The Application of Modern Molecular Biological Techniques in the Research of Microbial Community Structure and Diversity

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Abstract. The development and the prevalence of modern molecular biological techniques have provided revolutionary progress in microbial community studies. These molecular methods have made significant contributions to the research of microorganisms that cannot be detected by traditional culture-based methods. This paper summarized the development of molecular biological techniques applied in the research of microbial community diversity, then highlighted the principals and characteristics of denaturing gradient gel electrophoresis (DGGE), clone library analysis and high-throughput sequencing technique. Each method has its advantages and limitations, it’s important for researchers to select suitable methods in studies.

1. Introduction

With the rapid development of industry and the continuous improvement of living standards since the 1980s, a range of new environmental problems such as global warming, water shortage and ecosystem degradation have become increasingly prominent. The earth, the only home of human beings suffered widely polluted. People are increasingly aware of the severity and urgency to resolve problems of environmental pollution that promote the rapid development of pollution ecology. However, microorganisms play major roles in the pollution control and management [1]. Environmental microorganisms consist of various populations which affect each other, the relationships between different species are competition, predation and mutualism. Communities of microorganisms play a vital role in material recycling and energy transformation.

The research on microbial community structure has become a hot spot in the fields of environmental science and microbial ecology in recent years. The microbial community structure can be analyzed from different aspects of bacterial abundance, microbial metabolic activity and function, community structure and diversity. However, the community structure and diversity of environmental microorganisms are the core research [2]. The microbial
community structure, abundance and the characteristics of population distribution can be investigated through the research on the diversity of microorganisms in environment. The mechanisms and effects of bioremediation and technique and biological treatment technology can be analyzed and evaluated at community level [3]. The research on microbial structure will offer both theoretical significance and practical interest in the adjustment and optimization of microbial community structure as well as in finding the new microbial function species.

2. The Development of Microbial Diversity Research Techniques

The development of research techniques in bacterial community structure and diversity can be divided into following three stages: (1) Traditional isolation and culture methods were employed for the analysis of natural communities before 1970s, including microbial morphology observation method, pure strain separation, physiological and biochemical methods and selective culture counting [4]. In the nearly hundreds years, traditional isolation and culture methods promoted the development of microbiology. However, there were many shortcomings in traditional methods as the base for the development of microbiology. Cultured microorganisms only account for about 1 to 10 percent of the microbial world. Traditional methods severely hindered people’s objective understanding of the microbial communities and caused the selective and insufficient recognition of the microbial structure and diversity [5]. In addition, samples obtained from traditional methods were unrepresentative of time and space; (2) The researchers adopted biomarker methodology to classify and identify microorganisms according to their differences in chemical structure in the 1970s and 1980s [6]. It reduced artificial error and broke through a new approach to conduct quantitative research on species; (3) The modern molecular biotechnology used DNA as a target in the 1980s and 1990s, revealing microbial community succession, dynamic population changes, gene mapping and expression rapidly and accurately through 16S rDNA gene-sequencing technology and DGGE fingerprint technique [7,8]. With the development of molecular biology techniques, the next-generation high-throughput sequencing techniques developed rapidly and provided technical support for molecular techniques.

3. Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis was first proposed by Fischer and Lerman in 1979, and was used in the field of medicine [9]. In 1993, for the first time, the PCR-DGGE technique was applied to the research of microbial community structure by Muyzer. As a modern molecular technology, PCR-DGGE is a powerful tool for the analysis of diversity and dynamics of microbial community, which overcomes the limitations of traditional isolation and culture methods. In the process of DGGE experiment, double-stranded DNA with the same length but different base sequences need different denaturant concentrations to melt. The DNA fragment is denatured at its corresponding denaturant concentration, and the physical shape of the molecules is altered at the same time. Then electrophoresis rate decreases greatly, DNA strands with different base sequences can stay on different position in the gel due to the different electrophoretic mobility [10]. The separated bands appear on the gel after dyeing. Each separated band from the denaturing gel can represent a kind of microbe in theory, the number of bands reflect microbial diversity, and the staining intensity of bands indicate the abundance of microbial community.
PCR-DGGE is widely used in study of activated sludge, soil, biomembrane and sediment microbial diversity. The advantages of this method are its affordability for ordinary laboratories and significance on dynamic monitoring of community structure. However, PCR-DGGE is limited to the length of target DNA fragment, sequencing above 500bp might not be detected.

4. Clone Library Analysis

Sequencing of 16S rDNA clone libraries is commonly used in the study of prokaryotic communities in molecular microbial ecology. The clone library technique was first applied in research of diversity of microplankton Sargasso on the sea by Giovannoni in 1990 [11]. 16S rDNA clone libraries investigate microbial community and diversity from environmental samples by amplification and analysis of the full-length sequence 16S rDNA. Total DNA is extracted from the sample, 16S rRNA gene fragments are amplified using specific primers. And then the amplified products are cloned into plasmid vector. The obtained high-quality sequences are classified by constructing clone library, and compared to 16S rRNA gene sequences in GenBank, then assigned to phylum, class, order, family, genus or species [12]. Compared with PCR-DGGE, clone library has shortcoming of high cost, complex operation and time-consuming. However, clone library can provide more detailed analysis of genetic diversity, species richness, population structure and taxonomic and homologous evolution information of microorganisms. In addition, clone library can be used to design optimized method for FISH probes and PCR primers. It has the incomparable advantages in finding undiscovered species compared with traditional methods.

5. High-throughput Sequencing Technique

In the past decade, the first-generation sequencing, represented by Sanger sequencing, had been progressed to the new generation high-throughput sequencing. The sequencing platforms including 454 FLX (Roche), HiSeq/MiSeq (Illimina) and SOLiD (Applied Biosystems) possess the abilities to read millions of DNA fragments and analysis hundreds of different samples at the same time with high-efficiency and low-cost [13]. IlliminaHiSeq and MiSeq platforms have been commonly used to investigate the microbial community structures. Compared with clone library technique, high-throughput sequencing method don’t need to construct a clone library using E.coli and avoid introducing errors during the process of sub-clone. In the sequencing platforms, it can read hundreds of thousands to millions of sequences in one analysis, causing the sequencing cost to reduce greatly [14]. It overcomes the inherent limitations of conventional molecular biological techniques and provides high sequencing depth to investigate low-abundance microorganisms. As a promising method, it has been successfully applied to analyze microbial diversity and compositions in wastewater treatment plant (WWTP), marine water, sediment and pathogenic microorganisms.

6. Conclusions

Molecular biology techniques have advantages in accuracy and sensitivity and make it possible to research the microbial diversity in culture-independent methods. The modern molecular technologies have expanded the horizon of species diversity in gene level, and provided a favorable tool for studying the diversity and dynamic changes of microbial
communities. The principals and characteristics of each molecular technology are different and each of them possesses unique advantages. Researchers have to adopt multi combined method for their study, improving the accuracy and sensitivity of the detection. The culture-independent molecular technologies have become more realistic and accurate for people to study microbial ecology in environmental samples.

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**References**


