Molecular Genetic Analysis of Diversity in Populations of Edible Mushrooms

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1. INTRODUCTION

Over 25 years ago, Lewontin and Hubby (1966) first demonstrated high levels of molecular polymorphisms in the fruit fly *Drosophila pseudoobscura*. The discovery of allozymic protein variation in this species revolutionized our ability to study evolution. The availability of this technology stimulated molecular genetic studies of many other organisms including fungi.

Most early isozyme studies of fungi focused primarily on general protein patterns, utilizing some specific enzymes (Shechter *et al*., 1973; Mouches *et al*., 1979; Paranjpe *et al*., 1979). The electrophoretic phenotypes were evaluated on the presence or absence of particular bands without regard to the genetic bases of these bands. One early study, however, on an imperfect fungus (*Neurospora intermedia*) established genetic basis for the protein variants observed (Speith, 1975). With allozyme analysis, unambiguous codominant genotypes can be recognized-a must for successfully conducting genetic studies of fungi.

Relatively few population studies of edible fungi have been conducted using molecular genetic markers. Natural populations of *Agaricus campestris* (May & Royse, 1982), *Pleurotus* spp. (Boisselier-Dubayle, 1983). *Agaricus bisporus* (Kerrigan & Ross, 1989; Kerrigan, 1990; Kerrigan *et al*., 1993a), and *Morchella* spp. (Gessner *et al*., 1987; Yoon *et al*., 1990) have been examined by have been examined using DNA restriction site variation (Kerrigan *et al*., 1993a).

2. METHODS USED IN FUNGAL MOLECULAR GENETICS

2.1. Allozyme Analysis

Enzymes differing in electrophoretic mobility as the result of allelic differences in a single gene are called allozymes. Informative data sets are allowed by allozyme analysis because the phenotypic difference in electrophoretic banding patterns between individuals can be directly correlated to genotypic differences. Thus, allozyme electrophoresis provides unambiguous codominant genotypes that can be used successfully in conducting genetic studies of fungi.

The methods used for allozyme electrophoresis have been reviewed by Royse and May (1993) and May (1993). The review by Royse and May (1993) and May (1993). The review by Royse and May (1993) included a summarization of buffer systems. Enzymes, abbreviations. Enzyme Commission numbers, encoding loci, protein subunit composition, and a list and discussion of mushroom species where allozymes have been used for genetic studies.

More recently, Kerrigan *et al*., (1993a) have provided evidence that a wild California population of *A. bisporus* may comprise two or more ancestral elements. Using seven allozyme markers and 14 restriction fragment length polymorphisms (RFLPs) they were able to evaluate the markers and 14 restriction fragment length polymorphisms (RFLPs) they were able seven allozyme markers and 14 restriction fragment length polymorphisms (RFLPs) they were able to evaluate the California isolates relatives to "a comprehensive sample of cultivar isolates likely to be of European ancestry". Such a comparison revealed that the California isolates could be placed either in the cultivar group or in a group associated with a cypress habits. The discovery of an intermediate or less distinct group of isolates was suggestive of spontaneous hybrids between the two ancestral groups or of lines with other affinities. Studies such as the one cited above are crucial to our understanding of genetic behavior of isolated of a cultivated species in the wild. As natural habitats are lost and man-made habitats are created, genetic makers will be crucial in monitoring the changing population of this commercially important mushroom. Allozyme analysis will continue to play a role in this assessment.

2.2 DNA-DNA Hybridization

There are two basic methods used for DNA-DNA hybrid molecule tests: thermal stability and cross-hybridization. Almost all studies on fungi have tested the percentage of cross-hybridization between total DNA extracts rather than the thermal stability of hybrid molecules (Burns *et al*., 1991).

One study, however, by Horgen *et al*. (1984) in which some wild and cultivated mushrooms were examined by thermal elution, revealed a 7.7 to 11.6% mismatch

between unique DNAs of *A. bisporus* and *A. bitorquis*. This study confirms earlier contentions that these two species are closely related as inferred from cultivation and habitat studies.

2.3 Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs result from the presence or absence of particular restriction sites in DNA. Polymorphisms can be indentified because they are the length of characteristic restriction fragments. These DNA fragments can be separated by electrophoresis, in a manner similar to that used to separate allozymic proteins. RFLP analysis can be accomplished two ways: 1) by using radioactive probe DNA molecules containing the nucleotide sequences of interest, or 2) through enzymatic amplification of DNA from specific target in the genome followed by treatment with restriction enzymes.

2.3.1. RFLP analysis with prove DNA.

In 1975, Southern developed a procedure to identify DNA fragments through a special procedure known as a Southern blot (Southern. 1975). The key element in a Southern blot is the use of a probe DNA molecule. This probe molecule contains the nucleotide sequence of interest, which is obtained from a gene that has been cloned into bacterial cells. In the Southern procedure, DNA fragments are transferred to and immobilized on a filter, hybridized to the labeled DNA probe, and visualized by autoradiography. The number of observed bands depends on the number of loci in the genome homologous to the probe and the number of restriction sites within these DNA sequences.

This method has been used to analyze wild populations (Kerrigan *et al*., 1990, 1993a) and isolates (Summerbell *et al*., 1989; Loftus *et al*., 1988) of *A. bisporus* and *A. campestris* and *A. bitorquis* (Hintz *et al*., 1989). The number of RFLP markers is effectively unlimited because any cloned, low-copy-number piece of DNA can be used as a probe. This may be combined with several restriction enzymes to identify RFLPs with each probe.

2.3.2. RFLP analysis with enzymatic amplification of DNA.

Enzymatic amplification (via the polymerase chain reaction; PCR) of DNA is now a routine procedure in genetic studies of fungi (White *et al*., 1990; Kohn, 1992). Restriction analysis of PCR-amplified DNA allows analysis without Southern blotting and use of radioactive probes. Population studies on cultivated edible mushrooms using this approach have yet to be published. Vilgalys and Hester (1990) have shown that this

technology is not only faster but it avoids the potential complication of RFLPs caused by methylation differences (Burns *et al*., 1991). In addition, this methodology allows limitation of the population survey to a portion of the DNA that is evolving at the appropriate rate. The use of this methodology in systematic studies of edible mushrooms is likely to increase due to the relative ease of enzymatic amplification of DNA and the commercial availability of over 500 restriction endonucleases.

2.4. Random Amplified Polymorphic DNA (RAPD)

A PCR-based method, RAPDs produce fragments similar to RFLPs via random amplification of DNA with a single short primer of arbitrary sequence. These fragments have been demonstrated to behave as simple Mendelian loci (Williams *et al*., 1990; Kerrigan *et al*., 1993b). One of the major problems encountered with use of RAPDs, however, is that they are dominant. Alternative alleles are null so that in diploid fungi it is not possible to distinguish heterozygous and homozygous individuals. One other reported problem with this methodology is that multiple fragments of a single RAPD sometimes map to the same or nearly the same locus and so may not indicate independent loci (Williams *et al*., 1990). In the future, however, these problem should be overcome (Burns *et al*., 1991)because this methodology offers speed and convenience relative to many of the above methods.

2.5. Sequencing

Nucleic acid sequencing is a relatively new approach systematic studies (Hillis *et al*., 1990). Direct sequencing by PCR is more efficient than cloning techniques and thus, will probably increase for applications in fungal population genetics. Sequencing is expensive and more time consuming, however, than RFLP analysis and information generated may be difficult to analyze especially where nucleotide alignments are highly subjective.

Sequencing in cultivated edible fungi has already begun (Kwan *et al*., 1992). Ribosomal RNA genes(rDNA) and their intergenic spacer regions appear to be good targets for many systematic and population studies. Differences in the rate of evolution for each region in the rDNA repeat allows for selection of the appropriate region for the comparison of interest. Many of the sequences already determined for various organisms including fungi are available from several databases. GenBank, perhaps the best known of the databases, contains, contains over 108 base pairs and is doubling every 1 to 2 years (Hillis *et al*., 1990).

2.6. Electrophoretic Karyotyping

Pulsed-filed electrophoretic separation of chromosomal-length DNAs has been applied to many species of fungi (Mills & McCluskey, 1990). Most of these studies have revealed a high frequency of chromosomal-length polymorphisms within a species. In *A. bisporus*. Southern hybridizations to contour-clamped homogeneous field (CHEF) electrophoretic separations of chromosomal DNAs showed polymorphisms between parental homokaryons used in a cross (Kerrigan *et al*., 1993a). In addition, 13 chromosomes were observed with the rDNA repeat mapping exclusively to chromosome VII. These studies also help to clarify the pattern of marker transmission in *A. bisporus* and demonstrate that determinants of traits as homokaryon vigor can be mapped to chromosomal length DNAs.

3. OUTLOCK

As our knowledge continues to increase with regard to the evolutionary relationships among and between taxa, perhaps, in the future, more emphasis will be placed on estimates of a time frame for divergence. A phylogenetic tree with times of divergence for all cultivated edible fungi and their close relatives, would allow a new perspective on the way we view the evolutionary relationship of each species. Such a tree also might provide insight on species that are the most likely candidates for genetic and economic exploitation.

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