Review Article

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TAXONOMIC STUDIES ON EDIBLE MUSHROOM *LENTINUS* SP.: A REVIEW

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ABSTRACT

From a mushroom biodiversity study of the South India, seven *Lentinus* spp. were collected and identified as *Lentinus stupeus, Lentinus cladopus, Lentinus squarrosulus, Lentinus pseudotenebrosus*, *Lentinus cyathiformis, Lentinus radicus* sp. nov., *Lentinus tuberregium* were collected from the dead logs of rubber and mango trees of Keeriparai forest, Kanniyakumari District of the Western Ghats in Tamil Nadu, India. The local tribes ascribed many medicinal properties to these mushrooms. From the literature survey, it is clearly evident that scanty reports of *Lentinus* sp, are available from other countries. Further, not many studies were carried out on the indigenous *Lentinus* sp. Therefore, the present review was aimed at evolving suitable taxonomical study fungus, *Lentinus* sp.

Keywords: *Lentinus* sp, Taxonomy, ITS, Phylogenetic tree

INTRODUCTION

Mushrooms have been prominent in most of the terrestrial ecosystems and are endowed with a wide variety of ecological roles as saprophytes, mutualists and parasites. Taxonomy of mushrooms is traditionally relied on the morphological characteristics. However, the morphological characteristics are inconsistent and unstable sometimes as because they are strongly influenced by the environmental factors. Many mycologists still disagree on the taxonomic limits of the Agaricales and the identity of a natural groups within the order[1,2].

The genus *Lentinus* includes approximately 53 species *viz.*, *Lentinus australia, Lentinus levis, Lentinus anthocephalus*, *Lentinus araucariae*, *Lentinus aespiticola*, *Lentinus bertieri, Lentinus badius, Lentinus concinnus*, *Lentinus squamosus*, *Lentinus villosus*, *Lentinus copulatus, Lentinus courtetianus*, *Lentinus crinitus*, *Lentinus cyathiformis*, *Lentinus velutinu*, *Lentinus swartzii*, *Lentinus connatus, Lentinus cochleatus, Lentinus ciliatus, Lentinus lentinellus, Lentinus edodes, Lentinus lepidus, Lentinus panustigrinus, Lentinus cretaceous, Lentinus detonsus, Lentinus fusipes*, *Lentinus fastigatus, Lentinus tigrinus, Lentinus squamosus, Lentinus glabratus*, *Lentinus kauffmanii, Lentinus lamelliporus*, *Lentinus lepideus, Lentinus lecomtei, Lentinus strigosus, Lentinus martianoffianus*, *Lentinus zelandicus*, *Lentinus sajor-caju*, *Lentinus polychrous*, *Lentinus prancei, Lentinus praerigidus, Lentinus edulis, Lentinus striatulus*, *Lentinus squarrosulus, Lentinus strigellus, Lentinus scleropus*, *Lentinus zeyheri, Lentinus umbrinus*, *Lentinus stuppeus*, *Lentinus suavissimus*, *Lentinus subdulcis*, *Lentinus tuberregium* and *Lentinus ursinus.*

Taxonomy of Mushrooms

Taxonomy of mushrooms has traditionally relied on morphological characters that are known to be the subject to parallel evolution and phenotypic plasticity; as a result, many modern genera and families have been artificial[3]. The earliest classification of agarics and other basidiomycetes by[4] was most notable for its clarity, logical, simple and complete artificiality. Family and twenty genera of gilled (agaricoid) fungi[5]; Mycologists began to revise 'Fries' taxonomy and to recognize increasingly larger numbers of segregate genera based on ever-more-restricted sets of characters[6].

After a century, the modern taxonomic systems recognize up to 230 genera[7] that are classified under 80 families and 25 orders[8-11]. The most comprehensive modern taxonomy for the Agaricales was reported[7]. Interestingly, the mycological floristic treatments mainly follow the Singer's system[9,12,13].

However, several alternative systems were proposed against Singer's classification, including the systems proposed[10,11]. Till date, it has been difficult to evaluate the merits and demerits of each classification system in the levels of knowledge about the phylogenetic relationships. A growing number of phylogenetic studies have started to address the evolutionary relationships between mushrooms and their relatives by rRNA gene sequence[15-19]. Monophyly of Singer's Agaricales was rejected by several of these studies[20-22], which support the recognition of a clad of "euagarics" (gilled mushrooms) corresponding to the Singer's concept for the Agaricineae but also including a number of non-agaric fungi such as puffballs and coral mushrooms.

Taxonomic Distribution of *Lentinus* sp.

The mode of wood decay has been an important taxonomic character in the basidiomycetes, especially in the predominantly wood-decaying polyporus and corticoid fungi[23-28]. Wood decay mode has generally been regarded as a moderately conservative character and has often been used to segregate genera. For example segregated the brown rot gilled mushroom genus, *Neolentinus* from *Lentinus*, which is otherwise composed of white rot species[27]. Similarly, the brown rot polypore genus, *Antrodia* from the morphologically similar white rot genus, *Antrodiella*[29]. White rot is by far the most common form of wood decay in the basidiomycetes. The 1,568 species of wood-decaying basidiomycetes have been described from North America whereas; only 103 species (7 %) produce a brown rot[30].

Totally 71 species (70 %) of the North American brown rot fungi are in the Polyporaceae[26], which has long been regarded as an artificial taxon[30]. According to the dominant morphology-based classification of basidiomycetes[30], the remaining brown rot basidiomycetes are classified into six additional families: Coniophoraceae, Corticiaceae, Paxillaceae, Sparassidaceae, Stereaceae and Tricholomataceae. Except for the Sparassidaceae, which has only two species in the North America, each family that contains brown rot species also contains white rot species. Recent molecular studies reviewed[31] suggest that the families contain brown rot species (Corticiaceae, Paxillaceae, Polyporaceae, Stereaceae and Tricholomataceae) are polyphyletic. In contrast, the brown rot is the pleiomorphic form in the homobasidiomycetes[23,24] and that white rot has been repeatedly derived by elaboration of wood decay mechanisms (i.e., gaining the ability to degrade lignin). Most recent authors have supported Gilbertson's view that the brown rot fungi are derived from the white rot[32-34]. However, these inferences have not been based on phylogenetic analyses. Indeed, the lack of a broad phylogenetic classification of basidiomycetes has been the primary obstacle to the understanding of the evolution of decay modes[35]. The artificial nature of the Polyporaceae is especially limiting owing to the concentration of brown rot taxa in this family.

Molecular Systematic in Fungal Identification

The rapid development of DNA sequencing techniques, phylogenetic theory and bioinformatics has enabled systematists to envision a phylogenetic classification of all the branches of the tree of life. In fungi, the pace of discovery about natural relationships has also been greatly accelerated by new evidence from molecular systematic, mostly using rDNA sequence analysis[36,37]. With the current strategies in biotechnology, molecular genetic markers have been employed for rapid identification of different kinds of mushrooms[36-41,37]. The development of DNA-based PCR and taxon-specific primers[42] has made the detection and study of fungi increasingly feasible.

The Internal Transcribed Spacer (ITS) region has generally been considered a convenient target for the molecular identification of fungi at species[43,38,44]. Nuclear-encoded rRNA genes are primarily focused on investigation for new taxonomic approaches in fungal molecular systematic. These genes are arranged in tandemly repeated units with each unit contained genes for the small subunit 18S, 5.8S and large subunit 25S-28S. Each unit is separated by one or more Intergenic Spacer (IGS) regions and these IGS regions may contain separately transcribed coding region for 5S RNA[45-48].

The coding regions, 18S 5.8S and 28S of nuclear rDNA genes, are highly conserved among the fungi and they showed little sequence diversity between the closely-related species and are useful for phylogentic studies among distantly related organisms[49,50]. Within each repeat unit, the conserved regions are separated by two internal transcribed spacers, ITS I and ITS II, which show higher rates of divergence[16,51]. These ITS regions are now most widely sequenced DNA regions in fungi for molecular studies. Variable sequence regions in both the small (18S) and large (25S) subunits of rDNA genes have also led to numerous molecular approaches that provide rapid identification of fungal species[51].

The rDNA phylogenies support the monophyletic of many basidiomycete taxa, which also demonstrated the existence of several clades of distinct groups[49,21,18]. In the homobasidiomycetes, gilled mushrooms appear to have evolved along multiple lines from morphologically diverse ancestors[21,22], making the Agaricales polyphyletic. It has also been demonstrated that gasteromycetes (e.g., Puffballs and Sequestrate or Secotioid fungi) have evolved several times from gilled or poroid ancestors[52-54]. These findings open the way to reconstruct the artificial taxa (e.g., Gasteromycetes) and to redefine others in a phylogenetic order.

The core group of euagarics clades, encompassed the gilled mushrooms. It correspondent to the Agaricineae[7] but also includes taxa that were traditionally classified in the Aphyllophorales (e.g., *Clavaria, Typhula, Fistulina, Schizophyllum*, etc) and several Gasteromycetes (e.g., Hymenogastrales, Lycoperdales and Nidulariales). Phylogenic relationships within the euagarics are still poorly understood. However, an earlier molecular phylogentic study 107 on rDNA sequence from 152 diverse agaricoid taxa showed that many families and genera of the Agaricales did not match the natural groups[3].

Internal Transcribed Spacer (ITS) Region

The internal transcribed spacer (ITS) and intergenic spacer (IGS) domains have become important DNA fragments for the purpose of molecular characterization. ITS and IGS are rDNA domains that evolve at faster rate. The ITS domain is a molecular marker for comparing different species of a particular fungal genus[55]. IGS domain is a highly variable domain[56], usually containing the effective sequence for determining the interspecies or individual genetic relationship between fungi[57].

ITS and IGS are important molecular markers for extra species, comparison in the same fungal generic and for analysis of interspecies mutation and genetic polymorphism[58]. The highly conserved ribosomal genes, which flank the ITS regions, are ideal for universal primer targeting and amplification by Polymerase Chain Reaction (PCR). The sequences thus obtained are analyzed, compared and accordingly the evolutionary trees are constructed.

The ITS region in fungi are highly variable and are useful in distinguishing between the species[59]. There are specific segments in the ITS regions which have greater variability than the other segments. The frequency of nucleotide substitutions was similar in both the ITS regions but found that variations were mostly located in the central region of ITS I and close to the termini in ITS II[16]. They also reported that nucleotide divergence between recently diverged taxa was usually in the ITS II region. Similar observation has been reported, where a lower level of resolution of internal phylogenetic branches was obtained from the ITS I[60].

IT'S I and ITS 4 primers are used for most studies; several taxon-specific primers have been described that allow selective amplification of fungal sequences. Grades and Bruns (1993) described amplification of basidiomycetes ITS sequences from mycorrhiza samples. Many reports have been published on the analysis of the ITS regions to establish the taxonomic relationships within the *Ganoderma* species[16,60,61].

Phylogentic Analysis

Taxonomy aims to reflect the natural classification of taxa and molecular data offer a set of objective characters on which to base taxonomic decisions. The use of phylogenetic programmes to analyse such molecular data has rapidly become popular and it is the resulting phylograms (genetic evolutionary trees) that display monophyletic groups, the members of which share a common ancestor[62]. The practice of phylogenetic analysis should be conceived as a search for the suitable model, as much as a search for the correct tree[63].

The phylogenetic inference currently can be classified into three major groups: distance methods, maximum likelihood methods and parsimony methods. In distance method, an evolutionary distance is computed from all pairs of sequences and a phylogenetic tree is constructed from the pair-wise distances. In maximum likelihood methods, maximisation of the likelihood is

performed for each topology separately and the topology with the highest likelihood is chosen as an estimate of the true tree topology. However, in the maximum parsimony methods, the given set of nucleotide sequences are considered and the nucleotides of ancestral sequences for a hypothetical topology are inferred under the assumption that mutational changes occur in all directions among the four different nucleotides. The smallest numbers of nucleotide substitutions that explain the entire evolutionary process for topology are computed. The computation can be done for all topologies and requires the smallest number of substitutions chosen to be the best tree[64].

CONCLUSION

There are reviews available on the statistical methods developed for different models and how these models may affect data sets. Although numerous phylogenetic algorithms, procedure and computer programs have been devised, their reliability and practicality are, in all cases, dependent on the structure and size of the data. The field of phylogenetic Analysis is controversial. Some of the debates have been summarised in reviews. Of particular importance is the issue of phylogenetic Analysis of large molecular data sets and the suggestions that statistical reliability is sensitive to the sample size. Conversely, evidence from various studies suggests that increasing sample size generally increases phylogenetic accuracy.

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