DOI: https://doi.org/10.36036/MR.34.2.2026.151887
Mushroom Research 34 (2) : 257-266, 2025

Identification of oyster mushroom specimen [*Pleurotus ostreatus* (Jacq.) P. Kumm] from Junagarh (Gujarat), its cultivation using different agricultural waste and biochemical profiling

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ABSTRACT

A study was conducted for identification, biochemical profiling and biological efficiency with biological efficiency of *Pleurotus ostreatus* collected from Junagarh (Gujrat) on different agricultural waste. Identification was done based on morphological characters and confirmed by ITS sequencing. The ITS sequence was submitted to NCBI gene bank with accession number MW446165. The cultivation trials were undertaken using different agriculture and horticulture residues. Significantly higher yield was obtained on wheat straw (605.3 g/kg substrate) followed by paddy straw (588.6 g/kg substrate). However, highest protein content (26.155 mg/g), total sugar (23.670 mg/g), phenol content (1.892 mg/g), moisture (92.1 %) and carbohydrate (6.580 mg/g) were recorded in groundnut leaves and stalk, wheat straw, wheat straw, groundnut shell and banana leaves, respectively.

Keywords: Oyster mushroom, ITS sequencing, cultivation trial, yield, biochemical profiling

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 (Chang and Miles, 1992). Oyster mushrooms, scientifically known as *Pleurotus* species, belong to the class Agaricomycetes, the order Agaricales and the family Pleurotaceae. They are commonly found in temperate and tropical forests, occurring naturally on decomposing logs or occasionally on dried trunks of both deciduous and coniferous trees. These mushrooms have fruit bodies that are distinctively shell or spatula-shaped and come in various shades, such as white, cream, grey, yellow, pink, light brown and blue, depending on the specific species, also emit a unique sweet like anise or licorice (liquorice) scent. The popularity of Oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Aditya *et al*., 2024).

Morphological identification of mushrooms is often difficult as most of the morphological features varies due to the climatic variations such as temperature, relative humidity, substrate composition and light intensity while microscopical or taxonomic identification is highly technical. Thus, morphological identification coupled with molecular tools such as ITS sequencing or DNA barcoding serves as a

Received on 15-07-2025; Accepted on 22-05-2026
https://epubs.icar.org.in/index.php/MR/article/view/151887

confirmatory tool for confirmation of identification. Oyster mushroom (*Pleurotus* sp.) was differentiated on the basis of morphological (sporocarp, sporophore, colour, size), cultural, ITS sequences and Phylogenetic analysis (Krishnapriya *et al*., 2017; Jing *et al*., 2017). Cultivation of oyster mushroom has been reported on different substrate (cereal straws, dried leaves, banana leaves, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue) with varied biological efficiency and fruit body quality (Chadha and Sharma, 1995; Chang and Miles, 1989).

Previous studies showed that growth, yield, and nutritional composition of oyster mushrooms varied with different substrates. The results indicated that different substrate formulas gave a significant difference in total colonization period, characteristics of fruiting bodies, yield, biological efficiency (BE), nutritional composition and mineral contents of two oyster mushrooms PO and PC (Hoa *et al.*, 2015).

Present study is aimed to identify a oyster mushroom specimen from Junagarh, Gujrat on the basis of morphological & molecular characters, effect of different substrates on its yield potential and the nutritional properties of the fruiting bodies.

MATERIALS AND METHODS

Study area and experimental materials

The experiment was conducted at the Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh during 2020-21. Wheat straw, paddy straw, groundnut shell, banana leaves, coconut husk, mango leaves, agri. waste, cocopeat, groundnut leaves and stalk and waste paper were collected from different location of saurashtra region.

Biological material and culturing

Pure culture of *Pleurotus* sp. collected from mushroom production unit, Department of Plant Pathology, J.A.U., Junagadh and morphological characters were noted such as size of fruiting body, stipe length, stipe diameter, pileus diameter, microscopic view of mushroom spore and spore print. Pure culture of the mushroom was maintained on potato dextrose agar (PDA) medium.

Spore print and microscopy

Spore print of the mushroom was obtained by keeping a small piece of fruit body on butter paper keeping the gills downwards overnight. Mushroom spores were studied by observing cotton blue stained slides under compound light microscope 400 X magnification. After calibrating the microscope, the measurement of spore was taken with the help of ocular micrometre.

Molecular identification

Molecular identification of the specimen was done using ITS 5.8S sequencing. 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. The genomic DNA was extracted using DNA isolation kit. The ITS region was amplified using universal ITS1 (5'TCCGTAGGTG AACCTGCGG3') and ITS4 (5'TCCTCCGCTTT ATTGATATG3') primers (White *et al*., 1990). The amplification was performed in 30 µl reaction volume with 0.1 mM of each dNTP and 100pmol of both forward and reverse primer. PCR reaction 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. ITS amplified product was sequenced by ABI 3730 capillary sequencing by Eurofins Genomics India Pvt. Ltd., Bangalore.

Consensus sequence was generated from forward and reverse sequence data using aligner

software 6 and BLAST-n was carried out against NCBI GenBank database. 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 (Kumar *et al.,* 2018). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura and Kumar, 2004) and were in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985).

Cultivation trial

Sorghum grains were used to prepare the spawn of the oyster mushroom using standard package of practice (Upadhyay *et al*., 2004). A total of 10 substrates (Wheat straw, Paddy straw, Mango leaves, Groundnut shell, Groundnut leaves and stalk, Waste paper, Banana leaves, Agri. Waste (Mix Dry Leaves), Coconut husk, Cocopeat) were evaluated for the cultivation. Each substrate was taken separately (around 12 kg) in a plastic tub and sterilized chemically using carbendazim 50% WP @ 10 g/10 liters of water and 40% of formaldehyde solution (13.50 ml/10 liters of water) for a period of 24 hours. After 24 hrs, the substrates were washed twice with water and excess water was removed completely after washing. Tea waste was sterilized in autoclave. In case of office waste, first small pieces of paper, carton were taken into the plastic bag. The bag was sterilized in the autoclave.

Surface dried and sterilized substrates were filled in polythene bags. Layer spawning (3 per cent) was made at periphery of the bag between 2 layers of substrate. Bags were tied with strings and small holes were made in bags for gaseous exchange and better aeration. Bags were kept at 20 to 25 °C temperature and 80 to 90 per cent relative humidity. Same

Fig. 1. Flow chart of oyster mushroom cultivation

procedure was repeated for paper and carton in bag and in between the layer water spraying was carried out to maintain the moisture (Fig. 1).

Data collection and analysis

The experiment was carried out to find the effective substrate for yield, other morphological and nutritional characteristics. All the substrates were evaluated for total number of fruiting bodies, height of fruiting bodies (cm), diameter of stipe (cm), stipe length (cm), pileus diameter (cm) and yield (gm/kg) (Gopinath, 2015). Completely randomized block design with factorial concept was used for analysing data.

Biological efficiency of mushroom was calculated by using formula as recommended by Chang and Miles (1989).

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

Biochemical analysis

The various biochemical properties of fruiting bodies of *Pleurotus ostreatus* harvested from different substrates were analysed. After harvesting fresh fruiting body of mushroom dried in Hot air oven at a temperature of 50°C. The well-dried mushrooms were powdered in grinder and mixture and used for protein, total Sugar, phenol, carbohydrate and moisture estimation. Analysis of biochemical parameter were done by standard biochemical procedures mentioned below.

1	Protein	Protein content will be measured by Folin-Lowry's method (Lowry *et al.,* 1951).
2	Total Sugar	Total sugar will be measured by phenol sulphuric acid method (Dubois *et al*., 1956).
3	Phenol	Total phenol will be measured by phenol-folin method (Snell and Snell, 1953).
4	Moisture	Moisture content will be measured by method of Gaur *et al*. (2016).
5	Carbohydrate	Carbohydrate content will be measured by Dinitrosalicyclic acid (DNS) Method (Miller, 1959).

RESULTS AND DISCUSSION

Morphological characterization

Oyster mushroom (*Pleurotus* sp.) was characterized on the basis of their morphological and molecular characteristics. Morphologically characters such as mycelial growth on PDA plate, spore print, spore size, length & diameter of pileus and stipe of the collected sample were recorded. The details are given in table 1 and fig 2.

As per the finding of this study, the morphological characteristics of *Pleurtous sp.* collected was more or less similar with the characters of *P. ostreatus* reported by Chhetry and Pfoze (2007) as soft and smooth fruit bodies with light yellow colour 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. Ram (2007) reported 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, spathulate 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.* (2017) isolated Oyster mushroom from forest region and identified *Pleurotus* sp. based on microscopic view and phylogenetic tree.

Table 1. Characters of the oyster mushroom specimen collected

Parts of the specimen	Measurements	Characters
Cap (pileus)	5.6 - 11.2 cm in dimeter	Whitish to grey color, convex, smooth, soft, maturing to a shell shape
Stipe	4.7 - 7.2 cm in length; 1.3 – 2.2 cm in diameter	Cream and smooth surface
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.

Collected sample showing gills

Pure sulture of the sample

Collected sample cultivated

Close view of Pileus

Fig. 2. Collected sample and cultivated fruit body

Molecular characterization

The mushroom culture was identified based on ITS sequencing done by Eurofins Genomics India Pvt. Ltd. Evolutionary analyses were conducted in MEGA X involving 5 nucleotide sequences. There was a total of 703 positions in the final dataset. The consensus tree with the sum of branch length = 0.02507224 was shown in fig 3. The BLAST analysis of the ITS sequence data supported the morphological identification, whereby the closest match with NCBI sequence number MT261808 (94%) in the NCBI GenBank database was found to be the *Pleurotus ostreatus*. Further, the sequence was submitted to gene bank of NCBI and accession number MW446165 assigned.

Shnyreva and Shnyreva (2015) analysed ten *Pleurotus* sp. based on Internal Transcribed Spacer (ITS) sequences of rDNA. They showed divergence between commercial strains and natural isolates of *Pleurotus ostreatus* by phylogenetic analysis.

ITS based genetic diversity in *Pleurotus* sp. was also analysed by different workers (Imtiaz *et al*., 2011). Similarly, Liu *et al*. (2013) assessed the genetic diversity of *Pleurotus ostreatus* strains on the basis of the Internal Transcribed Spacers (ITS) sequence, translation elongation factor (EF1α) and the second largest subunit of RNA polymerase II (RPB2).

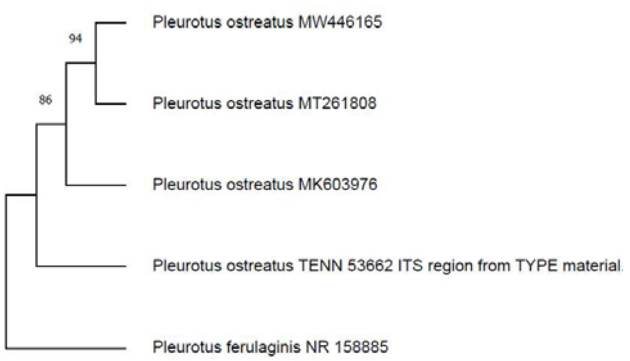

Fig. 3. Phylogenetic relationship of *Pleurotus ostreatus* isolate with other species

Yield attributes of *Pleurotus ostreatus* grown on different substrates

Results of the yield and yield related parameters of Oyster mushroom grown in each substrate are given in Table 2. The results showed that the maximum number of fruiting body was observed in wheat straw (32) which was statistically at par with paddy straw (31). While, lowest number of fruiting body was observed in waste paper (17). Similar results was reported by Kumar *et al*. (2019) and Bhatti *et al*. (2007) on different substrates i.e. maize, bajra, paddy and wheat on production of oyster mushroom.

The maximum height of fruiting body, stipe length, stipe & pileus diameter was observed in wheat straw followed by paddy straw. The stipe and pileus diameter were observed to close in banana leaves also. The height of fruiting body on different substrates ranged from 7.5-12.5 cm. Mandaviya *et al*. (2018) also showed the same result in which they reported that the wheat straw was best suitable among different substrates in highest number of fruiting body (31), stipe length (3.08 cm) and maximum yield (213.20 g/kg) with biological efficiency.

Besides yield relataed parameters, the maximum yield was obtained in wheat straw substrate (605.3 g/kg) with a biological efficiency of 60.53 % closely followed by paddy straw substrate (588.6 g/kg) and a biological efficiency of 58.86 %. Lowest yield was obtained in waste paper (317 g/kg) with a biological efficiency of 31.70%. The total dry weight of mushroom observed to be the maximum in waste paper (11.8 g/100g) and was statistically at par with the paddy straw and groundnut leaves and stalk (11.4 g/100g). The higher yield of oyster mushroom was also reported by different workers (Iqbal *et al.*, 2016; Kumari *et al*., 2018; Ahmed *et al.*, 2016). Kumar *et al*. (2019) also studied locally available plants wastes namely banana leaves, casurina, coir pith, sugarcane

Table 2. Effect of different substrates on growth of mushroom

Sr. No.	Substrates	Total no. of fruiting body	Height of fruiting body(cm)	Stipe length (cm)	Stipe diameter (cm)	Pileus diameter (cm)	Yield (g/kg)	Total dry weight of mushroom (g/100 g)	Biological efficiency (%)
T_1	Wheat straw	32	12.5	6.5	1.7	10.5	605.3	9.0	60.53
T_2	Paddy straw	31	12.0	6.4	1.7	10.3	588.6	11.4	58.86
T_3	Mango leaves	24	8.7	5.6	1.5	7.1	367.0	10.0	36.73
T_4	Groundnut shell	29	10.0	6.0	1.4	9.2	421.3	7.9	42.13
T_5	Groundnut leaves and stalk	19	7.5	4.8	1.3	6.4	329.0	11.4	32.90
T_6	Waste paper	17	7.5	4.7	1.3	5.6	317.0	11.8	31.70
T_7	Banana leaves	28	9.5	5.9	1.6	7.6	400.0	9.4	40.00
T_8	Mix Dry Leaves	22	8.5	5.5	1.4	6.9	363.6	9.4	36.36
T_9	Coconut husk	26	9.3	5.8	1.5	7.4	384.0	8.0	38.40
T_{10}	Cocopeat	21	8.0	5.3	1.3	6.5	347.0	10.2	34.70
	S.Em.±	0.77	0.26	0.09	0.04	0.11	10.04	0.21	1.00
	CD at 5 %	2.28	0.77	0.27	0.13	0.34	29.63	0.62	2.96
	CV %	5.39	4.87	2.85	5.27	2.61	4.22	3.72	4.22

trash, water hyacinth and paddy straw and reported highest biological efficiency in paddy straw.

Biochemical analysis

Oyster mushroom grown on different agricultural residues were evaluated for different nutritional parameters i.e. protein, total sugar, phenol, moisture and carbohydrate. Table 3 shows nutritional values of oyster mushroom with total protein ranging from 9.629 to 26.155 mg/g, total sugar from 6.571 to 23.670 mg/g, phenol from 1.023 to 1.892 mg/g, moisture from 88.2 to 92.1 %) and carbohydrate 2.055 to 6.580 mg/g. In the present investigation, the maximum content of protein (26.155 mg/g) was observed in groundnut leaves and stalk used as a substrate followed by wheat straw (25.680 mg/g) and paddy straw (24.482 mg/g). Lowest content of protein (9.629 mg/g) was observed in cocopeat used as a substrate. The maximum sugar content (23.670 mg/g) was observed in case of wheat straw followed by cocopeat and coconut husk. The maximum and minimum phenol content was obtained in the fruit bodies harvested in wheat straw (1.892 mg/g) and groundnut shell (1.02 mg/g), respectively.

Vanathi *et al*. (2016) reported chemical composition of oyster mushroom as protein content (22.7 mg/g), carbohydrate (10.1 mg/g), amino acid (9.1 mg/g), lipid content (4.3 mg/g) when paddy straw was used as a substrate. Kajendran *et al*. (2018) reported that 89.69 % moisture content in fresh oyster mushroom and the maximum carbohydrate content (6.580 mg/g) in mushroom grown on banana leaves substrate compared to the other substrates. Similar results were also reported by different workkers (Kumar *et al*., 2008; Pandey *et al*., 2008; Randive, 2012).

Table 3. Effect of different substrates on nutritional composition of mushroom

Sr. No.	Substrates	Protein (mg/g)	Total Sugar (mg/g)	Phenol (mg/g)	Moisture (%)	Carbohydrate (mg/g)
T_1	Wheat straw	25.680	23.670	1.892	91.0	4.853
T_2	Paddy straw	24.482	13.275	1.793	88.6	4.642
T_3	Mango leaves	20.900	17.626	1.531	90.0	4.991
T_4	Groundnut shell	23.253	13.459	1.023	92.1	4.075
T_5	Groundnut leaves and stalk	26.155	6.571	1.801	88.6	4.255
T_6	Waste paper	18.780	18.432	1.545	88.2	4.335
T_7	Banana leaves	22.160	12.933	1.661	90.6	6.580
T_8	Agri. Waste (Mix Dry Leaves)	12.660	10.359	1.363	90.6	4.442
T_9	Coconut husk	20.863	20.796	1.512	92.0	5.578
T_{10}	Cocopeat	9.629	21.359	1.421	89.8	2.055
	S.Em.±	0.425	0.381	0.044	0.89	0.093
	CD at 5 %	1.256	1.125	0.131	2.62	0.276
	CV %	3.610	4.170	4.970	1.71	3.540

ACKNOWLEDGEMENT

The authors are thankful to Department of Plant Pathology, College of Agriculture, JAU, Junagadh, and Authority of Junagadh Agricultural University for Funding and providing facility to conduct the research work.

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