measurements and the specific DNA (RNA) starting quantity, thus enabling quantitative detection.

The basic goal of RealTime PCR is to precisely measure and identify very small amounts of specific nucleic acids, so that the content of original target genes can be quantified by monitoring CT values. The greatest advantage of real-time fluorescent quantitative PCR is to overcome the large error of end-point PCR in Plateau or saturation period, and to realize the accurate quantification of DNA / RNA. This technique not only realizes the quantification of DNA / RNA template, but also has the characteristics of high sensitivity and specificity, multi-reaction, high automation, no pollution, real-time and accuracy, this technique is of great significance in clinical examination and research.

3.Expectation

In this paper, various experimental techniques of molecular biology for the study of soil microbial diversity are introduced. Based on the analysis of the microbial community structure in the past, the community structure based on functional genes will be analyzed in the future, from the traditional microbial culture method to the modern molecular biology experimental techniques, in particular, it is necessary to study the regulation of functional genes expression in the natural environment in order to understand the real state of microorganisms in the environment. With the development of various new technologies, especially the development of molecular biology, we can study soil microbial diversity and explore the unknown microorganisms in soil. We will gradually learn about the diversity of microbial communities in the natural ecological environment and their actual living conditions, and then we can adjust the types and quantities of soil microorganisms through various agricultural measures such as fertilization, artificially introducing some beneficial microorganisms, inhibiting the development of harmful pathogenic bacteria, improving soil ecological fertility, enhancing the supply of resources and the ability of waste absorption and purification of the soil ecosystem, and promoting the virtuous cycle of the whole terrestrial ecosystem, improve the living environment of human beings and realize the harmony and sustainable development between human beings and nature. However, the new experimental methods have their own characteristics and advantages, as well as their limitations. Therefore, most scholars believe that the data obtained from any experimental technique is a supplement to other methods. In order to study the characteristics of soil microecology deeply, a variety of experimental methods should be adopted. With the further improvement of various experimental techniques and methodologies, we have seen more and more mysteries of soil ecological microcosm. At the same time, it also provides a broader research space for soil microbial diversity.

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