

Original Research Article

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Characteristic Mycelial Phenotype of New *Pleurotus* spp. used as a Marker for Identification

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ABSTRACT

The present study was carried out to characterize two newly collected *Pleurotus* species viz., *Pleurotus djamor* isolate virutha1 and *Pleurotus djamor* isolate woody 1 compared with *Pleurotus florida* which is a commercial cultivar. All these *Pleurotus* spp. grew well in maize agar medium compared to potato dextrose agar medium. With regard to phenotypic characters of mycelia on the culture medium, mycelial growth of *P. florida* appeared thick, cottony white and rhizomorphic strands as generally noticed in most of the mushroom culture. Interestingly, *P. djamor* isolate woody 1 appeared thin, loosely grown, non-rhizomorphic mycelia and sparingly aggregated white mycelial strands. *P. djamor* isolate virutha appeared intermediate mycelial type such as moderately rhizomorphic mycelial aggregation and moderately thick and white growth. With regard to mycelia growth pattern on the spawn, *P. djamor* isolate woody1 and *P. djamor* isolate virutha 1 appeared loosely interwoven, low mycelial density and non-rhizomorphic mycelial strands on the paddy/sorghum grain substrates. Whereas, *P. florida* appeared thick, densely grown, cottony white and rhizomorphic mycelial strands on the paddy/sorghum grain substrates. The appearance of the thin/loose filamentous mycelial pattern can be used as phenotypic marker for the identification of these two new *P. djamor* isolates woody1 and *P. djamor* isolate virutha 1.

Keywords

Pleurotus spp,
Phenotypic
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Introduction

Oyster mushrooms (*Pleurotus* spp.) are suitable for commercial cultivation throughout the years in subtropical regions and winter seasons in tropical regions of the world. They grow naturally on dead and decaying but un-decomposed wooden logs and on dying trunks of wood trees in the temperate and tropical region. The oyster

mushrooms have two distinct parts such as oyster like fleshy shell or cap (pileus), a short or long lateral stalk known as stipe. Beneath the pileus, long ridges and furrows known as gills or lamellae are present.

Basidiocarps of oyster mushroom are praised for culinary purpose since they are rich in protein, fiber, vitamins (rich in vitamin B) and minerals (rich in potassium) (Chang and

Miles, 2004; Reis *et al.*, 2012). In addition to their nutritional value, these fungi produce vital and medicinally important biomolecules (Papaspayridi, 2011).

Mushroom mycelial growth is influenced by culture media, type of substrates and temperature and for basidiocarp production relative humidity plays important role in addition of type of substrates and temperature. Cereal grain based media are best suited for the culturing of oyster mushroom (Rajoriya and Gupta, 2015).

Temperature is the most important conditions for the mycelial growth and basidiocarp formation. Oyster mushroom requires 28°C for optimum growth (Wasantha Kumar and Edirimanna, 2009; Nwokoye *et al.*, 2010; Hoa and Wang, 2015). However, for basidiocarp formation, it requires 22 – 25°C with high relative humidity more than 80 %.

Thus growing of oyster mushroom is restricted to the winter months especially between October and February in India. Cropping period of oyster mushroom is 50 days.

In order to develop germplasms of oyster mushroom with great genetic diversity, earlier we started natural collection of several *Pleurotus* spp. and identified two isolates viz., *P. djamor* isolate woody1 and *P. djamor* isolate virutha1 (Viruthambigai, 2019). Typically, both *Pleurotus* sp. isolate virutha and *P. djamor* isolate woody 1 has no stipe (astipitate). Whereas *P. florida* had well developed stipe. Basidiocarp of *P. djamor* isolate woody 1 appeared as white whereas that of *P. djamor* isolate virutha appeared light pink in colour at primordial phase and later the color changes to white. *P. florida* appeared in creamy white in colour. Thus, in the present study, the mycelial growth pattern of the newly identified *Pleurotus* spp. were

analysed and found that the mycelia of *P. djamor* isolate woody1 typically appeared non-rhizomorphic, thin and loosely arranged mycelial strands both on the culture medium and in the spawn

Materials and Methods

Isolation and culturing of *Pleurotus* spp

Healthy and fully matured basidiocarp was collected and surface sterilized with 70% ethanol. The basidiocarp was longitudinally split open into two halves using new sterile blade. A small piece of plectenchymetous tissue was taken from the centre portion of the split mushroom at the junction point of the pileus and stipe.

Three tissue bits were placed on the PDA medium blended with 100 ppm of streptomycin sulphate in the Petri plates at equal-distance and incubated at 28°C until the medium was fully covered with mycelial growth. The pure culture of *Pleurotus* spp. was maintained on PDA slants for further use during this study. Three *Pleurotus* spp. viz., *P. florida*, *P. djamor* isolate virutha 1 and *P. djamor* isolate woody 1 were used in this study.

Mycelial growth phenotype on culture media

To study the mycelial phenotypic characters of *Pleurotus* sp., five millimetre culture discs were cut with sterilized cork borer from advancing margins of the colonies and inoculated on PDA and maize agar medium supplemented with streptomycin sulphate (100 ppm). The plates were incubated at 28°C. Five replications were maintained for each medium. Radial growth of the mycelium was recorded when the mycelial growth covered in any one of medium. Mycelial characters were observed visually.

Spawn running period and pattern of mycelial growth on the spawn substrate

The well grown mycelia of *P. florida*, *P. djamor* isolate virutha 1 and *P. djamor* isolate woody 1 were used for preparing spawn. For the preparation of spawn, paddy grains or sorghum grains were used as substrate. Disease free and healthy grains were soaked for 2 hours in water. The excess water was drained and grains were allowed to incubate in wet condition for 12-18 hours.

The well soaked and incubated grains were boiled for 1 hour and then shade dried. The calcium carbonate was added to the grains at the rate of 20 g per kg of seeds for absorbing excess moisture and retaining adequate moisture; maintaining the slightly alkaline conditions and to prevent sticking of grains with one another. The grains were filled upto three-fourths of the height of polypropylene cover. The PVC rings were placed on the top of the grain filled covers and the edges were folded down.

The mouths of the bags were plugged tightly with non-absorbent cotton and autoclave-sterilized at 20 lbs for the period of two hours. Mycelial discs of 8 to 10 mm diameter of pure mycelial culture of *Pleurotus* species were

taken from Petri plate and transferred to the sterilized grains and incubated at 28°C for the period of 15 days. The time taken for development of entire spawn and pattern of growth were noted.

Results and Discussion

Mycelial growth pattern of *pleurotus* spp. on culture media

Phenotypic characters on the mycelial growth of *P. florida*, *P. djamor* isolate virutha 1 and *P. djamor* isolate woody 1 were studied on PDA and maize agar medium. The maximum mycelial growth for all the three *Pleurotus* spp. was observed on maize agar medium. With regard to the phenotypic characters, mycelia of *P. florida* appeared thick, cottony white and rhizomorphic strands as generally noticed in most of the mushroom culture.

However, *P. djamor* isolate woody 1 appeared thin, loosely interwoven, non-rhizomorphic mycelia and sparingly aggregated white mycelial strands. *P. djamor* isolate virutha1 appeared intermediate mycelial type such as moderately rhizomorphic mycelial aggregation and moderately thick and white growth (Figure 1; Table 1).

Table.1 Phenotypic characters of mycelia of *Pleurotus* spp. on culture medium and spawn

<i>Pleurotus</i> spp	Days required for spawn development	Mycelial growth pattern	
		On culture medium	In the spawn
<i>P. florida</i>	15.6 ^b	thick white mycelium	thick white mycelium
<i>P. d</i> isolate virutha 1	13.6 ^a	Moderately thick white mycelium	Thin and sparse mycelium and non-rhizomorphic mycelium
<i>P. d</i> isolate woody 1	13.0 ^a	Thin, loose and sparse mycelium and,non-rhizomorphic mycelium	Thin and sparse mycelium and non-rhizomorphic mycelium

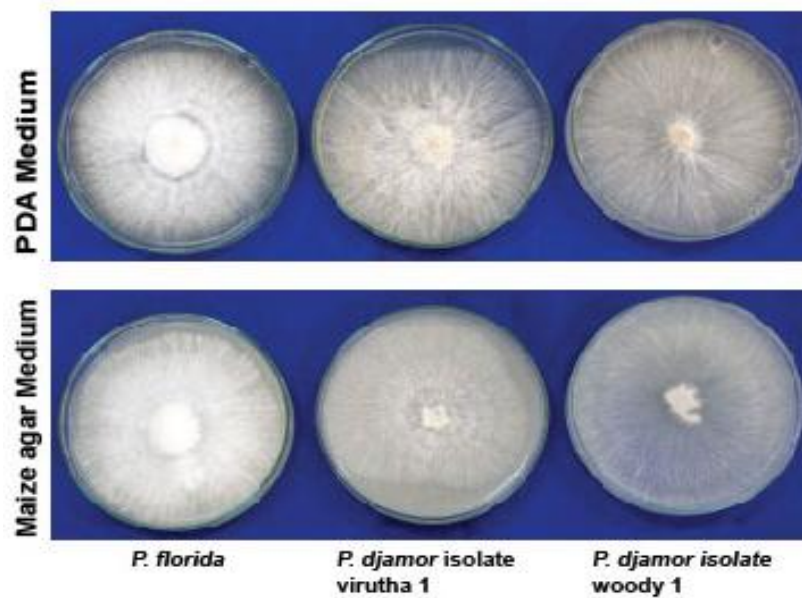


Fig.1 Effect of mycelial growth of different *Pleurotus* spp on PDA and Maize agar medium



Fig.2 Mycelial growth pattern of different *Pleurotus* spp on paddy grains as a spawn substrate

Mycelial growth pattern of different *Pleurotus* spp. on spawn substrate

P. florida grew as thick compact white mycelium and covers the spawn completely within 15 days on the paddy grains as a substrate for spawn. White mycelia of *P. florida* completely covers the grain substrate used in the spawn and the grains in the bags are not visible.

Whereas *P. djamor* isolate virutha 1 and *P. djamor* isolate woody 1 appears thin, loosely interwoven and non-rhizomorphic mycelia and complete mycelial growth occurred in 13 days (Figure.2; Table.1). Since, the mycelia

of both the new isolates appeared thin filamentous strands instead of thick, cottony white mycelium in other mushroom cultures, this mycelial morphological characters can be used as a phenotypic marker for identification of the *P. djamor* isolate woody1 and also can be used in breeding program to analyse the segregation patterns and progenies.

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