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Molecular characterization of wild mushroom

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ABSTRACT

Identification of wild mushrooms is difficult considering visual and / or metabolic approaches. Molecular markers, especially DNA markers are quick and reliable to establish the identities of wild collections and are helpful in mushroom taxonomy. Hence an attempt was made in the study to characterize the wild mushrooms by molecular methods. Fungal material used in the present study is the fruiting body of wild mushrooms. The genomic DNA of the mushroom was extracted, the rDNA - ITS fragment of the genomic DNA was amplified using ITS1 and ITS4 primers and subjected to nucleotide sequence determination. The sequence thus determined was aligned using Jukes-Cantor Corrected Distance model. The aligned sequence (559bp) revealed 88% match score with Perenniporia sp. (GQ982890.1). Hence this wild mushroom which belongs to Agaricomycetes is a new addition to the Indian mushroom biodiversity.

Keywords: Mushroom, Molecular Markers, Primers, Biodiversity.

INTRODUCTION

Mushroom is broadly defined by Chang and Miles [1] as “a macro fungus with a distinctive fruiting body which can be either epigenous or hypogenous and large enough to be seen with the naked eye and to be picked by hand”. Mushrooms appeal to different people in different ways. They are objects of beauty for artists, and for medical people they are the possible source of new drugs. There are many traditional methods for testing these fungi but they are unreliable [5].

In 1990 the magnitude of fungal diversity was estimated conservatively to be at least 1.5 million species [4]. Of the 1.5 million estimated fungi, 140,000 species produce fruiting bodies of sufficient size and suitable structure to be considered macro fungi, which can be called “mushrooms”. Of these, about 7000 species are considered to posses varying degrees of edibility, and more than 3000 species are regarded as prime edible mushrooms. To date, only 200 of them are experimentally grown, 100 economically cultivated, approximately 60 commercially cultivated, about 10 have reached an industrial scale of production in many countries [2].

Mushrooms mycelia and spores are often microscopic and usually filamentous with very few phenotypic markers that can be used to differentiate between individuals in a population. In Indian context, all edible mushrooms other than the common button mushrooms, *Agaricus* are grouped under the specialty mushrooms [13].

The use of molecular tools is almost essential to ensure that the inoculum used is from the correct species. Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features. Molecular markers, especially DNA techniques are quick and reliable to establish the identities of wild

collections and are helpful in mushroom taxonomy. All DNA markers other than RFLPs are based in some way or other upon the PCR. After the advent of cycle sequencing methodology [10], direct sequencing of PCR products became a routine matter at least in organelle DNA loci or repetitive nuclear DNA such as ribosomal DNAs [12]. This technology is considered to be one of the most powerful methods for phylogenetic studies [11].

The ribosomal RNA genes (rDNA) of fungi are located on a single chromosome and are present as repeated subunits of a tandem array of transcribed and non-transcribed stretches of DNA, which appeared highly conserved [18]. ITS (Internal Transcribed Spacer) refers to a piece of non-functional RNA situated between structural ribosomal RNAs on a common precursor transcript. Sequence comparison of the ITS region is widely used in taxonomy and molecular phylogeny because it is easy to amplify even from small quantities of DNA and has a high degree of variation between closely related species. This ITS region is most widely used to sequence the DNA region in fungi. It has typically been most useful for molecular systematics at the species level and within the species. In this study, a preliminary study on molecular characterization of wild mushroom was performed, and the results were used to assess the identity of the mushroom.

MATERIALS AND METHODS

The wild mushrooms were collected during November and December 2009 and stored in a polythene bag under refrigeration until processing. Using tissue culture technique, the mushroom was aseptically inoculated in Malt extract agar (malt extract 15 gl^{-1}, agar 20 gl^{-1}, HiMedia) and incubated under dark condition at 28°C for 5 days and were looked for the development of white mycelium. Pure cultures were raised in petriplates on Malt extract agar for 10 days to obtain uniform mycelia growth.

For DNA extraction, mycelia cultures were raised in liquid culture medium (malt extract 10gl^{-1}, glucose 5gl^{-1}) for eight days at 25°C. Total genomic DNA was extracted as described by Graham *et al*. [3].

Amplification of 5.8S rRNA gene for assessing ITS length variation was done using primer ITS1 **(**TCCGTAGGTGAACCTGCGG**)** and ITS4 (TCCTCCGCTTATTGATATGC) as described by White *et al.,* [16]. PCR amplification products were electrophoretically separated on 1.5% agarose gel prepared in 1X TAE. The gel was run for 2 hrs at 50V. Staining was done with ethidium bromide and photographed.

PCR product of the ITS - amplified region containing ITS1, 5.8S rDNA and ITS2 was directly sequenced using ITS1 (forward primer) and ITS4 (reverse primer) by Big dye Terminator method with ABI 3130 Genetic Analyzer at Chromous Biotech Pvt. Ltd., Bangalore. The sequence data were assembled and analyzed. A distance matrix was generated using the Jukes - Cantor corrected distance model. The phylogenetic tree was created using Weighbour with alphabet size 4 and length size 1000 [17].

RESULTS

Using genomic DNA isolated from the mycelial culture, an approximately 500 basepair fragment of the rDNA – ITS region was amplified using ITS1 and ITS4 primers (Fig.1) and subjected to nucleotide sequencing. The amplified product was found to have 559 basepairs (Fig. 2 & 3). The aligned sequence (Fig. 4) was deposited in GenBank and named as *Agaricomycetes* sp. India01 (Acc. No. HM167516.1). Based on Distance matrix table it was observed that the wild mushroom has homology score value of 88 with *Perenniporia* sp. (GQ982890.1). Mushrooms of medicinal importance like *Coriolopsis caperata* (GQ372861.1) and *Ganoderma pseudoferreum* (FJ374876.1) exhibited homology score value of 87 and 86, respectively (Table 1). The phylogenetic tree (Fig. 5) constructed also revealed that the test organism is closely related to *Perenniporia* sp. Based on the above results, it is assumed that this wild mushroom could be a member of the family Agaricomycetes with economic significance.

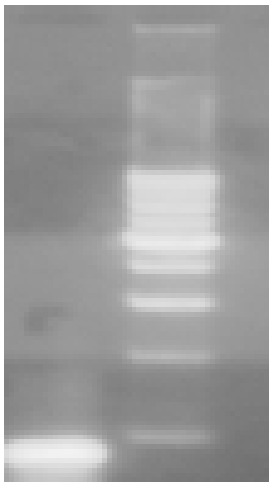
DISCUSSION

With increasing demand for edible and medicinal mushroom, it becomes a necessity to unravel the rich biodiversity of ectomycorrhizal fungi. Identification of those high-quality fungal species is not only necessary but has great economic significance as it will help in detecting fraudulent products being marketed.

Agaricomycetes function as degrading organism or pathogens, parasite, and ectomycorrhizal symbionts of forest trees. Majority of the edible mushrooms are Agaricomycetes and being collected from the garden, the mushroom taken up for the present study can possibly belong to Agaricomycetes.

Recently molecular characterization has been introduced to detect the living organisms as this procedure is specific, rapid and requires only a small amount of sample. However, this procedure does not directly reflect the pharmacological activity of the specimen being studied [7,9,15]. Although they have been proven to be efficient in taxonomic identification, the application of these methods is limited by the high cost of the fine quality template DNA that is required in these experiments.

Fig. 1 Amplified gene product on agarose gel



Lane 1 - amplified DNA of ~ 500 bp
Lane 2 - 500 bp DNA ladder

Table 1 : Alignment view and Distance matrix table
(With Sample Mushroom sequence taken as reference sequence)

NCBI Accession No.	Organism Name	Score
GQ982890.1	*Perenniporia sp.*	88
AJ132941.1	*Polyporus tricholoma*	86
GQ372861.1	*Coriolopsis caperata*	87
FJ711050.1	*Tinctoporellus epimiltinus*	87
AY216475.1	*Marasmius cladophyllus*	87
GU388303.1	*Tinctoporellus sp.*	86
FJ374876.1	*Ganoderma pseudoferreum*	86
FJ810520.1	*Coriolopsis byrsina*	86
AJ608713.1	*Ganoderma philippii*	86
FJ711056.1	*Earliella scabrosa*	86

Score: Sequence Match Score obtained based on nucleotide alignment

Singh *et al*., [14] performed molecular characterization of specialty mushrooms collected from germplasm accessions from Rajasthan using DNA fingerprinting and rRNA gene sequencing and added two new additions to the Indian basidiomycetes biodiversity. This is the first kind of report documented from India on molecular characterization of specialty mushrooms. In the present study, rDNA-ITS fragment sequencing was attempted to identify the biological identity of the wild mushroom, which again is the first report from India as far as wild mushrooms are concerned. By increasing the specificity, the results of amplification are less sensitive to changes in reaction conditions and are more reproducible [6]. Hence, *Agariciomycetes* sp.India01 can be characterized with primers constructed specifically so that it can help in molecular taxonomy of wild mushroom to a greater extent. Also, the present study validates the homology of the wild mushroom with medicinal mushrooms like *Ganoderma*, *Coriolopsis*, etc., (Table 1). Molecular identification results of the ITS sequences of 5.8S rRNA gene and published mushroom records of India validate that Agaricomycetes sp.India01 is a new addition to the Indian Agaricomycetes.

FIG. 2 DNA Sequence Using ITS1 Primer

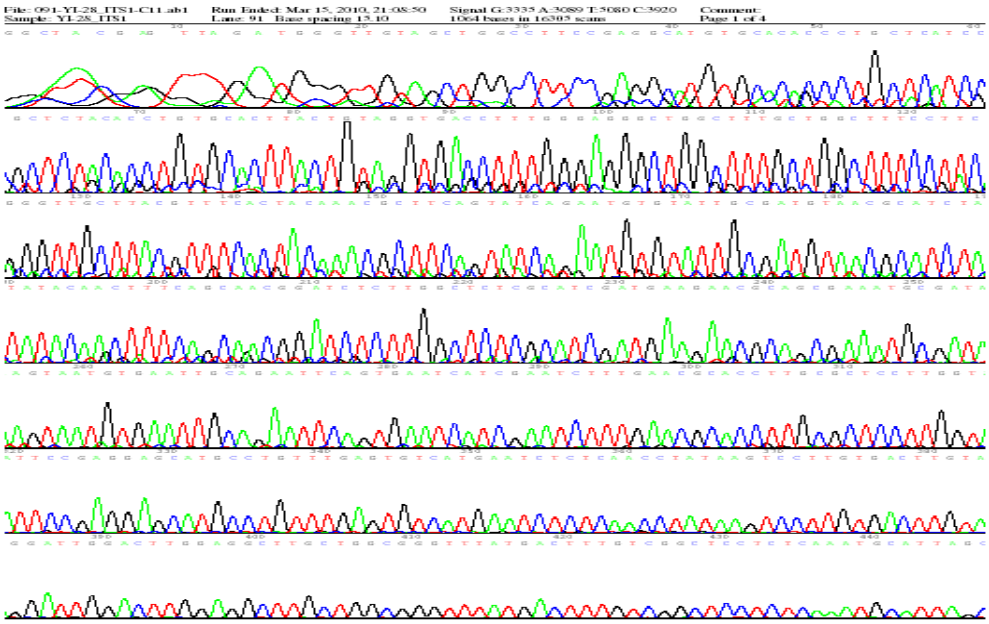


FIG. 3 DNA Sequence Using ITS4 Primer

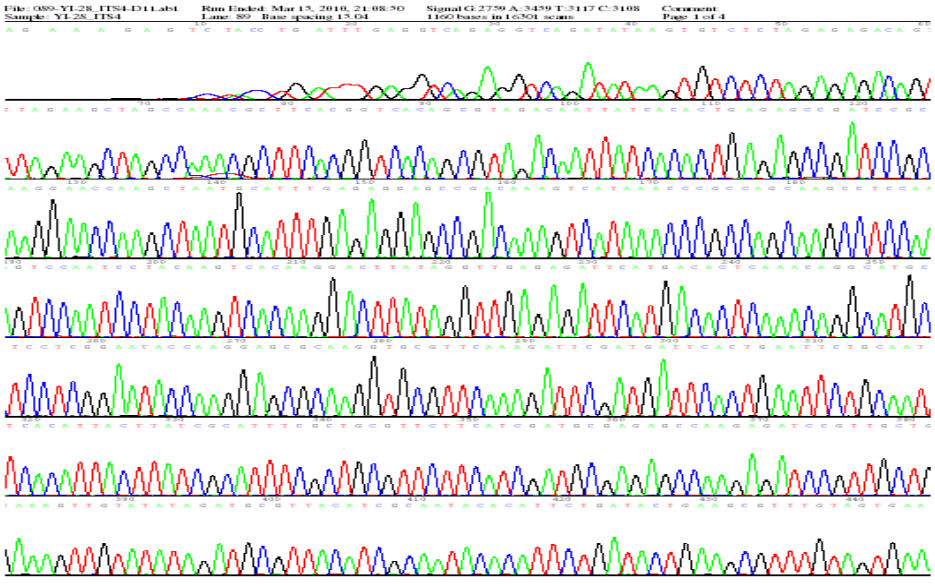
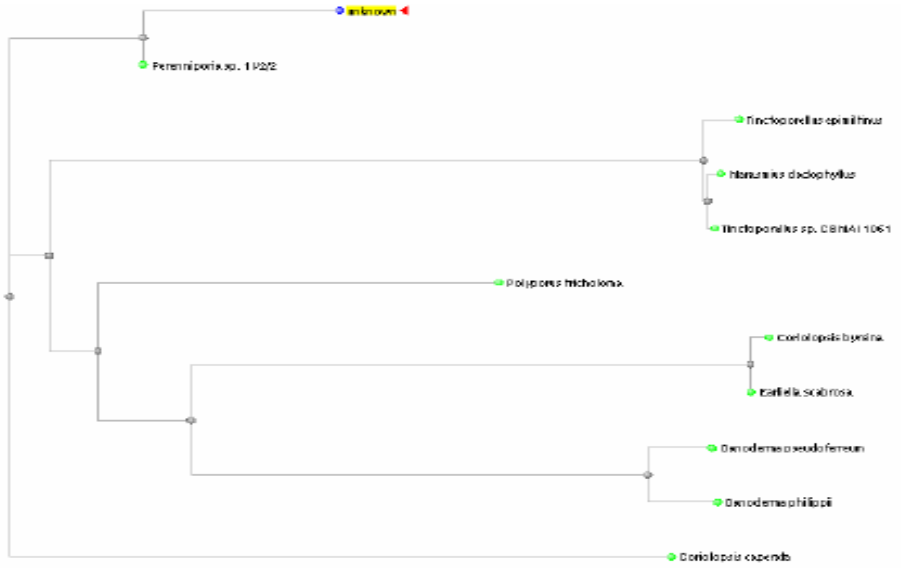


FIG. 4 Aligned Sequence Data: (559 bp)

ATGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACACCCTGCTCATCCGCTCTACACCTGTGCACTTACTGT
AGGTGACCTTTGGGAGGGCTGGCTTTGCTGGCTTTCCTTCGGGTTGCTTACGTTTCACTACAAACGCTTCA
GTATCAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCA
TCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGA
ACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAATCTCTCAACCTATAAG
TCCTTGTGACTTGTAGGATTGGACTTGGAGGCTTGCTGGCGGGTTTATGACTTTGTCGGCTCCTCTCAAAT
GCATTAGCTTGGTTCCTTGCGGATCGGCTCTCAGTGTGATAATTGTCTACGCTGTGACCGTGAAGCGTTTG
GCTAGCTTCTAACTGTCTCTCTAGAGACACTTATATCTGACCTCTGACCTCAAATCAGGTAG

FIG. 5 Phylogenetic Tree



CONCLUSION

In conclusion, an accurate and practical phylogenetic analysis establishes a theoretical foundation for defining a classified status of new edible or medicinal fungi. In addition, their evolutionary relationships could provide an important clue for further exploration of the active compounds. Furthermore, molecular characterization is an authentication of wild mushrooms.

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