

Morphological and Molecular Identification of Oyster Mushroom [*Pleurotus Ostreatus* (Jacq.) P. Kumm]

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Research Article

Keywords: Oyster mushroom, *Pleurotus ostreatus*, Morphology, ITS, NCBI

Posted Date: October 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-934342/v1>

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Abstract

Oyster mushroom (*Pleurotus ostreatus*) is fleshy, edible fungi and used throughout the world. Among the most well-known species of mushrooms, *Pleurotus* sp. is the third most popularly grown mushroom in the world and rank second in India. The most well-known species of *Pleurotus* are *P. ostreatus*, *P. florida*, *P. eryngii*, *P. cystidiosis*, *P. flabellatus*, *P. cornucopie*, and *P. sajor-caju*. According to the morphological, molecular character and ITS sequencing, the *Pleurotus ostreatus* was distinguish from the other species. The cap (pileus) of the oyster mushroom has whitish to grey color and fleshy. Stipe cream and smooth surface. Spores are whitish to lilac grey in mass, cylindrical to oblong shape. Spore print was white in color. Whitish mycelium growth in PDA plate. The ITS sequence was submitted to gene bank NCBI and it was accepted by NCBI with accession number MW446165.

Introduction

Oyster mushroom (*Pleurotus ostreatus*) is also known as 'Dhingri' belongs to the family *Pleurotaceae*. It is inevitable diet food in India. The word mushroom is derived from the Gallo Roman *mussiro* which is defined as a "macro-fungus" that has a fleshy and used throughout the world to describe the fruiting bodies of saprophytic, mycorrhizal and parasitic fungi. It is either epigenous or hypogenous and large enough to be seen with the naked eye and to be picked by hand [1]. The fruiting bodies of this mushroom are observed in different shades of white, cream, grey, yellow, pink or light brown depending upon the species. The oyster mushroom has three distinct parts - a flashy shell or spatula shaped cap (Pileus), a short or long lateral or central stalk called *stipe* and long ridges and furrows underneath the pileus called gills or *lamellae*. The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value.

The genetic study of mushroom has been worked out using molecular markers especially polymerase chain reaction (PCR). It is effective tools of plant biotechnology used for the assessment of genetic diversity. oyster mushroom (*Pleurotus* sp.) was differentiated on the basis of morphological (sporocarp, sporophore, colour, size), cultural, ITS sequencing (accession number) and Phylogenetic analysis [2, 3].

Materials And Methods

3.1 Morphological identification

The *Pleurotus ostreatus* was identified based on the various morphological characters such as mycelium growth, height of fruiting body, stipe length, stipe diameter, pileus diameter, microscopic view of mushroom spore and spore print.

3.2 Growth and pure culture of mushroom

Potato dextrose agar (PDA) medium was poured in sterilized Petri plate and inoculated with 10 mm disc of *Pleurotus ostreatus* under aseptic condition. Then, the Petri plate were incubated at $25 \pm 2^\circ\text{C}$. The

diameter of colony was measured after 6 days of growth. sub culturing was done on PDA slants as and when needed.

3.3 Microscopic view of mushroom spore

Mushroom spore studied by observing cotton blue stained slides under compound light microscope by using 40x objective and 10x eyepiece lenses. After calibrating the microscope, the measurement of spore was taken with the help of microscopic measuring slide [4].

3.4 Spore print

While a single mushroom spore can't be seen by the naked eye, a pile of many spores and color of a mushroom's spores is an easily identify by obtaining a mushroom's "spore print".

To make a spore print first remove the stem from smaller mushroom and place the cap, gills or pores downward, on a piece of paper or glass. For white color oyster mushroom using a black paper while white paper used for pink oyster mushroom. In case of large mushroom, slices off a section of the cap and use only the section. Place a cup or glass upside-down on top of mushroom, to keep in air tight condition. After overnight, when remove the cap and lift the mushroom cap, we got a print like the ones illustrated to the left [5].

3.5 Molecular identification

Mushroom species was identified based on molecular method. In molecular method, genome sequencing, was done for ITS region. For genome sequencing the pure culture of *Pleurotus* sp. was sent to Eurofins Genomics India Pvt. Ltd., Bangalore. The fungal genomic DNA was extracted from mycelia grown in 250 ml of PDB at 28°C for 5 days. The mycelia were harvested from broth and lyophilised and stored at -20°C for further process.

3.6 DNA extraction

The genomic DNA for PCR was extracted by using DNA isolation kit. The ITS region of fungi, including ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTTATTGATATG3') were amplified. The amplification was performed in 30 µl reaction volume with 0.1 mM of each dNTP and 100pmol of both forward and reverse primer. ABI Veriti pcr was programmed for initial denaturation at 94°C for 4 min, and 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The amplification was completed with a final extension at 72°C for 5 min. Further it was sequenced by ABI 3730 capillary sequencing [6].

3.7 Sequencing

Consensus sequence of the PCR amplicon was generated from forward and reverse sequence data using aligner software. 6. The ITS region sequence was used to carry out BLAST with the database of NCBI GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Cluster W. Distance matrix was generated and the phylogenetic tree was constructed using MEGA X [6].

Results

Oyster mushroom (*Pleurotus* sp.) was distinguished on the basis of their morphological and Molecular characteristics. Morphologically identified by mushroom mycelial growth on PDA plate, spore print, spore size and also identified by measuring length and diameter of pileus and stipe from different collected samples of oyster mushroom (Fig. 1).

4.1 Cap (pileus): 5.6 - 11.2 cm in diameter, Whitish to grey color, convex, smooth, soft, maturing to a shell shape.

4.2 Stipe: 4.7 - 7.2 cm in length; 1.3 – 2.2 cm in diameter, Cream and smooth surface.

4.3 Spore and spore print: Spores are whitish to lilac grey in mass, cylindrical to oblong shape, Spore print was white in color. Whitish mycelium growth in PDA plate.

4.4 Molecular identification

The identification sequence was BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and similarity with other species computed. The evolutionary history (Fig. 2) was inferred using the UPGMA method [7]. The optimal tree with the sum of branch length = 0.02507224 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) shown next to the branches [8]. The evolutionary distances were computed using the Maximum Composite Likelihood method [9] and were in the units of the number of base substitutions per site. This analysis involved 5 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 703 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [6].

The BLAST analysis of the ITS sequence data, (Table-1) supported the morphological identification, whereby the closest match with NCBI sequence number MT261808 (100 %) in the NCBI GenBank database was found to be the *Pleurotus ostreatus*. Further, the sequence was submitted to gene bank NCBI and it was accepted by NCBI with accession number MW446165. So, based on the morphological and molecular methods the oyster mushroom was identified as *Pleurotus ostreatus*.

Discussion

5.1 Morphological studies

As per the finding of this study, the morphological characteristics of *Pleurtous ostreatus* was more or less similar with Chhetry and Pfoze [10] who reported that the pileus of *Pleurotus* spp. was soft, smooth, light yellow with 4.5-11 cm diameter. It possessed white, broad decurrent gills with often lateral *stipe*, usually elongated from 5-8 cm with white, solid, several fruit bodies joined at the base to form a large common base. The fresh sample was soft, white with mild odour. Ram [11] showed that *Pleurotus* sp. was morphologically characterized by white spores with an eccentric or lateral stem of fleshy texture. Cap was

2-15 cm wide with 3-11 cm long and upper surface smooth with white, spathulae to kidney shaped, margin decurved or inrolled. Stem was usually short or stem poke base, imbricate in groups of 5-20 cm. Gills were 18-20 cm at margin, 5-15 mm wide, decurrent, sometimes uniting to form a net or pore like pattern on the stem, white when fresh, yellowish when dried. Murugesan *et al.* [12] isolated oyster mushroom from forest region and identified *Pleurotus* sp. based on microscopic view and phylogenetic tree.

5.2 Molecular identification

The study confirmed that the *Pleurotus ostreatus* MW446165 sequence was shown 94 per cent similarity with *Pleurotus ostreatus* MT261808. Similarly, it was 86 per cent match with *Pleurotus ostreatus* MK603976. Shnyreva and Shnyreva [13] analyzed ten *Pleurotus* sp. based on Internal Transcribed Spacer (ITS) sequences of rDNA. A phylogenetic tree was constructed on the basis of 31 oyster fungi strains of different origin and 10 reference sequences from Gene Bank to fungal species identification. They were revealed the divergence between commercial strains and natural isolates of *Pleurotus ostreatus* by phylogenetic analysis.

Oyster mushroom (*Pleurotus ostreatus*) was molecular identified by using ITS primer, which was closest match with result of Imtiaz *et al.* [14]. They were collected twenty strains of *Pleurotus* spp. from different region and used DNA sequences of the ITS (Internal Transcribed Spacer) region to analyse the genetic diversity of *Pleurotus* strains. Result indicated that the among the 20 strains, the major one included *P. ostreatus*, *P. cystidiosus*, *P. eryngii*, and *P. pulmonarius*. Similarly, Liu *et al.* [15] assessed the genetic diversity of *Pleurotus ostreatus* strains on the basis of the Internal Transcribed Spacers (ITS) sequence, translation elongation factor (EF1 α) and the second largest subunit of RNA polymerase II (RPB2). The polygenetic tree constructed using combined results of the ITS, EF1 α and RPB2 sequence analyses showed the genetic relationship between studied strains. They were provided valuable information on its relationship and identified *Pleurotus ostreatus* on molecular basis.

Conclusion

Mushroom is a type of macro fungus that form large fructification visible without the help of microscope. The collected samples of mushroom fungus were further identified based on various morphological characters and molecular features. On the basis of morphological characters *viz.*, whitish mycelium growth, spore print, length and diameter of stipe and pileus, the fungus was identified as *Pleurotus ostreatus* and it was also matched with sequencing and BLAST results.

Declarations

7.0 Acknowledgement:

I am thankful to Department of Plant Pathology, College of Agriculture, JAU, Junagadh, and Authority of Junagadh Agricultural University for providing facility to conduct the research work.

8.0 Conflict of Interest

Do not have any conflicts of interest to declare.

9.0 Authors' Contribution

A. U S Kotadiya: Conducted the research and written the paper

B. J R Talaviya: Technical guidance in conducted experiment and corresponding author

C. K D Shah: Helped in analysis.

D. S V Lathiya: Helped in written the research paper and conducting experiment.

10. Data Availability statements

ITS sequence was sent to NCBI and it is available with nucleotide database number MW446165.1

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Tables

Table-1 Sequence data

Oyster mushroom	Sequence	NCBI Accession number
<i>Pleurotus ostreatus</i>	<pre> 1 caaccacctg tgaactttg atagatctgt gaagtcgtct ctcaagtcgt cagacttggt 61 tgctgggatt taaacgtctc ggtgtgacta cgcagtctat ttacttacac accccaaatg 121 tatgtctacg aatgtcattt aatgggcctt gtcctttaa accataatac aactttcaac 181 aacggatctc ttggctctcg catcgatgaa gaacgcagcg aaatgcgata agtaatgtga 241 attgcagaat tcagtgaatc atcgaatctt tgaacgcacc ttgcgccctt tggtattccg 301 aggggcatgc ctgtttgagt gtcattaaat tctcaaactc actttggttt ctttccaatt 361 gtgatgtttg gattgttggg ggctgctggc cttgacaggt cggtcctctt taaatgcatt 421 agcaggactt ctcattgcct ctgcgcata tgtgataatt atcactcacc aatagcacgc 481 atgaatagag tc </pre>	MW446165

Figures



Spore print



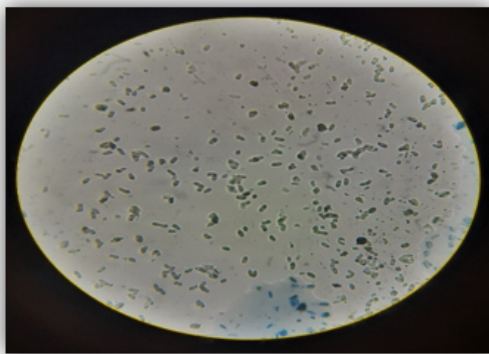
Pure culture



Gills



Pileus



Microscopic view

Figure 1

Morphological identification of *Pleurotus ostreatus*

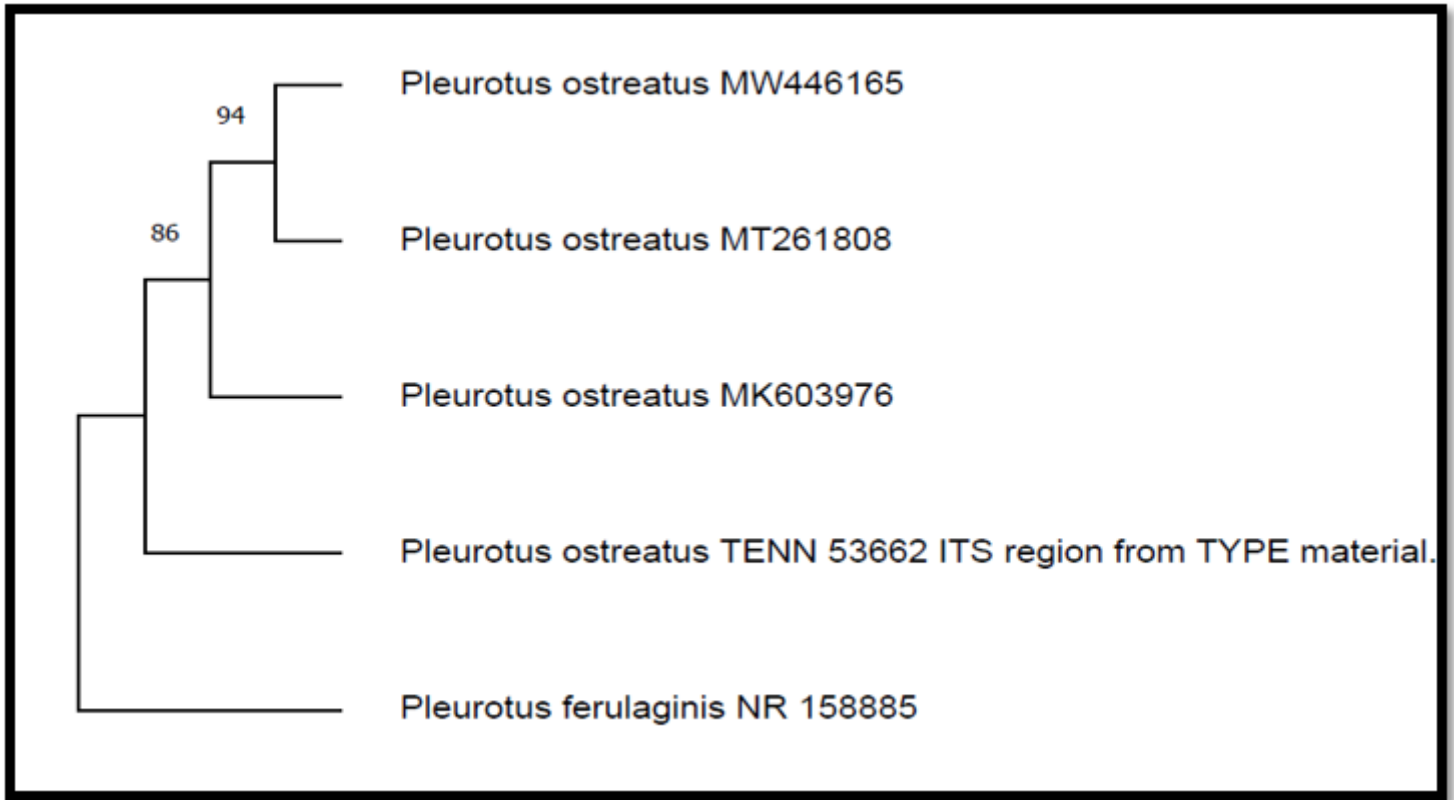


Figure 2

Phylogenetic relationship of *Pleurotus ostreatus* isolate with other species