Genetic fingerprinting of plankton community provides new insights into aquatic ecology*

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Abstract Over the past two decades, molecular techniques have been widely used in ecological study and molecular ecology has been one of the most important branches of ecology. Meanwhile, genetic fingerprinting analyses have significantly enhanced our knowledge of the diversity and evolutionary relations of the planktonic organisms. Compared with conventional approaches in ecological study (e.g., morphological classification), genetic fingerprinting techniques are simpler and much more effective. This review provides an overview of the principles, advantages and limitations of the commonly used DNA fingerprinting techniques in plankton research. The aim of this overview is to assess where we have been, where we are now and what the future holds for solving aquatic ecological problems with molecular-level information.

Keywords: genetic fingerprinting, plankton community, aquatic ecology, genetic diversity.

Plankton, composed of different functional groups, is the basis of the entire aquatic food chain. It also gives off oxygen and absorbs carbon dioxide from the earth’s atmosphere. Additionally, plankton exhibits complicated and sensitive responses to environmental variability through changes at the individual, population, and community levels of organization. These processes have important consequences for the dynamics of aquatic systems and could be used as valuable indicators of the environmental conditions. So, plankton research as a whole plays a key role in aquatic ecological study. Traditional methods for plankton research are mainly based on morphological identification. But the absence of conspicuous morphological features for multitudes of minute planktonic organisms and the requiring of sophisticated expertise in taxonomy make morphological classification onerous[1-3].

Molecular approaches to investigate the genetic information in the extracted DNA of environmental communities are called “community DNA analysis”[4]. Over the past 20 years, various genetic fingerprinting techniques have been developed to describe microbial communities and study their ecological roles[3,5,6]. Compared with conventional approaches, the fingerprinting techniques are much more magnetic, as they are simpler and much more effective for ecological study. Estrada et al.[7] found that diversity of planktonic organisms described by morphological identification and genetic fingerprinting showed a good agreement. Furthermore, Savin et al.[8] suggested that molecular techniques could reveal many unidentified but possibly ubiquitous planktonic organisms. So, our understanding of the structure and function of eukaryotic communities will continually be improved with the application of molecular methods. Yu and co-workers[8] applied DNA fingerprinting to the whole plankton community. Most recently, Yan et al.[9,10] studied the relationships between DNA polymorphism of plankton community and species composition and physico-chemical factors. These studies together with others have shed a new light on fingerprinting analysis of the plankton community.

This review presents the current status of the application of genetic fingerprinting techniques in plankton research and gives an outlook into the future. The aim of this review is to highlight the power of genetic
fingerprinting techniques for the study of aquatic ecology.

1 Genetic fingerprinting techniques commonly used in plankton research

Genetic fingerprinting involves the display of a set of DNA fragments from a specific DNA sample based upon the physical separation\footnote{[11]}. A variety of DNA fingerprinting techniques are available, and these molecular techniques have been used in diverse environments (e.g., ocean, lake, reservoir, river, artificial niche; water, soil, etc.) and numerous aspects of ecological study (e.g., biodiversity, productivity, competition, food web structure, population biology, biogeography, gene flow, biogeochemical cycles, symbiosis and adaptation\footnote{[12]}). The choice of the fingerprinting technique to be used is dependent of the application and the organisms under investigation. Here we will present the most commonly used genetic fingerprinting approaches in plankton research (Fig. 1) and will discuss the advantages and limitations of these methods.

![Flow diagram showing the molecular approaches commonly used in plankton research. RT-PCR, reverse transcription polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; TGGE, temperature gradient gel electrophoresis; RAPD, random amplified polymorphic DNA; SSCP, single-strand conformation polymorphism; AFLP, amplified fragment length polymorphism; RISA, ribosomal inorganic spacer analysis; T-RFLP, terminal-restriction fragment length polymorphism; ARDRA, amplified ribosomal DNA restriction analysis.](image)

1.1 Denaturing gradient gel electrophoresis and temperature gradient gel electrophoresis

In 1993, Muyzer et al.\footnote{[13]} introduced the denaturing gradient gel electrophoresis (DGGE) into microbial ecology. They showed that it was possible to identify constituents which represent only 1% of the total population. This technique greatly enhanced our understanding of the genetic diversity of uncharacter-
ized populations. Within a short period this method has become very popular in microbial ecological studies, and it is now routinely used to assess the diversity and dynamic of communities. DGGE as well as the related technique called temperature gradient gel electrophoresis (TGGE) can separate DNA fragments of the same length but with different sequence by polyacrylamide electrophoresis through a linear gradient of chemical denaturants of urea and formamide, or through a linear temperature gradient. DGGE and TGGE approaches have been used in a huge number of studies about the planktonic organisms, especially the bacterioplankton and picoplankton. A big advantage of DGGE/TGGE is that they make it possible to obtain taxonomic information by excising, re-amplifying and sequencing of specific DNA fragments or by hybridization analysis with taxon-specific oligonucleotides probes\(^\text{[14]}\). With the help of these approaches an extraordinary diversity of previously undetected planktonic lineages have been revealed\(^\text{[21]}\). However, almost all of these investigations focused on the small planktonic organisms. Until recently, Savin et al.\(^\text{[11]}\) started applying the DGGE to study the diversity among communities of large plankton.

DGGE and TGGE also have their own limitations, for instance, only short DNA fragments (up to 500 bp) can be optimally separated\(^\text{[15]}\). However, by using group-specific primers, primers for functional genes, or fluorescent dye labeled primers, DGGE/TGGE could provide valuable information for understanding of community structure and function.

1.2 Randomly amplified polymorphic DNA

Randomly amplified polymorphic DNA (RAPD), also known as arbitrarily primed PCR, allows the detection of polymorphisms without prior knowledge of the nucleotide sequences. The polymorphism may be used as genetic markers and may also be used for construction of genetic maps. Additionally, RAPD is also a technique ideally suited to fingerprinting applications because it is fast, requires little material and is technically easy. Xia et al.\(^\text{[16]}\) used RAPD to follow the response of soil microbial communities to the application of 2, 4-dichlorophenoxyacetic acid (2, 4-D). Regarding the plankton community as a whole, Yu et al.\(^\text{[8]}\) explored the feasibility of RAPD fingerprinting on community life system, and Yan et al.\(^\text{[17]}\) investigated the relationship between DNA polymorphism of the plankton community and eutrophication. Most recently, they realized that the DNA polymorphism of the plankton community is closely related to the species composition\(^\text{[10,18]}\) and physico-chemical factors\(^\text{[10]}\).

The main disadvantage of RAPD is the concern of its reproducibility, which may be affected by the quality and quantity of template DNA or the concentration of Mg\(^{2+}\) and primer used. However, comparatively steady banding patterns can be obtained by optimization of RAPD conditions (e.g. PCR cycle parameters, primer screening, concentrations of DNA template, Mg\(^{2+}\), dNTP, Taq DNA polymerase, primer, etc.)\(^\text{[19]}\).

1.3 Single strand conformation polymorphism

Another fingerprinting technique used in plankton research is single strand conformation polymorphism (SSCP). Separation in this method is based on difference in folded conformation of single-stranded DNA, which influences the electrophoretic mobility in a non-denaturing polyacrylamide gel. Similar to DGGE/TGGE, PCR fragments with the same size but different sequences will be separated into different bands. Bands of the SSCP profiles could also be excised and subsequently sequenced as previously highlighted for DGGE/TGGE analyses, but there have relatively fewer studies using the SSCP method. Lee et al.\(^\text{[20]}\) firstly applied this technique to study the natural bacterial communities in aquatic ecosystem. They demonstrated that SSCP was sensitive enough to detect population that made up less than 1.5% of the community. Servais et al.\(^\text{[21]}\) investigated the cell-specific activity (CSA) and biomass-specific activities (BSA) of high nucleic acid (HNA) and low nucleic acid (LNA) cells from different aquatic ecosystems.

The detection sensitivity of SSCP tends to be decreased as fragment length increases\(^\text{[22]}\), and the optimally separated length is between 150 and 400 bp. The reproducibility problem, which may be influenced by electrophoretic conditions (e.g. temperature), also should be under consideration before using the technique. However, the addition of glycerol to 5%—10% in SSCP gels could improve the sensitivity of the method.

1.4 Amplified fragment length polymorphism

Amplified fragment length polymorphism (AFLP) is based on the selective PCR amplification of restriction fragments from a total digest of genomic
DNA. The technique, developed by Zabeau and Vos \cite{23}, was patented by Keygene NV (Wageningen, The Netherlands) at first. Two years later, Vos et al. \cite{24} published a paper describing the procedure and made it public. The technique involves three steps: (i) restriction of the DNA and ligation of oligonucleotide adapters; (ii) selective amplification of sets of restriction fragments; (iii) gel analysis of the amplified fragments \cite{24}. It is well known that AFLP has combined the advantages of RAPD (e.g. no sequence information necessary, only small amounts of DNA is required) with that of restriction fragment length polymorphism (RFLP) (e.g. high reproducibility). Furthermore, the AFLP is very powerful and reliable for DNAs of any origin or complexity. Franklin et al. \cite{25} applied AFLP to compare the overall diversity, considering richness, evenness, and taxonomic relatedness of community members. John et al. \cite{26} used AFLP for analyzing the population structure of the Alexandrium tamarense species complex. The applications of AFLP are reviewed in Ref. \cite{27}. The major limitation of AFLP is in need of expensive equipments such as automated gene sequencers for electrophoretic analysis of fluorescent labels. Traditional electrophoretic methods can also be employed but radioactive labels or special staining techniques are required.

1.5 RFLP and terminal-restriction fragment length polymorphism

Restriction fragment length polymorphism (RFLP), developed by Grodzicker et al. \cite{28} in 1974, has also been used to characterize microbial communities. The RFLP fingerprinting is simple, straightforward, and no expensive equipments are required. The related technique terminal-restriction fragment length polymorphism (T-RFLP) is based on the restriction digest of double-stranded fluorescently end-labeled PCR fragments. In this method, one primer is labeled at the 5' terminus with a fluorescent dye. The fluorescent signals are converted into electrophoregrams, in which the peaks represent the size of fragments, and the areas under the peaks indicate the relative proportions of fragments. The fluorescently labeled terminal restriction fragments were precisely measured by using an automated DNA sequencer. T-RFLP is a high-throughput, reproducible and sensitive method that can be used to carry out both qualitative and quantitative analyses. T-RFLP was originally developed to identify mycobacteria by Avaniss-Aghajani et al. \cite{29}. Just one year later, Liu et al. \cite{30} employed it to characterize microbial community structure. They distinguished all bacterial strains in a model bacterial community, and the pattern was consistent with the predicted outcome. Matz and Jürgens \cite{31} used this technique to examine the impact of nutrient conditions and grazing by protozoa on the phenotypic community structure of freshwater bacteria in continuous culture systems.

The limitations of T-RFLP include formation of pseudoterminal restriction fragments, which result in the overestimation of the diversity \cite{32}. In addition, sequencing of excised bands for further analysis is not possible.

2 Discussion

The application of genetic fingerprinting techniques in plankton research has significantly enriched our knowledge of the biodiversity and continually renewed our views about the evolution relations of the planktonic life \cite{33,38}. In general, fingerprinting analyses are simple and straightforward; we just need to extract total community DNA from environmental samples and subsequently analyze them with different procedures as shown in Fig. 1. So, the traditional cultivating and taxonomic classification, generally regarded as the major obstacles of conventional ecological approaches, are not required. Since the first shot in genome revolution by RFLP \cite{38}, various genetic fingerprinting techniques have been developed and introduced to natural community study. Recently, they are frequently used in studying community complexity and dynamics, studying niche differentiation, monitoring the enrichment and isolation of bacteria, etc. In a word, the genetic fingerprinting techniques are powerful tools for assessing the diversity of communities and for rapidly comparing the community structure and function of different ecosystems.

It must be emphasized that genetic fingerprinting techniques also have some drawbacks. Apart from the general potential biases (e.g. those produced by nucleic acids extraction and PCR), every method has its own limitations (Table 1). However, numerous publications showed that, over the past two decades, considerable amounts of molecular information about the structure and function of planktonic organisms have been obtained, and many novel planktonic lineages have been identified \cite{33,38,39}, providing new in-
sights into the study of aquatic ecology. The application of molecular methods will continue to enlarge our knowledge about the genetic diversity of communities. Besides, it also could help us to elucidate the molecular mechanisms of some ecological problems.

Table 1. The advantages and limitations of the above mentioned genetic fingerprinting techniques

<table>
<thead>
<tr>
<th>Technique</th>
<th>Developers</th>
<th>Advantages</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>DGGE</td>
<td>Fischer and Lerman, 1976[33]; Rosenbaum and Riesner, 1987[34]</td>
<td>Bands can be excised and subsequently sequenced; possibility of identifying community members; community profiles can be hybridized with specific probes to detect presence of particular populations; reliable, rapid and inexpensive</td>
<td>Only suitable to separate relatively small fragments (up to 500 bp); influence of double bands and heteroduplex molecules</td>
</tr>
<tr>
<td>RAPD</td>
<td>William et al., 1990[35]; Welsh and McClelland, 1990[36]</td>
<td>Prior sequences knowledge does not needed; special primer design does not required; the least technical demandings; fast and easy</td>
<td>Problems of reproducibility; cannot provide phylogenetic information</td>
</tr>
<tr>
<td>SSCP</td>
<td>Orita et al. 1989[37]</td>
<td>Possible to identify community members; bands can be excised and sequenced; can be hybridized with taxon-specific probes</td>
<td>Only short fragments (150~400 bp) can be optimally separated; problems of reproducibility</td>
</tr>
<tr>
<td>AFLP</td>
<td>Zabeau and Vos, 1993[33]; Vos et al., 1995[34]</td>
<td>Sets of restriction fragments could be visualized without prior knowledge of nucleotide sequence; number of fragments can be analyzed simultaneously; could generate fingerprints of any DNA regardless of the origin or complexity; reliable</td>
<td>Expensive; require a high level of technical skill</td>
</tr>
<tr>
<td>T-RFLP</td>
<td>Avani-Aghajani et al. 1996[38]</td>
<td>Can be used to carry out both qualitative and quantitative analyses</td>
<td>Formation of pseudoterminal restriction fragments; bands cannot be excised for further analysis; expensive</td>
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Although genetic fingerprinting of plankton community has significantly improved our understanding of aquatic ecology and will go further with stronger power, the full realization of the potential of molecular techniques for ecology is only in its infancy. Along this line, future studies should combine molecular techniques with classical ecological approaches to address both structural and functional problems of the whole plankton community. Statistical analysis in the resulting process of molecular analysis should also be taken into account. Furthermore, novel approaches (e.g. real-time quantitative PCR) and new applications of technologies “borrowed” from other areas will significantly enhance the capability of molecular approaches, and will make it possible to unlock some mysteries of aquatic ecology.

References


