

Mairi Hope explores the new genetic fingerprinting technique of DGGE and predicts it will become a core facility within every microbial ecology lab

Density Gradient Gel Electrophoresis



IT IS NOW ACCEPTED THAT the vast majority of microbes have not yet been isolated, identified or characterised. This is largely due to a lack of knowledge of how these organisms survive and grow in natural habitats. When one also considers that a bacterium is often part of a larger more complex community or ecosystem with possible co-dependence on other members, it is understandable why traditional culturing techniques fail to accurately reflect the large microbial diversity in an environmental sample.

The advent of culture-independent techniques has transformed the field of microbiology and microbial ecology in particular. PCR-based techniques allow the classification of microorganisms based on particular genetic markers and the profiling of complex microbial communities on the basis of sequence diversity (including the uncultured majority). One technique that is now routinely used is denaturing gradient gel electrophoresis (DGGE) (and the analogous temperature gradient gel electrophoresis (TGGE)).

DGGE is a genetic fingerprinting technique that is used to separate individual sequences from a complex mixture. DNA sequences with differing base composition have different melting properties when passed through an acrylamide gel containing an increasing gradient of chemical or thermal

denaturant. The melting temperature of a double stranded DNA fragment is influenced by hydrogen bonds formed between complementary base pairs and also by the attraction between neighbouring bases on the same strand (known as stacking interactions). The order of bases on a strand determines the degree of stacking. A DNA molecule may therefore have several melting domains with characteristic melting temperatures (T_m) determined by the nucleotide sequence. Changes in base sequence as small as a single base may alter the stacking significantly enough to modify the T_m by over 1°C. When separated by electrophoresis through a gradient of increasing temperature (TGGE) or chemical denaturant (DGGE — usually formamide and urea), the mobility of the molecule is retarded at the concentration at which the DNA strands dissociate, forming a partially single stranded molecule with no further movement in the gel. Complete denaturation is prevented by the presence of a high melting domain, which is usually artificially created at one end of the molecule by incorporation of a GC clamp. This is produced by using a primer with a 5' tail consisting of a GC-rich sequence of around 30-50 base pairs in the PCR (Myers *et. al.*, 1985). In principle, this means that DNA fragments of the same length are separated on the basis of differing sequences, even if only by a single base. The separation of the

DNA fragments is then visualised by staining. The most sensitive method of detection is silver staining, however this prevents any further analysis of bands by techniques such as sequencing or hybridisation analysis. Alternative staining methods are ethidium bromide or SYBR green.

The most commonly used marker for profiling bacterial communities is the small sub-unit or 16S rRNA gene. The size of this gene (1.5 kilobases) is large enough for reliable phylogenetic information. Ribosomes are evolutionary and functionally conserved and are found in all living cells. Due to functional constraints, the sequence over certain areas of this gene remain conserved throughout all evolutionary lineages but interspersed with variable or hypervariable regions of sequence. This allows for the design of hierarchical primers, which can target broadly (e.g. the domain bacteria) or with high specificity (e.g. the genus *Pseudomonas*). Different functional genes can also be used in order to target specific groups of bacteria, for example the *amoA* gene present in ammonia oxidising bacteria and the *iap* gene for *Listeria* species (Avrahami & Conrad 2003; Ercolini 2004).

Profiles generated by DGGE (figure 1) can be analysed by comparing the presence or absence of individual bands as well as measuring the intensity of a

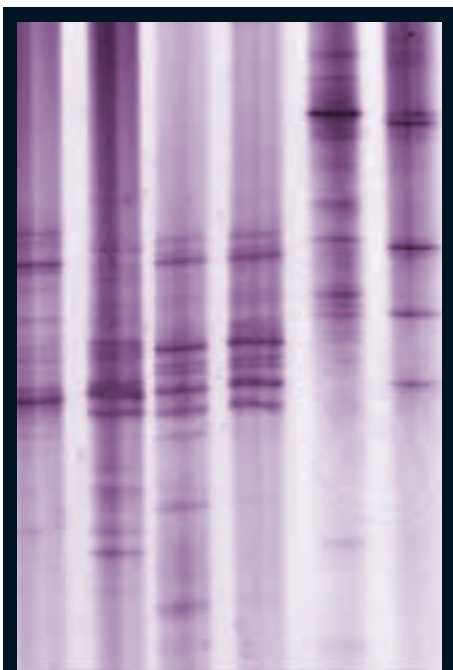


Figure 1: DGGE of PCR products of the V6 to V8 region of 16S rDNA from various human colonic biopsy samples.

band within a profile, where the intensity can relate to the relative abundance of a sequence within a sample, although at best this is considered a semi-quantitative measure. By performing RT-PCR and subsequent DGGE analysis on the same sample, one can also determine expression levels and compare activity of the more prominent bacteria within the profile (Zoetendal, Akkermans, & de Vos 1998). Single bands may also be excised and sequenced in order to ascertain the identity. Furthermore, statistical analysis of the banding profile can be performed using computer software packages to generate similarity indices leading to more refined results (Fromin *et al.*, 2002).

It should be noted that as with all PCR-based analyses, DGGE is not without its limitations. In general, for optimal resolution the fragment size of the PCR product generated prior to DGGE analysis is limited to a maximum of around 500bp. This means that definitive species identification from databases may not be possible. Difficulties have also been reported in attempting to resolve fragments that differ by only two or three bases, yet co-migration of non-related sequences has also been reported. Microheterogeneity in rRNA encoding genes present in some species must also be taken into consideration where

References

- Avrahami, S. & Conrad, R. 2003, "Patterns of community change among ammonia oxidizers in meadow soils upon long-term incubation at different temperatures", *Appl.Environ.Microbiol.*, **vol. 69**, no. 10, pp. 6152-6164.
- Ercolini, D. 2004, "PCR-DGGE fingerprinting: novel strategies for detection of microbes in food", *J.Microbiol.Methods*, **vol. 56**, no. 3, pp. 297-314.
- Felske, A., Rheims, H., Wolterink, A., Stackebrandt, E., & Akkermans, A D. 1997, "Ribosome analysis reveals prominent activity of an uncultured member of the class Actinobacteria in grassland soils", *Microbiology*, **vol. 143** (Pt 9), pp. 2983-2989.
- Fromin, N., Hamelin, J., Tarnawski, S., Roesti, D., Jourdain-Miserez, K., Forestier, N., Teyssier-Cuvelle, S., Gillet, F., Aragno, M., & Rossi, P. 2002, "Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns", *Environ.Microbiol.*, **vol. 4**, no. 11, pp. 634-643.
- Hold, G. L., Smith, E. A., Birkbeck, T. H., & Gallacher, S. 2001, "Comparison of paralytic shellfish toxin (PST) production by the dinoflagellates *Alexandrium lusitanicum* NEPCC 253 and *Alexandrium tamarense* NEPCC 407 in the presence and absence of bacteria", *FEMS Microbiol.Ecol.*, **vol. 36**, no. 2-3, pp. 223-234.
- Muyzer, G., de Waal, E. C., & Uitterlinden, A. G. 1993, "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA", *Appl.Environ.Microbiol.*, **vol. 59**, no. 3, pp. 695-700. ■
- Muyzer, G. & Smalla, K. 1998, "Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology", *Antonie Van Leeuwenhoek*, **vol. 73**, no. 1, pp. 127-141.
- Myers, R. M., Fischer, S. G., Lerman, L. S., & Maniatis, T. 1985, "Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturing gradient gel electrophoresis", *Nucleic Acids Res.*, **vol. 13**, no. 9, pp. 3131-3145.
- Nicol, G. W., Glover, L. A., & Prosser, J. I. 2003, "Spatial analysis of archaeal community structure in grassland soil", *Appl.Environ.Microbiol.*, **vol. 69**, no. 12, pp. 7420-7429.
- Zoetendal, E. G., Akkermans, A. D., & de Vos, W. M. 1998, "Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria", *Appl.Environ.Microbiol.*, **vol. 64**, no. 10, pp. 3854-3859.
- Zoetendal, E. G., von Wright, A., Vilpponen-Salmela, T., Ben Amor, K., Akkermans, A. D., & de Vos, W. M. 2002, "Mucosa-associated bacteria in the human gastrointestinal tract are uniformly distributed along the colon and differ from the community recovered from feces", *Appl.Environ.Microbiol.*, **vol. 68**, no. 7, pp. 3401-3407.

multiple bands for a single species can subsequently lead to an overestimation of community diversity (Muyzer & Smalla 1998).

One of the main advantages of DGGE is that it allows the simultaneous analysis of multiple samples, making it feasible to monitor shifts in populations over periods of time or different environmental conditions. Before the arrival of DGGE this was more commonly achieved by cloning and sequencing - an approach that is both labour intensive and relatively costly, especially when dealing with numerous samples.

The DGGE method was first used to profile communities of a microbial mat and bacterial biofilms (Muyzer, de Waal, & Uitterlinden 1993) and since then has been used to analyse microbial communities from extremely diverse microbial environments too numerous to mention. A few examples include profiling of microbial soil communities, marine

environments, hydrothermal vents, the gastrointestinal tract of humans and even the study of a microbial community resident in a medieval wall painting (Felske *et al.*, 1997; Hold *et al.*, 2001; Nicol, Glover, & Prosser 2003; Zoetendal *et al.*, 2002). More recently, DGGE has been introduced into food microbiology for the identification of microorganisms isolated in food and for evaluation of the microbial diversity during food fermentation.

In conclusion, DGGE has proved to be an exceptional tool to study species diversity and bacterial community dynamics. Despite the fact that it is still a relatively new technique within microbial ecology, its relative simplicity and reproducibility means that DGGE will no doubt become a core facility within every microbial ecologists laboratory.

Mairi Hope
University of Aberdeen