DENATURING GRADIENT GEL ELECTROPHORESIS FOR STUDYING MICROBIAL POPULATIONS

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Denaturing gradient gel electrophoresis (DGGE) is a new genetic fingerprinting technique for examining a microbial population based on genetic markers. The vast majority, or 80%, of microbes are not yet isolated and identified due to lack of knowledge on how they survive and grow in their habitats.

The purpose of DGGE is to monitor changes in the microbial community to identify the species responsible for certain processes (Avrahami *et al*., 2003; Ercolini, 2004; Zeotenal *et al*., 2002).

PRINCIPLES

Using DGGE, DNA fragments of the same length but with different sequences can be separated. Separation is based on the decreased electrophoretic mobility of a partially melted double stranded DNA molecule in polyacrylamide gels containing a linear gradient of DNA denaturants or a linear temperature gradient (Saiki *et al*., 1988).

Once a domain reaches its melting temperature (*Tm*) at a particular position in the denaturing or temperature gradient gel, a transition of a helical to a partially melted molecule occurs, and migration of the molecule will halt. Sequence variation within such domains causes the melting temperatures to differ, and molecules with different sequences will stop migrating at different positions in the gel.

PROCEDURES FOR STUDYING MICROBIAL COMMUNITY

Bacterial genomic DNA was extracted from natural samples using the method of Yates *et al.* (1998). Then segments of bacterial 16S rDNA and fungal 18S rDNA genes were amplified using the polymerase chain reaction, PCR technique (Saiki *et al*., 1988), as in *Figure 1*. The rDNA from the different microbes in the sample were separated by DGGE (Ercolini, 2004). The result was a pattern of bands (*Figure 2*), the number of which corresponded to the number of

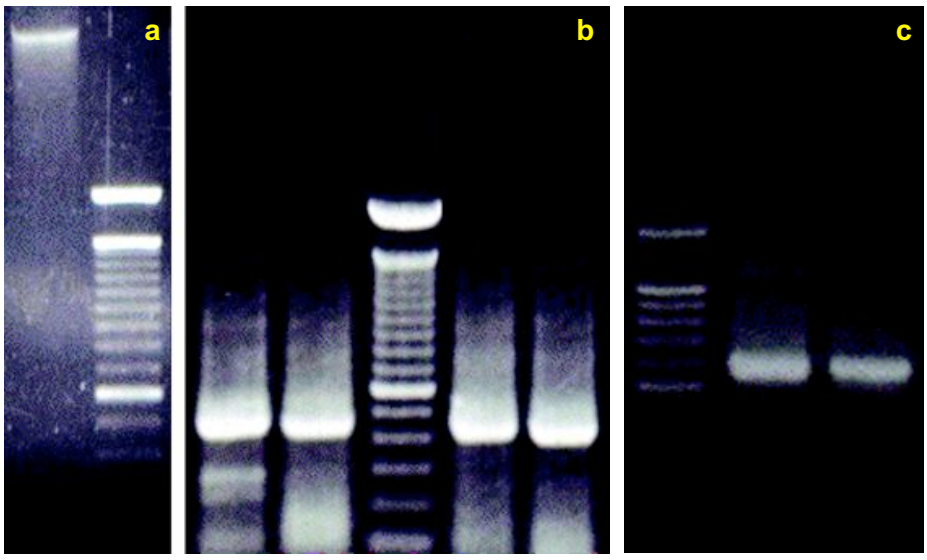

Figure 1. Total microbial DNA from a soil sample (a) with an estimated size of 20 kb. The PCR product was approximately 430 bp for 16S rDNA (b) and 580 bp for 18S rDNA (c).

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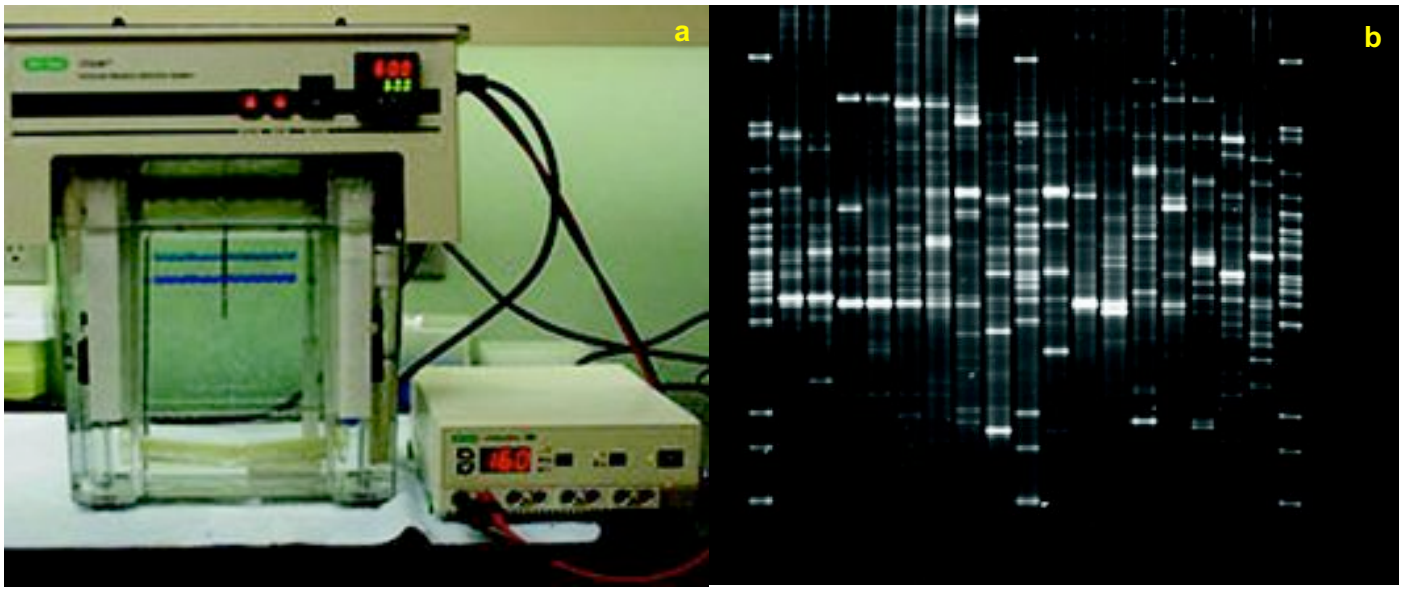

Figure 2. Denaturing gradient gel electrophoresis apparatus (a) and the separated PCR amplicons for individual microbe (b).

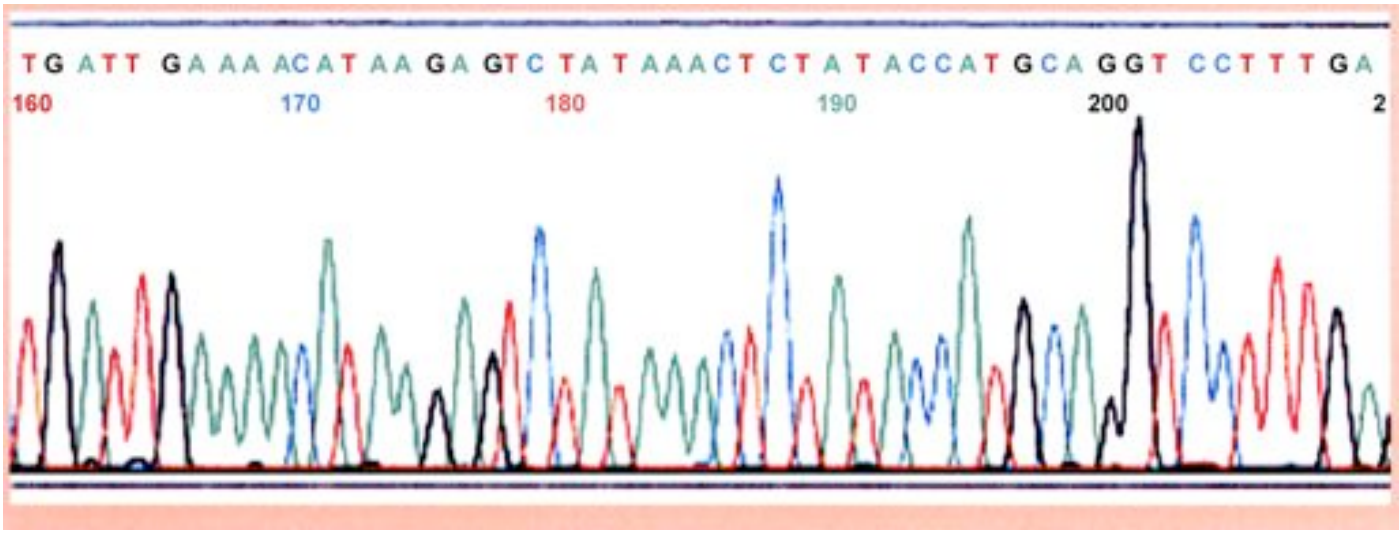

Figure 3. DNA sequencing determines the precise sequence of nucleotides in the microbial ribosomal DNA for identification of species.

predominant members in the microbial communities (Janssen, 2006). The individual band can be sequenced to identify the microbe within the sample (*Figure 3*). The banding pattern itself can be used to monitor the population changes over time. The sequences will be analysed using the bioinformatics technique for identification of microbial genus and species in the samples as in *Figure 4*.

COST

MPOB will charge a minimal cost per species or strain of microbe identified.

BENEFITS

- This is a rapid and economical way of comparing large number of samples to one another without having to culture, isolate and analyse each sample individually.
- The progression of specific microbes within the community or the overall community itself associated with different oil palm management practices can be catalogued and compared with that of the nature very rapidly.

Figure 4. Microbial phylogenetic tree constructed based on the DNA sequence and bioinformatics.

- DGGE eliminates the need for many expensive media, minimizing the sample volumes, and able to detect the unculturable microbes and that which perished from the sample over time and unfavourable conditions.

- DGGE also allows the researcher to examine which organisms are forced out of a community over time, which organisms are stable, and what new organisms may be appearing.

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