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Morphological and Molecular Characterization of Oyster Mushroom (*Pleurotus cystidiosus*)

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A B S T R A C T

Mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand. The oyster mushroom (*Pleurotus* sp.) is highly suitable for commercial cultivation in subtropical regions of the world. They occur seasonally all over the world in various habitats such as humus rich soils, decaying plant litter and wood logs in forests as well as in meadows. The current study deals with the studies on survey, morphological and molecular characterization. The collected samples were further identified as *Pleurotus cystidiosus* on the basis of morphological characters like pileus colour, diameter, shape, stipe colour, length, diameter, gills attachment and spore print. The molecular identification methods gave the similar results, in which strains of oyster mushroom was similar with *Pleurotus cystidiosus* strain P-24.

Introduction

The Indian sub continent is blessed with diverse agroclimatic zones that harbour a treasure trove of fungal diversity. Though, the occurrence of mushrooms is of diverse nature in India. Oyster mushroom (*Pleurotus* sp.) belongs to class: *Agaricomycetes* and family: *Pleurotaceae* is popularly known as 'dhingri' in India and grows naturally in the temperate and tropical forests on dead and decaying wooden logs or sometimes on dying trunks of deciduous or coniferous woods. It may also grow on decaying organic matter.

The oyster mushrooms have three distinct parts- a fleshy 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 gills stretch from the edge of the cap down to the stalk and bear the spores. The spores are smooth, cylindrical and germinate very easily on any kind of mycological media within 48-96 hrs. The mycelium of *Pleurotus* is pure white in colour. There is almost need of advance technique used for species identification beyond morphological and physiological criteria, because these characteristics are highly influenced by environmental conditions. The expression of particular gene is a cumulative outcome of environment and genetic makeup of a specie/strain (Kumar, 1999; Astarini *et al.*, 2004). Biochemical markers can be a source to reflect the genetic study because they are

direct product of genes. DNA finger printing is also one of the efficient tools of plant biotechnology used for the assessment of genetic diversity (Mehmood *et al.,* 2008). For long, different DNA markers along with morphological traits have been used for the determination of species at molecular level (Sajida *et al.,* 2009).

The genetic study of mushroom has been worked out using molecular markers especially polymerase chain reaction (PCR).

Materials and Methods

Survey

Surveys are particularly sensitive to timing and location. Therefore, present survey was done during period of July 1st 2016 to October 31st 2016 from south Gujarat.

Sampling

Sampling was done using opportunistic sampling method (Mueller *et al.,* 2004).

Collection and Observation

Specimen was collected along with substratum to facilitate identification. The specimens were collected in the box for further identification in the laboratory (Afyon *et al.,* 2005). The collected specimen was brought to the lab and preceded further for detection. The habitat and morphological characteristics of macrofungi was noted and photographed for detection during the collection (Kaya, 2005).

Molecular studies

DNA extraction

The protocol suggested by Sambrook *et al.,* (2001) was followed for DNA isolation from fungal species.

DNA was isolated from the culture *Pleurotus cystidiosus*. Quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Isolated DNA was amplified with *18S rRNA* Specific Primer (1Fand 4R) using Veriti® 99 well Thermal Cycler (Model No. 9902). A single discrete PCR amplicon band of 900 bp was observed.

PCR amplification

The PCR amplicon was enzymatically purified and further subjected to Sanger Sequencing. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with 1F and 4R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Sequencing

Consensus sequence of 839 bp of *18S* gene in SSU region was generated from forward and reverse sequence data using aligner software. The *18S* gene in SSU region sequence was used to carry out BLAST alignment search tool of NCBI gen bank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 5.

Results and Discussion

To find the distribution and occurrence of different species of oyster mushroom in particular area, survey was conducted during July 1st to October 31st 2016 at the different selected areas of Navsari district (Navsari and Vansada). Samples of oyster mushroom were collected from selected area of Navsari district. They were also recorded for their presence and absence from different selected places.

A single sample of oyster mushrooms was collected from Navsari district. The collected oyster mushroom found to be associated with bark of tulip tree (*Spathodea campanulata*) at the height of 1.5 m. A single sample of oyster mushroom was collected during the survey. This sample was labelled properly and carried to the place of study for further identification.

The distribution pattern varied with the collected locations. This may be due to climatic conditions, natural habitat and human disturbance (Borkar *et al.*,2015).

A collected sample was further processed for their detection based on molecular and morphological characters like pileus colour, diameter, shape, stipe colour, length, diameter and gills colour based on the molecular and morphological identification, the collected samples were identified as follows:

Pleurotus cystidiosus

Pileus

140-150 to 75-80 mm

Pleurotoid surface brown to grayish when young with numerous punctiform squamules formed by surface cracking, more numerous towards to margin.

Stipe

Brown to grayish, lateral 35-45 to 20-30 mm, tapered to the base context white, 15 to 28 mm broad, fleshy when fresh, compact, corky when dry.

Spore

12-16 × 4.0-6.0 µm hyaline, cylindrical-oblong, thin walled, smooth

Spore print: Whitish

Gill

Lamellae white when fresh, yellow when dry, 4-10 mm broad, thinner towards the stipe, decurrent and forming pseudoreticulum.

Based on morphological identification of the collected sample of oyster mushroom, it was *Pleurotus cystidiosus*.

Molecular studies

The culture, which was labeled as PC showed similarity with Pleurotus cystidiosus strain P-24 (Accession Number: FJ379283.1) based on nucleotide homology a phylogenetic analysis.

Information about other close homolog's for the microbe can be found from the Alignment View table (Table 1).

Consensus sequence of (839 bp)

CTCCTCATTGCCGTATATTAAAGTTGTT
GCAGTTAAAAAGCTCGTAGTTGAACTT
CAGACCCGGCTGGGCGGTCCGCCTAAC
GGCGTGTACTGTCTGGCTGGGCCTTAC
CTCTTGGTGAGCCGGCGTGCCCTTTAT
TGGTGTGCGTTGGGGAACCAGGACTTT
TACCTTGAGAAAATTAGAGTGTTCAAA
GCAGGCCTATGCCTGAATACATTAGCA
TGGAATAATAAAATAGGACGTGCGGT
TCTATTTTGTTGGTTTCTAGAGTCGCCG
TAATGATTAATAGGGATAGTTGGGGGC
ATTGGTATTGAGTCGCTAGAGGTGAAA
TTCTTGGATTGACTCAAGACCAACTAC
TGCGAAAGCATTTGCCAAGGATGTTTT
CATTAATCAAGAACGAAGGTTAGGGG
ATCGAAAACGATCAGATACCGTTGTAG
TCTTAACAGTAAACTATGCCGACTAGG
GATCGGGCAATCTCAAA**CATGATGTG**
TTGCTCGGCACCTTACGAGAAATCAAA
GTCTTTGGGTTCTGGGGGGAGTATGGT
CGCAAGGCTGAAACTTAAAGGAATTG
ACGGAAGGGCACCACCAGGTGTGGAG
CCTGCGGCTTAATTTGACTCAACACGG

GGAAACTCACCAGGTCCAGACATAAC
TAGGATTGACAGATTGATAGCTCTTTC
ATGATTTTATGGGTGGTGGTGCATGGC
CGTTCTTAGTTGGTGGAGTGATTTGTC
TGGTTAATTCCGATAACGAACGAGACC
TTAACCTGCTAAATAGCCAGGCCGGCT
TTCGCTGGTCGCCGGCTTCTTAGAGGG
ACTGTCAGCGTCTATCCTGCCCGGAAG
TTACCA

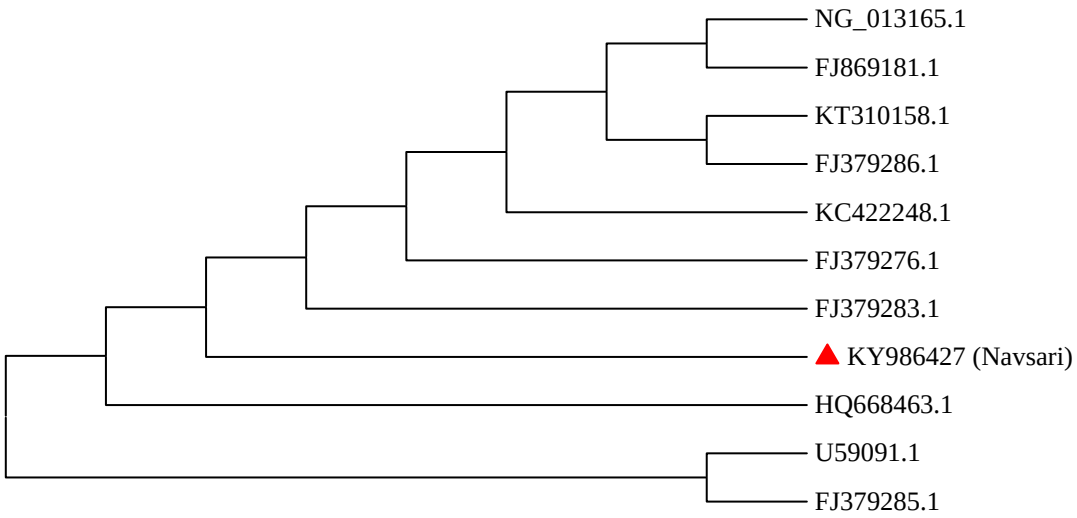
Phylogenetic tree

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. The rate variation among sites was modeled with a gamma distribution. The analysis involved 11 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}+$ Noncoding (Fig. 1).

Table.1 Sequence alignment view

Accession	Description
FJ379283.1	*Pleurotus cystidiosus* strain P-24
FJ379276.1	*Pleurotus nebrodensis* strain BL-1
NG_013165.1	*Pleurotus ostreatus* voucher TENN:53662
KT310158.1	*Pleurotus* sp. 8 YY-2015
FJ379286.1	*Pleurotus eryngii* strain X-102
FJ869181.1	*Pleurotus cornucopiae* strain P-38
HQ668463.1	*Pleurotus* sp. XDX-2011 voucher HMAS 199629
KC422248.1	*Pleurotus* sp. M2 ZM-2012
U59091.1	*Pleurotus tuberregium*
FJ379285.1	*Pleurotus salmoneostramineus* strain TH

Fig.1 Evolutionary relationships of taxa



All positions containing gaps and missing data were eliminated. There were a total of 800 positions in the final dataset. Different people conducted experiment on the survey, morphology oyster mushroom. The present results of survey are on the same line with the findings of Ravat and John (2016) carried out a survey on distribution of fleshy fungi from Dangs district, Gujarat, India. They reported a total of 37 species of fleshy fungi, among them 2 species of oyster were seen. Similarly, Bernardo *et al.,* (2004) collected six different species of oyster mushroom, namely *P. albidus, P. cystidiosus, P. ostreatus, P. pulmonarius, P. rickii* and *P. djamor* from the field and different national herbaria of Argentina. The present investigation of detection of oyster mushroom morphology was in agreement with earlier of several scientists. Bernardo *et al.,* (2004) studied the macro- and micromorphological characters of specimens of the genus *Pleurotus* obtained from the field and different national herbaria of Argentina. Based on morphological features they were identified as *P. albidus, P. cystidiosus, P. ostreatus, P. pulmonarius, P. rickii* and *P. djamor*. Dung *et al.,* (2012) studied the molecular identification methods gave the similar results, in which two strains of white oyster mushrooms were *Pleurotus floridanus* and one strain of Japanese oyster mushroom was *Pleurotus cystidiosus*.

References

Afyon, A., Konuk, M., Yagiz, D. and Helfer, S. 2005. Study of wood decaying macrofungi of the western black sea region of Turkey. *Mycotoxon.*, 93: 319-322.

Astarini, A.I., Plummer, A.J., Lancaster, A.R. and Yan, G. 2004. Fingerprinting of cauliflower cultivars using RAPD markers. *Aust. J. Agri. Res.*, 55: 112-124.

Bernardo, E.L., Jorge, E.W. and Edgardo, A. 2004. The genus *Pleurotus* in Argentina. *Mycologia,* 96: 845–858.

Chang, S.T. and Miles, P.G. 1992. Mushroom biology—a new discipline. *The Mycologist*, 6: 64- 65.

Dung, N.T.P., Tuyen, D.B. and Quang, P.H. 2012. Morphological and genetic characteristics of oyster mushrooms and conditions effecting on its spawn growing. *Int. Food Res. J.,* 19: 347-352.

Kaya, A. 2005. Macrofungi determined in Globasin (Adiyaman) District. *Turk. J. Bot.*, 29: 45-50.

Kumar, L.S. 1999. DNA markers in plant improvement. An overview *Biotechnol. Adv.*, 17: 143-182.

Mehmood, S., Bashir, A., Ahmad, A. and Akram, Z. 2008. Molecular characterization of regional sorghum bicolor varieties from Pakistan. *Pak. J. Bot.*, 40: 2015-2021.

Mueller, G.M., Bills, G.F. and Foster, M.S. 2004. Biodiversity of fungi-inventory and monitoring methods. Elsevier Academic Press, London.

Ravat, H. and John, P. 2016. Macrofungal diversity in Dangs district, Gujarat, India. *I.J.S.N.*, 7: 477-482.

Sajida, B., Imtiaz, A.K., Bughio, H.R., Iftikhar, A.O., Muhammad, A.A. and Abdullah, K. 2009. Genetic differentiation of rice mutants based on morphological traits and molecular marker (RAPD). *Pak. J. Bot.*, 4: 737-743.

Sambrook, J.D., Russell, W. and Sambrook, J. 2001. Molecular cloning: a laboratory manual, 3rd ed. NY: Cold Spring Harbor Laboratory Press.

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