MODERN APPROACHES TO THE TAXONOMY OF ASPERGILLUS

Brian W. Bainbridge

Microbiology Group, Life Sciences Division
Kings College London, Campden Hill Road
London W8 7AH, UK

INTRODUCTION

Techniques from molecular biology have provided a series of new tools for the analysis of diversity in the fungi. These techniques have been applied to a variety of fungal groups but only rather limited work has been done on the genus Aspergillus, which is surprising considering the economic importance of species within the genus. The availability of a detailed molecular genetic systems in Aspergillus nidulans has been of considerable help in providing a scientific base, but it appears on the whole that molecular geneticists have not been very interested in the taxonomy of the genus. However, a need to study the epidemiology, detection, diagnosis, identification, classification, characterisation and quantification of Aspergillus has resulted in an increasing interest in the taxonomic basis for differences within the genus. This has made it essential that closer links are forged between molecular biologists and taxonomists.

Recent techniques are based on variation in the nucleotide sequence of nucleic acids which is frequently analysed indirectly by hybridisation with nucleic acid probes. These may be based on repetitive DNA such as the ribosomal RNA gene complex (rDNA) which has both conserved and variable sequences (see below). Hybridisation or annealing of short oligonucleotide primers to a target sequences is also the basis of amplification using the polymerase chain reaction (PCR) which can be used to allow the specific detection of DNA or to produce a genetic fingerprint. Hybridisation is often combined with restriction enzyme analysis to produce restriction fragment length polymorphisms (RFLPs) but the reliability of this method for phylogenetic analysis has been questioned (Swofford and Olsen, 1990). This is because the fragments obtained may not fulfil the crucial test that characters are independent of each other. A new site may evolve between two pre-existing sites and one longer fragment may disappear and two new fragments will appear. Thus two organisms may have two restriction sites in common with only a single base pair different and yet have no fragments in common. Combined with other analyses, however conserved RFLPs have some value but further discussion of the value of RFLP data will not be given as it covered in the chapter by Croft.

Alternatively, complete or partial nucleotide sequences of target molecules can provide data for a more accurate phylogenetic comparison. Such sequences also provide useful data for the development of general or specific nucleic acid probes which can be used for RFLP
analysis, for slot blot hybridisation or for specific amplification. Analysis of chromosomal structure by use of pulse field gel electrophoresis (PFGE) can also reveal information about taxonomic relationships and some details of this technique will be found in the chapter by van der Bos.

Other taxonomic methods have been based on ubiquinones, antigenic properties, monoclonal antibodies, cell wall structure, isoymes polymorphisms and analysis of metabolic pathways. A detailed analysis of these methods is not possible in a chapter of this length although reference will be made to some examples.

Ideally a good classification system should be based on evolutionary descent and therefore a decision may need to be made on which characters are primitive and which are derived. This would allow a cladistic analysis based on natural relationship and assumptions about ancestors but such an approach is difficult in a filamentous fungus like *Aspergillus* where questions about whether imperfect species are primitive or advanced may be unhelpful. A more useful approach may be to apply phenetic or numerical analysis on a number of characters and to look for similarity making no assumptions about evolution. Total or partial base sequences of nucleic acids can however be analysed by cladistic methods. It is important, however, that molecular data are used as an extra character combined with conventional taxonomic methods, such as morphological and metabolic properties. Analysis of, for example nucleic acids alone, should not be expected to produce a reliable classification. A molecular phylogenetic analysis of the genus *Aspergillus* should provide us with data for use in a variety of new and exciting approaches to the analysis of genome diversity in industrial, agricultural, medical and environmental isolates of *Aspergillus*.

The scope of this chapter will include current and possible future applications of these approaches to the classification of *Aspergillus* into subgenera and species, as well as to methods for producing fine distinctions between strains, races and isolates using fingerprinting techniques.

**CHOICE OF A MOLECULAR METHOD**

A variety of factors will affect the choice of a method for the analysis of the taxonomic status of a range of *Aspergillus* strains. Ideally as many characters as possible should be analysed and molecular techniques should be combined with an analysis using a standard key as used by Klich and Pitt (1988). The approach then may be to make an epidemiological study of a large number of closely related isolates, or the requirement may be to analyse the phylogenetic relationships between a range of subgenera or species. Other factors such cost, speed, sensitivity and specificity will also influence the choice of methods. Fine distinctions between closely related strains will obviously need a fingerprinting techniques which shows subtle differences between strains. These techniques may be based on repetitive DNA or an amplification techniques using mismatch priming of polymerase chain reactions. Genetic fingerprinting is often an extension of RFLP analysis and this may be based on repetitive DNA or random probes. Attempts have been made to repeat the success of human genetic fingerprinting (Jefferys et al., 1991) using bacteriophage M13 probes and synthetic repeat oligonucleotides but only limited data is available for *Aspergillus* spp. (Meyer et al., 1991). These will need to be correlated with functional characters such as pathogenicity, vegetative compatibility and toxin production. Alternatively a fundamental phylogenetic study should analyse sequence data from an appropriate molecule which is expected to correlate with natural relationships between taxa. An example is the 16S-like ribosomal RNA subunit which has been analysed extensively in bacteria (Pace et al., 1986). Disagreement between the two methods would need to be resolved by the accumulation of further data as well as checking of the data already obtained.

Another approach depends on targeting a single gene or a pathway which has been shown to correlate with taxonomic groupings. A good example of this is the potential use of a probe based on a cloned gene for an enzyme in the pathway for the synthesis of aflatoxin