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Research Article

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A New Mushroom species *Tricholosporum purpureolilacinus* Recorded from Western Ghats of Karnataka

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ABSTRACT

Western Ghats of Karnataka is one of the richest biodiversity hotspot in India. In the present study, a new mushroom species in the Genus Tricholosporum, collected from Agumbe forest in Western Ghats region during monsoon season (June-September) with the help of Siddi and Adivasi tribal community and identified by internal transcribed spacer (ITS) region sequence homology using NCBI data base.

Key words: *Western Ghats, Mushroom, ITS, Molecular Identification.*

INTRODUCTION

Mushroom growing in wild are picked up by mankind from the time immemorial. Cool and humid climate of evergreen forest canopy of Indian Western Ghats is a favourable habitat for rich mushroom biodiversity. Mushrooms are efficient degraders of lingo-celluloses; hence they play a vital role in biodegradation. Some mushrooms have pharmaceutical value such as antimicrobial, anticancer, antioxidants etc[1].

Shimoga region is in the heart of Western Ghats, which is one of the hot-spots of biodiversity in India. This district comes under south-eastern transitional zone and receives an average annual rainfall of 2869 mm [2] making an ideal habitat for blooming variety of mushrooms. Defining the number and kinds of fungi on earth has been a point of discussion and several studies have focused on enumerating the world fungal diversity. Only a fraction of total fungal wealth has been subjected to scientific scrutiny and mycologists continue to unravel the unexplored and hidden wealth. One third of fungal diversity of the globe exists in India and of this only 50 % are characterized so far[3].

Mushrooms are ephemeral and disappear within a day. Therefore, documentation of mushrooms needs constant survey during appropriate season. Mushrooms can be identified based on their morphological and molecular characters. The Phenotypic characters include the shape, size, texture, colour and odour of the fruiting body. Molecular tools such as 18S rRNA/ITS (Internal transcribed spacer) region can be used to identify mushrooms at any stage[4].

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Several wild fungi were documented elsewhere and identified using ITS sequence[5]. Unfortunately no molecular analysis has included in species of *Tricholosporum* genus and it appears to be a widespread genus as members known from North and South America, Africa, Asia, Canary Islands and Europe. In this study, we report molecular characterization of *Tricholosporum purpureolilacinus* mushrooms documented from Shimoga region of Western Ghats for the first time.

MATERIALS AND METHODS

Collection and documentation of mushrooms

Field survey was made to document the wild mushrooms in forest area of Shimoga district (Shimoga, Siddapura, Agumbe and Theertha halli) of Karnataka from June to September 2013. The survey was carried out with the help of information provided by tribal communities like Adivasis, Halakki vokkals and Siddis in the locality during the visits as they were familiar with mushroom types and season of their appearance. The mushroom samples were collected in paper bags and field notes like date, weather condition, abundance, habitat and phenotypic characters were recorded.

Molecular characterization

Genomic DNA Isolation

Total genomic DNA from cap tissue was extracted using CTAB method (Sambrook *et al*.[6]). The DNA obtained was stored in Tris-EDTA (10:1) buffer at -20°C. The DNA concentration was measured using nano drop (Eppendorff) and then PCR amplification was carried out in 40 µl reaction mixture containing 4.0µl of 10 X PCR Taq. Buffer, 4.0 µl of 10 mM dNTP's mix, 2.0 µl of ITS primers (ITS1 - 5'TCCGTAGGTGAACCTGCGG3' and ITS4- 5'TCCTCCGCTTATTGATATGC 3'), 0.6 µl of Taq. DNA polymerase, 2.0 µl of Template DNA (~50 ng) and 27.4 µl of sterile distilled water.

PCR amplification and elution

The PCR reaction was carried out in a Thermal Cycler (Applied Biosystems). Programmed as initial denaturation at 96°C for 3min, 40 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30 sec and extension at 72°C for 1 min and final extension at 72°C for 10 min. The amplified products were separated by agarose gel electrophoresis. The gel was visualized under UV light and documented using Alpha Innotech Gel documentation unit. The amplified product was eluted using Gene JET™ Gel Extraction Kit (Thermo Scientific) following manufacturer protocol. The eluted product was cloned into pTZ57R/T cloning vector using Ins T/A clone PCR product cloning kit [MBI, Fermentas Life Sciences, USA (#K1214)] after determining the appropriate vector: insert ratios[6]. The ligation reaction was performed in a 10µl reaction volume at 16°C overnight. The ligated product was transformed in to *E. coli* (DH5á) cells using heat shock method[6] and plated on Luria Berton (LB) agar medium containing antibiotic (ampicillin, 100 µg/ml). The recombinant clones were initially screened by blue white selection, followed by colony PCR using M13 forward and reverse primers[6]. The transformed colony was multiplied in LB broth containing 100µl ampicillin for overnight and the recombinant plasmid was isolated using GenElute^{TM}HP Plasmid MiniPrep Kit (Sigma, USA) following the manufactures protocol. The isolated plasmid was sequenced at Sci Genome Labs Private Ltd. Kerala, INDIA using M13 forward and reverses primers.

Sequence analysis and homology search

Sequence results were analysed with Vec Screen online software from NCBI for removing the vector contamination. Forward and reverse primer sequences were checked against each other by generating the reverse complement of the "reverse" sequence using Fast PCR Professional (Experimental test version 5. 0. 83) and aligning it with the "forward" sequence with the help of CLUSTAL W Multiple Sequence Alignment Programme using the online software SDSC Biology Workbench (San Diego Supercomputer Center). The full length gene homology search was performed with blast programme of National Centre for Biotechnology Information (NCBI) (http:// www.ncbi.nlm.nih.gov/BLAST) [7].

RESULTS AND DISCUSSION

Man has been hunting wild mushrooms for food since antiquity. Thousands of years ago, fructifications of higher fungi have been used as a source of food due to their attractive flavor and taste. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors[8]. Mushrooms are placed in a separate division called Eumycota (the true fungi). The fungal class, Basidiomycetes comprises larger group of mushroom fungi compared to Ascomycetes. Mushrooms have been used as food and medicine by the ancient Egyptian, Greek, Roman and Chinese civilizations. These fungi had attained the status of a regular crop in France and China by 17th and 19th centuries, respectively, spreading gradually to the other countries in few years. There are about 69 thousand known mushroom species of which 2000 species from more than 30 genera are regarded as prime edible mushrooms but 80 of them are grown experimentally and around 20 are cultivated commercially. To understand the occurrence, abundance, locality or habitat and edibility of the mushrooms, traditional knowledge of the tribal folks was very much essential, therefore, we sought villager's knowledge and accompanied them during the survey for collection of mushrooms.

Field information of the mushroom species was recorded during collection (Table 1). Then the sample was labeled as WGM-6. The habitats were varied from soil to tree stump and leaf litter as it is a versatility of the forest ecosystem which provides diversified niche for different types of mushrooms under same umbrella. Literatures on molecular characterization of mushrooms are limited, as earlier classification of mushroom was done only on the basis of morphological and phenotypic characters that will leads to confusion in identifying mushrooms with in the same species. However, in the 20th century scientist identified mushrooms species by using 18s RNA/ITS genes. Prakasam *et al*.[9] collected two milky mushroom (*Calocybe indica)* strains- Ci (P), Ci (N), and *Tricholoma giganteum* from Coimbatore and Erode districts of Tamil Nadu. The mushrooms were pure cultured from the cap using tissue culture method and maintained on Potato Agar slants and stored. Then they isolated genomic DNA from the pure culture and sequencing was done using ITS-1(forward) and ITS-4 (reverse primer), the nucleotide sequence were performed using Blast Multiple Alignment Tool (BLAST) network sequence against the National Centre for Biotechnology Information (NCBI) database shows 91% homology with *Tricholoma giganteum* and is given with Gene bank accession number 120872.

Table 1: Field characters of mushrooms collected from Western Ghats region of Karnataka

S. No	Mushroom collected	Date of collection	Place of collection	Vernacular name	Habitat
1	WGM -6	22/10/2013	Agumbe forest of shimogha district	Nayee anabe	Humus

Table 2: List of Mushroom species identified by ITS region sequence and their DNA amplicon size

S. No	Mushroom Species Designation	Size of amplified DNA(bp)	Mushroom species identified	Blast search homology (%)
1	WGM-6	719	*Trycholosporum porpyrophyllum*	87

Molecular tools provide more accurate methods for identification of both prokaryotes and eukaryotes. The eleven mushrooms were identified up to species level by using ITS region sequence. PCR amplification of genomic DNA of the mushroom in this study yielded amplified product sizes of 719 bp (Table, 2) which were corresponding to almost full length gene sequence of ITS. The sequence homology of WGM-6 was matched 83% homology with *Triholosporum porphyrophyllum*.

TCCTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAGTCCTACCTGATTTGAGGCCAAGTC
AAAAAAGTATAGTCTATAATAGTGACTAAATAAGTTAGAAGCTAAATATGAAAAAGGAT
TCTAAGCAAAAGGCGTAGATAATTATCACACCAAAAGCTTTGTATCCACAAAGTCTAGCT
AATGCTTTTTAGAAGAGCTGACTATAAAAGCCTGCAACTCCCATAATCCAATACTAACTT
TTGTTCAATAAAAACAAAAGCAGATTGAGAAATTTAATGACACTCAAACAGGCATGCTC
CTCGGAATACCAAGGAGCGCAAGGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGC
AATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCC
ATTGTTGAAAGTTGTATTTTATTACAAGTAACCCAAACATTCAGTTACATTCGTGATATA
ATATAATACATAGATACCCCAGAGAGAGAAATTGAATAAAGGAAAGCTGACTTTCGCAC
AGCAAACCTTCAACTCAGGCGCATATATGCCTGAAATAATAAATCCATCTAAGGAGATA
TCTACAGATAAGTGCACAAGTGGTAATTGGAATGAAGGTCAAAGTGTGCACATGCTCCT
AGGAGCCAGCAACAACCTAACCAGGTTCATTCATTAATGATCCTTCCGCAGGTTCACCTA
CGGA

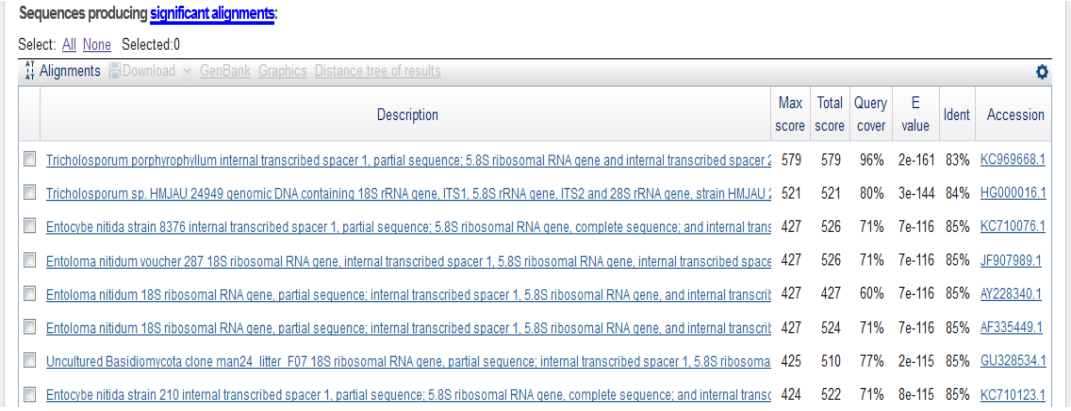

Fig. 1: Full length sequence and homology search of *Trycholosporum porpyrophyllum* (The above figure is representative of molecular identification)

Fig. 2: Mushrooms collected from Shimoga regions of Karnataka.

The ITS region/18S rRNA gene sequence are the most widely used techniques in molecular phylogenetics of mushroom as these sequences are conserved irrespective of life history and evolution[4]. An edible mushroom from the Theertha halli forest area of Western Ghats of Shivamoga district of Karnataka was identified using ITS region of ribosomal DNA sequences as *Termitomyces* sp.[10]. Our study documented the abundance of the mushroom flora from the Western ghats region (Shimoga) of Karnataka.

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