International Journal of Agriculture Sciences
ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 10, Issue 6, 2018, pp.-5551-5553.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

MOLECULAR IDENTIFICATION OF *TERMITOMYCES* SPECIES FROM WESTERN GHATS OF KARNATAKA

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Received: March 23, 2018; Revised: March 27, 2018; Accepted: March 28, 2018; Published: March 30, 2018

Abstract- Western Ghats of Karnataka is one of the richest biodiversity hotspot in India. In the present study, mushroom species in the Genus *Termitomyces,* collected from Shivamogha region of Western Ghats during monsoon season (June-September) with the help of Siddi and Adivasi tribal community. During collection, the field information was recorded and the samples were designated as Sample -1, Sample -2, Sample -3 and Sample -4. Further, these mushrooms were identified by internal transcribed spacer (ITS) region sequence homology using NCBI data base. The mushrooms identified based on sequence homology are *Termitomyces micro carpus* (Sample-1), *Termitomyces Sp* (Sample-2), *Termitomyces Sp* (Sample-3) and *Termitomyces Sp* (Sample-4). All are edible mushrooms belong to Genus *Termitomyces.*

Keywords- Western Ghats, Mushroom, ITS, Molecular identification, Genus *Termitomyces.*

Citation: Santhosh D.B., *et al*., (2018) Molecular Identification of *Termitomyces* species from Western Ghats of Karnataka. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 6, pp.-5551-5553.

Introduction

Termitophilic fungi are a monophyletic group of gilled mushrooms belonging to the genus Termitomyces. They are usually growing on termite mounds. Recently, Nobre and Aanen (2012) [2] argued that the termites harvest asexual spores of fungus along with lignocellulolytic enzymes of bacterial and fungal origin. Two-thirds of the species of the genus Termitomyces recorded worldwide occur in six states of the Western Ghats and on the west coast of India. Termitomyces micro carpus has a wide distribution in six states of the Western Ghats, while *T. cylindricus* (synonym of *T. aurantiacus*; see Tang *et al*. 2006 [3]), *T. fuliginosus* (synonym of T. robustus; see Pegler 1977), T. indicus (synonym of *T. microcarpus*). 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 (Rajarathnam and Thiagarajan., 2012 [4]). Several wild fungi were documented elsewhere and identified using ITS sequence (Oyetayo., 2012 [5]). In this study, we recorded 4 species of Termitomyces species from the Shivamogha region of Western Ghats. Where 9 species of Termitomyces recorded from Karnataka.

MATERIALSAND METHODS

Collection and documentation of mushrooms

Field survey was made from June to September 2013 from Western Ghats (Shimoga, Siddapura, Agumbe and Theerthahalli districts) of Karnataka. The survey was carried out with the help of information provided by tribal communities like Adivasis, Halakkivokkals and Siddisin 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 [6]. The DNA obtained was stored in Tris-EDTA (10:1) buffer at -20°C. The DNA concentration was measured using nanodrop (Eppendorff) and then PCR amplification was carried out in 40µl reaction mixture containing 4.0µl of 10X PCR Taq. Buffer, 4.0µl of 10m MdNTP'smix, 2.0µl of IT Sprimers (ITS1 - 5'TCCGTAGGTGAACCTGCGG3' and IT S4-5'TCCTCCGCTTATTGATATGC3'), 0.6µl of Taq. DNA polymerase, 2.0 µl of Template DNA (~50ng) 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 3 min, 40 cycles of denaturation of 94°C for 1 min, annealing at 60°C for 30sec and extension at 72°C for 1 min and final extension at 72°C for 10min. 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 GeneJET™ Gel Extraction Kit (ThermoScientific) following manufacturer protocol. The eluted product was cloned into pTZ57R/T cloning vector using InsT/A clone PCR product cloning kit [MBI, Fermentas LifeSciences, USA(#K1214)] after determining the appropriate vector: insert ratios [6]. The ligation reaction was performed in a centrifuge tube of 10 µl reaction volume at 4°C overnight in refrigerator.

Th eligated 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,100µg/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 TMHP Plasmid Mini PrepKit (Sigma, USA) following the manufactures protocol. The isolated plasmid was sequenced at SciGenome Labs Private Ltd. Kerala, IndiausingM13forwardandreversesprimers.

Sequence analysis and homology search

Sequence results were analysed with VecScreen 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 (Experimentaltestversion5.0.83) 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 (Rai [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. 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, tradition acknowledge 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].

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

S. No	Mushroom collected	Date of collection	Placeofcollection	Vernacular name	Habitat
1	Sample - 1	20/09/2013	Siddapura, Shimoga district.	Nayikode	Humus soil
2	Sample - 2	10/09/2013	Theerthahalli Shimoga district.	Nayikode	Humus soil
3	Sample - 3	17/08/2013	Siddapura Shimogha district	Huthadaanabe	Termite mound
4	Sample - 4	17/08/2013	Agumbe forest of Shimogha district	Huthadaanabe	Termitemound

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	Sample - 1	684	*Termitomyces microcarpus*	91
2	Sample - 2	737	*Termitomyces sp.*	99
3	Sample - 3	735	*Termitomyces sp.*	99
4	Sample - 4	773	*Termitomyces sp.*	99

TTAAGTTCAGCGGGTATCCTACCTGATTTGAGGTCAAATGGTCAA
AATGATTCCCCCTTATAAATCCCGATGATACACGTTAAAATCAAA
AAGGCCCCATTATTCAACCGACTGCACGCGATGTAGATAATTATC
ACACCACGAGCAAGTCAACAAAGGGTTCCACTAATGCATTTAAG
GGGAGCTGACTTCGAAATGAAGCCGGGGAAACCCCCACAATCC
AAGCCTATCCAAGCTCGCAAAAGCTGGTTAGGTTGAGAATTTAAT
GACACTCAAACAGGCATGCTCCTCGGAATACCAAGGAGCGCAAG
GTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACAT
TACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAG
AGATCCGTTGCTGAAAGTTGTATTTGATTAAAGGCACCAAAGGG
CCAATAACAAAACATTCTAATACATTCTTTACGGGGTATAATAAAA
TGCATAGACCGGAAATGCAGGGAAAGCCGGCTGCTTTGGCAGC
GCAGCAACCCCCCAAACCGAGGGTTTGACCCCTCGAGAGGTAT
GCTTGCATGCAGGCCTCTGCAGTCGA

Fig-1 Full length sequence and homology search of *Termitomyces microcarpus* (The above figure is representative of molecular identification).

a) Sample 1

b) Sample 2

c) Sample 3

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d) Sample 4

Fig-2 Mushrooms collected from Shimoga regions of Karnataka. a) *Termitomyces sp,* b) *Termitomyces sp,* c) *Termitomyces sp.,* d) *Termitomyces sp.*

The habitats were termite mound, humus soil 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. They isolated genomic DNA from the pure culture and sequencing was done using ITS-1(forward) and ITS-4 (reverse primer), the nucleotide sequence was performed using Basic Local Alignment Search 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.

Application of research: We can correctly identify the particular species without confusion and easily identify new edible mushroom flora in the vicinity.

Research Category: Molecular diversity identification

Abbreviations:
BLAST: Basic Local Alignment Search Tool

Acknowledgement / Funding: Author thankful to University of Agricultural sciences, Bengaluru, 560065, Karnataka, India. Author thankful to Department of Biotechnology (DBT), Govt of India for financial support for this research.

***Major Advisor of Research Guide:** Dr Earanna N.
University: University of Agricultural sciences, Bengaluru, 560065, Karnataka
Research project name or number: Ethnobotanical survey and molecular characterization of wild mushrooms from Western Ghats of Karnataka.

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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