EDITORIAL

PCR fingerprinting microbes by random amplification of polymorphic DNA

Technical simplicity combined with sensitivity and speed generated interest in the application of the polymerase chain reaction (PCR) to the diagnosis of infectious diseases. Now these same merits have led to the development of a PCR-based typing system. Initially this involved amplification between paired primers derived from previously characterised sequences. For example, a rapid method of fingerprinting cytomegalovirus (CMV) was developed by amplifying the hypervariable L-S junction region by PCR between consensus oligonucleotide primers to produce strain-specific variably sized PCR products.¹ The PCR products were detected by gel electrophoresis and Southern blot hybridisation against a ³²P-labelled CMV junctional fragment. The PCR profiles obtained were unique for unrelated strains whereas similar patterns were observed for epidemiologically related strains isolated from members of the same family. In some studies, such as that carried out on human herpes virus 6 with primers from known viral DNA sequences, the amplified products were analysed by a combination of Southern blot hybridisation, digestion with restriction endonucleases and partial nucleotide sequencing.²

For many organisms genetic maps are not available and relatively little is known of their molecular biology. In such situations the DNA markers most commonly used have been restriction fragment length polymorphisms (RFLPs). Fragments are usually generated by frequent-cutting enzymes and separated by conventional agarose gel electrophoresis, but occasionally rare-cutting enzymes are used and larger fragments are separated by pulsed-field gel electrophoresis. RFLPs have been used successfully to generate numerous microbial typing systems, but for some organisms discrimination is suboptimal because there is a tendency for one or two genetic types to predominate amongst an apparently heterogeneous population.³ Better discrimination between isolates can be achieved by the secondary step of Southern blot hybridisation with radiolabelled probes recognising repetitive DNA sequences. However, this adds a rather laborious, expensive second step which is incompatible with large scale epidemiological studies.

Recently, an alternative DNA polymorphism assay has been established, based on the amplification of genomic DNA with a single primer of arbitrary nucleotide sequence.^{4,5} The PCR is carried out at low annealing temperatures (36°C) under conditions that

favour relatively non-specific binding of the primer to multiple sites of the template DNA. This then results in multiple PCR products which are separated out according to size by conventional agarose gel electrophoresis. These polymorphisms have been termed RAPD markers,⁴ after random amplified polymorphic DNA, or arbitrary primed PCR (AP-PCR).⁵ Unlike PCR fingerprinting based on amplification of a specific gene, RAPD requires no sequence information and it scans the whole genome rather than relying on hypervariability within one specific gene. Each primer gives a different pattern of PCR products, each with the potential of detecting polymorphisms between strains. Therefore, discrimination between isolates can be maximised by combining the RAPD results obtained with several primers. Some primers may not generate polymorphic patterns with a particular microbe or they may generate a conserved PCR profile that is species-specific. DNA polymorphisms amplified with primers of nine nucleotides in length or longer result in relatively few amplification products, which on gel electrophoresis give rise to simple fingerprint patterns. With arbitrary primers as short as five nucleotides in length, more detailed and complex DNA profiles are generated which are usually resolved by polyacrylamide gel electrophoresis and silver staining rather than agarose gel electrophoresis visualised by ethidium bromide.⁶ Another variation is to use two short arbitrary primers in combination for each PCR instead of separately.⁷ In the latter study, primer annealing was performed at temperatures as low as 30°C and amplification products were separated on agarose 1.5% gels.

RAPD typing has been applied successfully to the genetic fingerprinting of man,⁴ crops such as soyabean,⁴ rice⁵ and banana,⁷ and bacteria including staphylococci and *Streptococcus phytoenes*.⁵ The method has been applied to two fungal plant pathogens, *Fusarium solari*⁸ and *Leptosira maculens*.⁹ We have found this technique particularly useful when applied to typing fungal pathogens, namely *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*. All isolates are typable and reproducibility is comparable to RFLP. Discrimination tends to be considerably better than that obtained by RFLP analysis with frequent-cutting enzymes such as *EcoRI*. One particular primer generated 11 distinct fingerprints with 15 isolates of *C. albicans*,¹⁰ six fingerprints with 20 isolates of *A. fumigatus*¹¹ and 12 fingerprints

with 12 isolates of *Cr. neoformans*.¹² The simplicity, versatility and economy of RAPD-based typing systems make them an ideal means of genetically fingerprinting isolates, suitable for large scale epidemiological studies of these fungi. Whether it will

prove to be equally valuable for typing other microbes is the subject of further investigation.

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