

# Morphological and Molecular Identification of Wild Mushroom Associated with Roots of Date Palm (*Phoenix sylvestris* L Roxb)

B. Usha Rani\*<sup>1</sup> and B. Angel Jenefa<sup>2</sup>

<sup>1,2</sup> Department of Microbiology, Annai Violet Arts and Science College, Ambattur, Chennai - 600 053, Tamil Nadu, India

Received: 22 Jun 2024; Revised accepted: 23 Aug 2024

## Abstract

The world population is increasing at a faster rate. Shortage of food and diminishing of human health can occur due to increase in population. Consumption of edible fungi to fulfill human nutritional needs has been a common denominator in the history of mankind. Identification and cultivation of warm-weather (30~38°C) varieties of edible mushrooms has been scientifically challenging. Milky white is one of such mushroom varieties, where complete commercial production techniques have been standardized. This type of mushroom have more medicinal values and uses and easy to cultivate. Macroscopic and microscopic evaluation is the preliminary step used for identification of this wild mushroom species. The genomic DNA of wild mushroom associated with roots of roots of Date palm tree was isolated its quality was assessed spectrophotometrically. The sequence was obtained using ITS as the marker, the sequence was converted to barcode and the sequences were submitted to GenBank. Based on the ITS region sequences submitted to GenBank for sequence. Alignment shows 98.90% similarity to *Calocybe indica* DMRO303 strain.

**Key words:** SDA culture, LPCB staining, KOH mount test, CTAB method, FASTA and BLAST *Calocybe indica*, *Phoenix sylvestris*

Globally there are 14000 different species of mushrooms present in the world. Out of these 2000 species are edible and also used for therapeutic formulation and human consumption [1-2]. *Calocybe indica*, mushroom variety was first identified in the Eastern Indian State of West Bengal and can be cultivated at a high temperature range (30~38°C). However, no commercial cultivation was made until 1998. Krishnamoorthy 1997 rediscovered the fungus from Tamil Nadu, India and standardized the commercial production techniques for the first time in the world. It is mainly a grassland species, saprophytic (organisms which obtain nutrients from dead organic matter) in nature and sometimes ectomycorrhizal [3-5]. It has over 100 medicinal functions [6]. These mushrooms composed of vital phytochemicals [7-8] and the nutritional, pharmaceutical, bioremediation, and biodegradation qualities of mushrooms are expanding by the day, and have accelerated in recent years [9].

*Phoenix sylvestris* is commonly known as Indian date. It is a rich source of carbohydrate, phytochemicals, dietary fibers, essential vitamins and minerals. Each parts of the plant exhibit diverse medicinal properties [10]. Trees can reach a height of up to 40 feet [11]. Therefore, keeping in view the importance of milky mushroom in India, identification of wild mushrooms on roots of date palm *Phoenix sylvestris* were carried out.

## MATERIALS AND METHODS

**Source of the organisms:** Mushroom sample was collected from Annai Violet Arts and Science College, Menambedu, Ambattur, Chennai 53.

**Macroscopic identification:** The length and breadth of mushrooms cap and stem are measured with measuring tape in centimeters. The weight of the mushroom is measured with weighing machine in kilograms. The mushrooms colour, shape, size, texture, odour are noted.

**Microscopic identification:** The basidiocarp is air dried and split open longitudinally from center and vegetative mycelial bits are cut from the collar region and placed in sterile Petri plates having SDA culture media. Plates were incubated at room temperature and new mycelium growth over the tissue is observed within 4-5 days.

**Staining:** A drop of LPCB stain was placed on the clean glass slide. The mycelia and spores were taken with the help of the sterile loop and placed on the slide to be uniformly teased. The slide was then covered with a cover slip and observed under the microscope.

**Staining for observation of basidia:** To visualize fungi and their structure 10% KOH solution is used as a reagent for the KOH mount test.

**Genomic DNA isolation:** The genomic DNA was isolated from the fungal tissue sample by CTAB extraction method. The quality and concentration of genomic DNA were checked by running the DNA sample on 1% agarose gel. The DNA concentrations were rechecked by visual assessment of band intensity under UV-trans-illuminator. The quantity of 1 µl

\*Correspondence to: B. Usha Rani, E-mail: ushaanusha9901@gmail.com; Tel: +91 9361354486

**Citation:** Rani BU, Jenefa BA. 2024. Morphological and molecular identification of wild mushroom associated with roots of date palm (*Phoenix sylvestris* L. Roxb). *Res. Jr. Agril. Sci.* 15(4): 1092-1095.

of isolated DNA was checked using Nanodrop. The isolated DNA was used as a template for PCR reaction and carried out in a thermocycler. The PCR products were then loaded onto 1% agarose gel and the amplification was confirmed.

#### Sequence analysis and submission in NCBI

FASTA format of the nucleotides were obtained using Finch TV from the chromatogram. The FASTA was fed into Basic Local Alignment Search Tool (BLAST) algorithm of NCBI to identify the closest matching sequence in the nucleotide database of GenBank. After confirmation of the species, the sequence was submitted to NCBI with the necessary details to obtain GenBank ID. The sequence was converted to Barcode using the software BioRad barcode generator.

## RESULTS AND DISCUSSION

Macroscopic and microscopic evaluation is the preliminary step for identification of mushroom species. The wild mushroom associated with roots of roots of Date palm tree is white in colour, umbrella-shaped, or convex in appearance, while further flattening occurs as the mushroom ages while the

stipe is bulbous and both the ring and the volva are absent. They possess a distinctive farinaceous odour. The mushroom cap measures 46 cm length and 36 cm in breadth which is high as compared to a local isolate of *Calocybe indica* (DMRO-1199) which was 14.23 cm with convex shape. The cylindrical stem is approximately 10-20 cm in length while most other species wild species show length of 3-7 cm [12]. The weight of the mushroom *Calocybe indica* is about 4.1 kg which is high as compared to a local isolate of *Calocybe indica* (DMRO-1199) which was 1.85 Kg. The length of our isolate cap was large, and stem was short as compared to other isolates. The spore print is white, and the spores shape is oval. The basidia is clavate has two sterigma on the top of the basidium. The hyphae are long and elongated. White cottony mycelia with clear margin were observed in SDA plate. The genomic DNA was isolated from the authenticated sample and its quality was assessed spectrophotometrically followed by agarose gel electrophoresis. The genomic DNA concentration was 1873.6 ng/μl. The sequence was obtained using ITS as the marker, the sequence was converted to barcode and the sequences were submitted to GenBank. Based on the ITS region sequences submitted to GenBank for sequence Alignment shows 98.90% similarity to *Calocybe indica* DMRO303 strain.

### SCRIPCOGCF07



### SCRIPCOGCF08

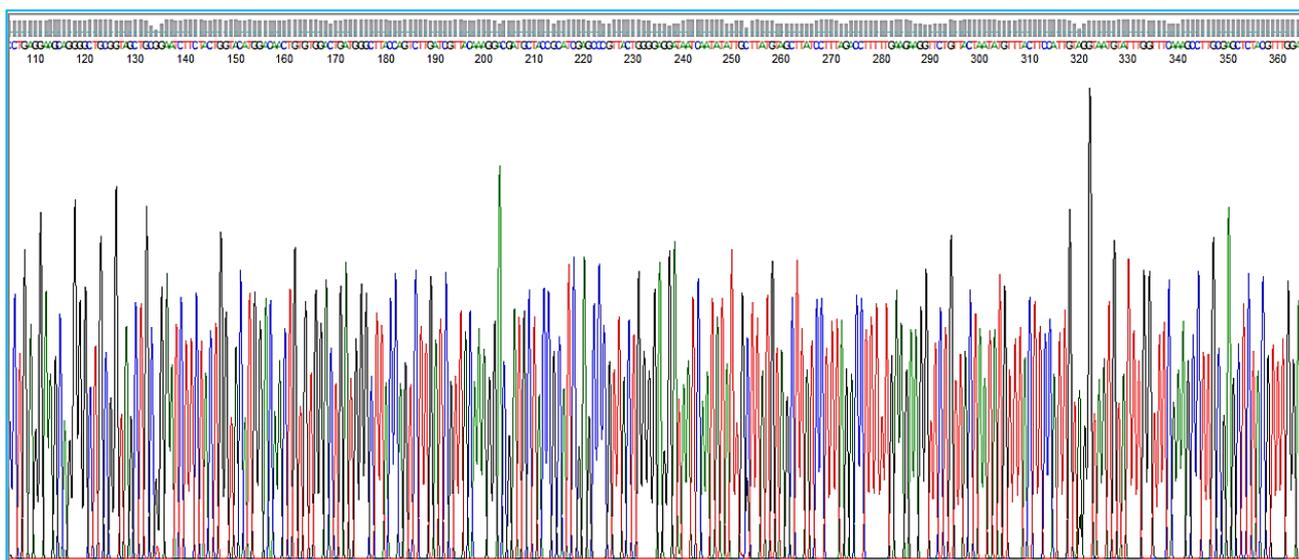


Fig 2 Chromatograms of *Calocybe indica*

Table 2 Sequence obtained

Sample code	Sequence with Barcode
>SCRIPCOGCF 13 (Forward)	tcaagttgattcgtggctctctcagagcatgtgcacactggctctgttttctaaaccccttgggcacctttgtatacattggaaaagattgagacta gagagagctcgggccccacacatcccagactatgtcttttcatatcattttactctgtgtgtaaaaaagtgttctcaagcgttattaatgccctaataca tatacaactttcaacaacggagatcttggctctctcatctataaaaaaaaaacccgaaatgcgataaaaaatgtgaattgtgcaaatctctgagt
>SCRIPCOGCF 13 (Reverse)	ataatcaagacagtttgggttctcgttagaagctgaaccacacaatatcgtgatgagccagcgtagataactatcacaccaggagactaa tcaacgatgggtcccactaatgtatttcagaggagccgactctttcaacaaaagccagcaactccactccagactctttga

Table 3 GenBank submission details

Voucher no.	Submission Id	Accession No. (Gen Bank link under processing)	Authors
SCRIPCOGCF13	SUB14353316	PP574457 PP574458	Usha Rani B, Angel Jenefa B, Sunil Kumar K. N., Divya, K. G, Remya A. and Pushkar K.



Fig 1



Fig 2



Fig 3 Mushroom (*Calocybe indica*)



Fig 4 Mycelium growth on SDA



Fig 5 Microscopic structure of mycelium



Fig 6 Collection of spore print

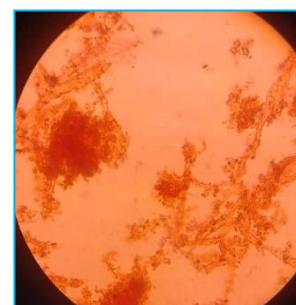


Fig 7 Microscopic structure of mycelium



Fig 8 Length = 46cm



Fig 9 Breadth = 36cm



Fig 10 Stem of mushroom (*Calocybe indica*)



Fig 11 Weight = 4.1kg

## CONCLUSION

In the present study, the milky mushroom *Calocybe indica* is associated with roots of Date palm tree (*Phoenix sylvestris* L Roxb). Macroscopic and microscopic evaluation is done as the preliminary step for identification of mushroom species. The molecular techniques used for precise

identification suggests that DNA isolated from wild mushroom was *Calocybe indica*. This mushroom variety can be suitable for commercial production in humid tropical and subtropical regions. Still today no studies have been done on growth of mushroom on roots of Date palm *Phoenix sylvestris*. Further purification, identification and characterization of the active compounds would be our priority in future studies.

## LITERATURE CITED

1. Mleczek M, Rzymiski P, Budka A, Siwulski M, Jasińska A, Kalač P, Niedzielski P. 2018; Elemental characteristics of mushroom species cultivated in China and Poland. *Jr. Food Compos. Anal.* 66: 168-178.
2. Garofalo C, Osimani A, Milanović V, Taccari M, Cardinali F, Aquilanti L, Clementi F. 2017; The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. *Food Microbiology* 62: 15-22.
3. Vikineswary S, Chang ST. 2013. Edible and medicinal mushrooms for sub-health intervention and prevention of lifestyle diseases. *Tech Monitor.* 33-43.
4. Purkayastha RP. 1984-1985: Cultivation of *Calocybe indica* (P&C). *Indian Jr. Mushrooms* 10-17.
5. Doshi A, Sidana N, Chakravarti BP. 1989; Cultivation of summer mushroom *Calocybe indica* (P&C) in Rajasthan. *Mushroom Science* 12: 395-400.
6. Valverde ME, Hernández-Pérez T, Paredes-López O. 2015. Edible mushrooms: Improving human health and promoting quality life. *Int. Jr. Microbiology* 376387.
7. Chelladurai G, Yadav TK, Pathak RK. 2021. Chemical composition and nutritional value of paddy straw milky mushroom (*Calocybe indica*). *Nat. Environ. Pollution Technology* 20: 1157-1164.
8. Maurya AK, John V, Murmu R, Simon S. 2019. Impact of different substrates for spawn production and production of milky mushroom (*Calocybe indica*) *Int. Jr. Pharma. Bio. Science* 10: 5-10.
9. Chaturvedi VK, Agarwal S, Gupta KK, Ramteke PW, Singh MP. 2018. Medicinal mushroom: Boon for therapeutic applications. *3 Biotech* 8: 1-20.
10. Jain P, Jain S, Sharma S, Paliwal S. 2018. Diverse application of (Phoenix Sylvestris) A potential herb. *Agriculture and Natural Resource* 52: 107-114.
11. Perveen, S, Khan AM, Yasmin T. 2016. Antifungal and antibacterial potential leaves extract of *Nannorrhops ritchiana* (Griff), *Phoenix sylvestris* (Linn) and *Olea ferruginea* Royle. *Bulletin of Environment, Pharmacology* 5(12): 35-43.
12. Bhupathi P, Subbaiah KA. 2019. Comparison of colony morphology, sporophore characters and yield performance of wild and cultivated milky mushroom isolates. *Jr. Pure Applied Microbiology* 13(4): 2405-2419.