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Denaturing Gradient Gel Electrophoresis (DGGE) an Overview

Charoskar D. N.[1] and Arun Y. P.[2]

[1]M.Sc. Scholar, Department of Biotechnology, University of Agricultural Sciences Dharwad, Karnataka.

[2]Ph. D. Scholar, Department of Biotechnology, University of Agricultural Sciences Dharwad, Karnataka.

SUMMARY

Denaturing gradient gel electrophoresis (DGGE) is a commonly used molecular technique for rapid fingerprinting of microbial community diversity and composition. The method is rapid and affordable which allows multiple samples to be processed simultaneously. DGGE is a form of gel electrophoresis that uses either a chemical gradient to separate samples as they move across an acrylamide gel. Sequence variations within the melting domains cause the melting temperatures to vary, and molecules with different sequences will stop migrating at different positions in the denaturing gradient gel, therefore becoming separated. DGGE has extended the knowledge of the diversity and dominant microorganisms in different environmental samples. Eliminating its limitation, DGGE is a rapid and efficient method in analysing the microbial community in their natural environment.

INTRODUCTION

Within a short period, this method has attracted the attention of many environmental microbiologists, and the technique is now used in many laboratories. PCR DGGE is been widely used for microbial diversity analysis for both bacteria and fungi. DGGE was developed to study the single mutation-based studies in DNA sequences (Muyzer and Smalla, 1998). Later, it was used to study the microbial diversity of environmental samples. The GC clamp of 45 to 50 bp oligonucleotide is a unique feature of DGGE specific primers added to either of the primers that allows PCR amplified products to remain renatured or clamped double stranded when passed through acrylamide denaturing gradient gel of chemical denaturants of urea and formamide (Muyzer and Smalla 1998).

How does DGGE work?

PCR technique is used to amplify the DNA fragment from a sample containing multiple organisms. These PCR products have conserved sequences that are already present in different organisms for example, 16S rDNA regions are a common in bacteria. This PCR product is then subjected to the DGGE analysis in which samples are loaded on polyacrylamide gel with increasing denaturing chemical concentration and also provided with constant heat of 60 °C which helps in the unwinding of double stranded DNA molecules. A quick glimpse at electrophoresis tells us that this is a separation technique based on the electrical charge, shape and molecular weight of particulates such as DNA, proteins and RNA (Fakruddin and Mannan, 2013). In DGGE, DNA, which is negatively charged, is attracted by the positive electrode and forced to migrate through the pores of a polyacrylamide gel. As the PCR product progress through the gel, their melting domains will denature at different points in the gel, depending on their Tm. Denaturing of these melting domains creates single stranded DNA which slows the migration of the PCR fragment within the gel matrix. In other words, base pairs formed by nucleotides A (adenine) and T (thymine), and those formed by C (cytosine) and G (guanine) are chemically melted apart. The complete dissociation of PCR product is protected by GC clamp which is added within the PCR primers thus allowing analysis of practically any DNA sequence. In a mixed population of samples, this change in migration rate creates a banding pattern similar to that seen for varying size fragments in traditional gel electrophoresis. Comparison of these bands with a marker of known sequences can then be used to identify the species present in the sample, and bands can be excised for DNA sequencing for confirmation. This provides DGGE with the power to distinguish between mutated and wild type sequences without prior knowledge of what these sequences are, justifying why this method is used to detect mutations within closely related organisms. (Hoshino and Matsumoto, 2012).

Applications of PCR-DGGE:

The DGGE has been widely used to study the dynamics and structure of complex microbial communities and is classified as part of the new discipline of molecular microbial ecology. This Microbial ecology aims at studying interactions among and between the microorganisms and their environment. This Molecular approach

has been very useful and has resulted in significant progress in understanding the microbial diversity of natural ecosystems. However, conventional cloning, hybridisation and culture methods are not always practical for such investigations. Moreover, these techniques do not provide any information on the dynamics of the microbial populations in complex ecosystems and the potential effects of environmental changes on such populations. However, both the over and underestimation of real diversity may occur due to inherent methodological limitations of this PCR-dependent method (Martínez-Alonso *et al*., 2010). The PCR DGGE analysis does not require prior knowledge of microbial populations. The molecular fingerprinting technique can generate different DGGE patterns in complex microbial ecosystems such as gastrointestinal tracts, soils, sediments, deep seas, rivers, hot springs and biofilms. A major advantage of this method is its potential to visually profile and monitor changes occurring in various microbial communities that are undergoing different treatments or modifications. It is a rapid and efficient separation technique of same length DNA sequences (amplified by PCR), which may vary as little as a single base pair modification. Furthermore, PCR-DGGE is a flexible method that allows a unique combination of different approaches for more accurate identification of, for example, functional genes present in particular bacterial populations or specific bacterial species by using hybridization of species-specific probes. The fragment separated on DGGE can be excised, cloned and sequenced the identification of prominent members of the community. This methodology can be utilized in diverse subject areas such as clinical and environmental microbiology and food safety.

- **Advantages –** Very sensitive to variation in DNA sequence, allows simultaneous analysis of multiple samples, useful in monitoring shifts in microbial community structure over time and can be applied to phylogenetic and functional genes
- **Limitations** – Time consuming, Microheterogeneity in rRNA encoding genes present in some species may result in multiple bands for a single species and Works well only with short fragments (<600 bp), thus limiting phylogenetic characterization

CONCLUSION

DGGE is undoubtedly a valuable approach in screening complex ecosystems on a large scale and in analysing various environmental samples in a reduced amount of time. A very suitable technique for the identification of novel and unknown organisms. This approach was found to work well in several cases, but can be only used for a relatively limited number of bands per sample. The limitation can be overcome by coupling the DGGE with the sequencing, which can help in avoiding the incorrect interpretation of the profiles due to the presence of different phylogenetic affiliations at the same gel position.

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