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Short Communication

Cultural and physiological studies of strain DMRP-330 of *Pleurotus ostreatus* suited to Kerala conditions

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ABSTRACT

Experiments were conducted to assess the optimum conditions *viz*., media, temperature and pH for mycelial growth of a strain of *Pleurotus ostreatus* suited to Kerala conditions. The media tested for mycelial growth were PDA (Potato Dextrose Agar), MEA (Malt Extract Agar), OMA (Oatmeal Agar), CA (Carrot Agar) and YEMA (Yeast Extract Mannitol Agar). All the media were found suitable for mycelial growth while the maximum growth was observed in PDA. Liquid broths of the above said media were also tested for optimal mycelial biomass production of the oyster mushroom. The optimum temperature for the mycelial growth of the *P. ostreatus* strain was recorded to be 30°C. The maximum mycelial growth of the *P. ostreatus strain* was supported by PDA at pH 7 and 7.5 while the least supportive pH was 4.

Keywords: *Pleurotus ostreatus*, mycelial growth, growth factors, media, temperature, pH

Pleurotus ostreatus belongs to the class Basidiomycetes, order Agaricales and family Pleurotaceae. It is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* also known by common names oyster mushroom, hiratake (in Japan) and Dhingri (in India) The nutrient content of fresh *P. ostreatus* mushroom *viz.*, protein, lipid, fiber, and carbohydrate content were 3.0-3.8 g, 0.63-0.73 g, 3.2-3.6 g and 5.0-5.4 g respectively; while in dried mushroom it was 22.0-26.0 g, 4.4-4.8 g, 23.0-26.0 g and 35.0- 40.0 g respectively (Alam *et al.*, 2008). *P. ostreatus* also contains more folacin, vitamin B1 and vitamin B3, but less vitamin B12 than other mushroom species (Deepalakshmi and Mirunalini, 2014). The presence of antitumor polysaccharide â-glucan called pleuran and an anti-lipidemic compound, lovastatin imparts therapeutic properties to this mushroom. The nutritional and medicinal value of oyster mushroom cultivation along with its simple low cost technologies, short crop duration and higher yield makes it suitable for reducing the gap of protein malnutrition among the population in developing countries.

Cultivation of mushroom involves tissue culturing, spawn preparation and cultivation on lignocellulosic substrates. The physiological and cultural characters of mushroom are generally influenced by culture media, temperature and pH of the agro-ecological regions. The study aims to standardize the culture conditions for a strain of the *P. ostreatus* suiting to Kerala conditions so that the strain can be popularized in the rural masses of the area. Hence, the study was carried out to evaluate and standardize the optimal physiological and cultural requirement for maximum mycelial growth of the *P. ostreatus* strain.

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Effect of different media on the growth of the *P. ostreatus* strain *in vitro*

In vitro evaluation of the cultural and physiological characters of the *P. ostreatus* strain in different media revealed that there was difference in the nature and rate of mycelial growth among the various treatments. There was variation in pattern of mycelial growth in media used *viz*., PDA (Potato Dextrose Agar), MEA (Malt Extract Agar), OMA (Oatmeal Agar), CA (Carrot Agar) and YEMA (Yeast Extract Mannitol Agar. The mycelial growth pattern ranged from fluffy to sparse to cottony. After three days of inoculation, the mycelium shows sparse growth with a fluffy centre in PDA and MEA, cottony growth with regular margin in OMA and YEMA, in CA the mycelium

Table 1. Colour and nature of mycelial growth in different media

Media	Colour	Nature of mycelial growth (DAI)		
		3rd	5th	9th
PDA	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
MEA	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
OMA	White	Cottony with regular margin and regular margin	Fluffy centre with sparse	Fluffy with regular margin
CA	White	Fluffy with irregular margin	Fluffy with regular margin	Fluffy with regular margin
YEMA	White	Cottony with regular margin and regular margin	Fluffy centre with sparse	Sparse with irregular margin

DAI- Days after inoculation

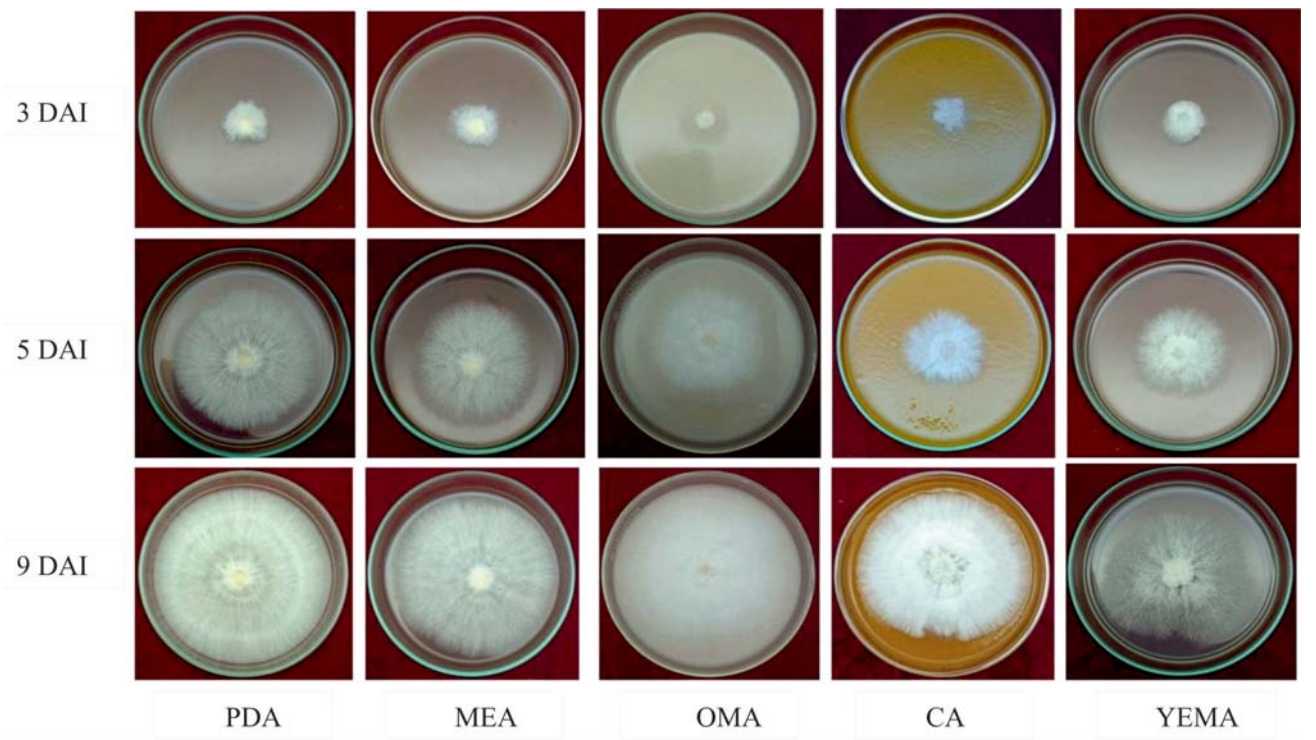

Fig. 1. Comparative growth of *The P. ostreatus strain* in different media

shows white fluffy centre with irregular margin. The mycelial growth turned fluffy with regular margin after nine days of inoculation in all the media except YEMA where the growth was sparse with irregular margin (Table 1, Fig. 1)

Three days after inoculation the maximum radial mycelial growth was observed in OMA followed by PDA, MEA and CA with the least growth in YEMA (Fig. 1). The radial growth ranged from 1.44 - 2.55 cm (3 DAI), 2.75 - 4.84 cm (5 DAI) and 6.88 - 8.76 cm (9 DAI). The maximum growth rate of the *P. ostreatus strain* was found in PDA (1.11 cm day^{-1}) followed by OMA (0.87 cm day^{-1}) and MEA (0.82 cm day^{-1}) which were at par with each other. The lowest growth rate was found in CA (0.77 cm day^{-1}) followed by YEMA (0.82 cm day^{-1}). The *P. ostreatus* strain took nine days for completing growth in PDA and OMA, 10 days in MEA and 12 days in CA and YEMA (Table 2). The variation in mycelial growth on different media may results from the variation in the availability and utilization of different carbon source and other essential nutrients. PDA have high carbon and nutrients essential for mycelial growth. The experiment indicated that all the five media were suitable for culturing the *P. ostreatus* strain with PDA being the best. Similar observations were made by Sardar *et al*. (2015), Maurya *et al*. (2019) and Fletcher (2019) who also reported PDA as the suitable media for growing *Pleurotus* sp. The easy availability of nutrients and other growth promoting conditions adds up to its suitability for mushroom growth under *in vitro* condition.

Effect of different liquid broth on the growth of the *P. ostreatus* strain *in vitro*

Five mm disc of seven day old culture of the *P. ostreatus* strain was inoculated into broth of potato dextrose, malt extract, oatmeal, carrot and yeast extract mannitol. Mycelial biomass was estimated at 21DAI and 30DAI. The maximum mycelium biomass (fresh weight) was observed in carrot broth (6.50g). The biomass in the other media ranged from 5.00-6.30g with minimum in PD. The dry weight of mycelial biomass was maximum in malt extract broth (0.60g) and lowest in yeast extract mannitol broth (0.40g). One month after inoculation, mycelial biomass from different treatments were found to be uniform or non-significant suggesting that after threshold point , the *P. ostreatus* strain utilized all the carbon sources equally for mycelial biomass (Table 3, Fig 2.) Kupradit *et al*. (2020) identified potato dextrose broth as an ideal media for maximum biomass production of *P. ostreatus, Lentinus* and *Volvariella.*

Effect of different temperatures on the growth of the *P. ostreatus* strain

Temperature is one of the most important environmental factor affecting the mycelial growth

Table 2. Radial growth and growth rate of mycelium of the *P. ostreatus* strain in different media

Media	Radial growth of mycelium in Petri dish (DAI) (cm) *			Rate of growth (cm day^{-1})	DTCP **
	3rd	5th	9th		
PDA	2.33 ± 0.37^{a}	4.78 ± 0.81^{a}	8.76 ± 0.26^{a}	1.11	9
MEA	2.23 ± 0.26^{ab}	4.64 ± 0.41^{a}	8.10 ± 0.41^{a}	0.82	10
OMA	2.55 ± 0.27^{a}	4.84 ± 0.33^{a}	8.43 ± 0.59^{a}	0.87	9
CA	1.87 ± 0.09^{b}	3.50 ± 0.50^{b}	6.88 ± 0.61^{b}	0.77	12
YEMA	1.44 ± 0.23^{c}	2.75 ± 0.19^{c}	6.93 ± 0.47^{b}	0.82	12
CD(0.05)	0.39	0.74	0.73		

* Mean ± SD of four replications; **Days taken for completion of growth in petri dish; Values followed by similar superscripts are not significantly different at 5%

Table 3. Mycelial biomass of the *P. ostreatus* strain in different broth

Treatment	21 DAI		30 DAI	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
PD	5.00± 0.14[b]	0.45	8.80	0.60
ME	6.30± 0.28[a]	0.60	10.10	0.70
OM	6.20± 0.42[a]	0.55	10.30	0.80
CB	6.50± 0.28[a]	0.50	11.60	0.60
YEM	5.30± 0.28[b]	0.40	9.90	0.50
CD (0.05)	0.763	Treatments found to be Non-Significant		

Values are mean ± SD of four replications; Values followed by similar superscripts are not significantly different at 5%

Fig. 2. a) Comparative growth of the *P. ostreatus* strain in different broth b) Dried mycelial biomass

since growth occurs over a wide range of temperature. The ideal temperature for the mycelial growth of the *P. ostreatus* strain was assessed at different temperature (20°C, 25°C and 30°C). Five mm disc were inoculated in PDA media and kept at 20°C, 25°C and 30°C in BOD incubator. The temperature has significant effect on the mycelial growth of the *P. ostreatus* strain. The mycelial colour was white. At a low temperature of 20! the mycelium had sparse growth. While at 25! and 30! initially, mycelium was sparse with a fluffy centre, later turning very fluffy with regular margin (Table 4, Fig.3). The mycelium grew best at 30ºC with 3.68 cm growth 3DAI, 6.19cm 5DAI and full growth 7DAI (Table 5). The rate of mycelial growth was 0.67, 1.17 and 1.2 cm day^{-1} respectively at 20°C, 25°C and 30°C. At 20°C, mycelium took 17 days for complete growth in petri dish while at 25! and 30! it took 9 and 7 days, respectively. The study revealed that the optimum temperature for mycelial growth was 30! indicating the suitability of the the *P. ostreatus* strain for tropical region. The result is in agreement with Hoa and Wang (2015) who reported that the mycelial growth was affected by temperature and the optimum temperature for oyster mushroom was 28!.

Effect of different pH on the growth of the *P. ostreatus* strain

Initially at 3DAI at different pH, the mycelial growth was sparse with fluffy centre. The treatments

Table 4. Colour and nature of mycelial growth at different temperatures

Temperature	Colour	Nature of mycelial growth (DAI)		
		3rd	5th	9th
20°C	White	Sparse	Fluffy centre and sparse towards margin	Sparse with irregular margin
25°C	White	Fluffy centre and sparse towards margin	Fluffy centre with regular sparse margin	Fluffy with regular margin
30°C	White	Fluffy centre and sparse towards margin	Fluffy centre with regular sparse margin	Fluffy with regular margin

DAI- Days after inoculation

Table 5. Radial growth and growth rate of mycelium of the the *P. ostreatus* strain at different temperature.

Media	Radial growth of mycelium in Petri dish (DAI) (cm) *			Rate of growth (cm day^{-1})	DTCP **
	3rd	5th	9th		
20°C	0.00 ± 0.00^{c}	1.14 ± 0.11^{c}	2.72 ± 0.30^{c}	0.67	17
25°C	1.96 ± 0.14^{b}	4.67 ± 0.17^{b}	8.80 ± 0.23^{b}	1.17	9
30°C	3.68 ± 0.68^{a}	6.19 ± 0.98^{a}	9.00 ± 0.00^{a}	1.20	7
CD(0.05)		0.795	0.296		

*Values are mean ± SD of four replications; **Days taken for completion of growth in petri dish; Values followed by similar superscripts are not significantly different at 5%

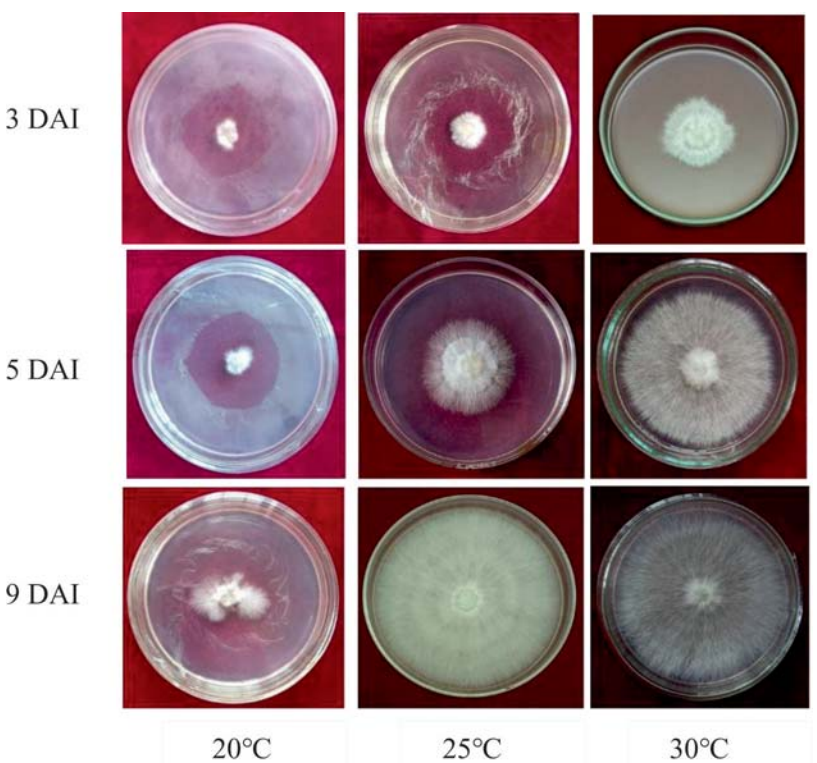

Fig. 3. Comparative growth of the *P. ostreatus* strain in different temperatures

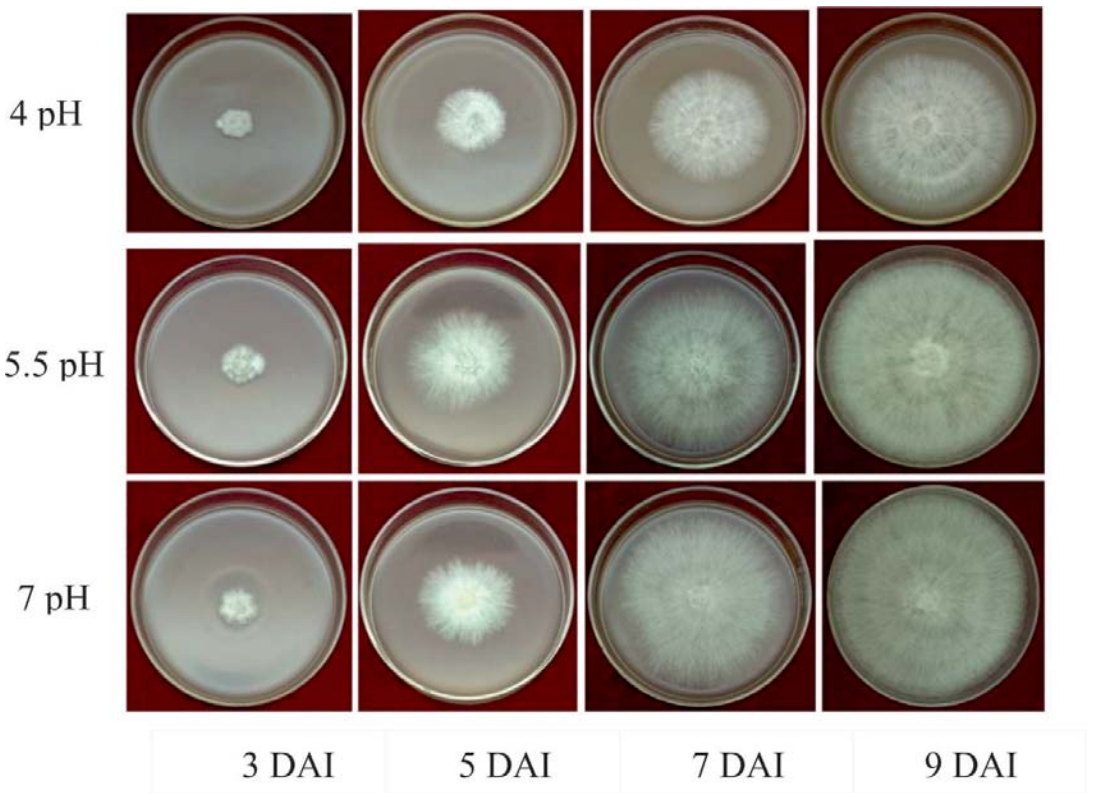

Fig. 4. Comparative growth of the *P. ostreatus* strain at different pH

Table 6. Colour and nature of mycelial growth in different pH.

pH	Colour	Nature of mycelial growth (DAI)		
		3rd	5th	9th
4.0	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
4.5	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
5.0	White	Fluffy centre and sparse towards margin	Fluffy centre and sparse towards margin	Fluffy with regular margin
5.5	White	Sparse with regular margin	Fluffy centre and sparse towards margin	Fluffy with regular margin
6.0	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
6.5	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
7.0	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin
7.5	White	Fluffy centre and sparse towards margin	Sparse with regular margin	Fluffy with regular margin

Table 7. Radial growth and growth rate of mycelium of the *P. ostreatus* strain in different pH.

pH	Radial growth of mycelium in Petri dish (DAI) (cm) *			Rate of growth (cm day^{-1})	DTCP **
	3rd	5th	9th		
4.0	1.59 ± 0.10^{c}	3.35± 0.36^{d}	7.23 ± 0.58 ^{c}	1.06	10
4.5	1.84 ± 0.19 ^{b}	3.91 ±0.42 ^{bc}	8.47 ± 0.52 ^{b}	1.02	9
5.0	1.87 ± 0.10 ^{ab}	3.97 ±0.19 ^{bc}	8.94 ± 0.13 ^{a}	1.20	9
5.5	1.84 ± 0.22 ^{b}	3.82 ±0.60 ^{bcd}	8.70 ± 0.28 ^{ab}	1.16	9
6.0	1.63 ± 0.18 ^{c}	3.71± 0.43 ^{cd}	8.67 ± 0.57 ^{ab}	1.21	9
6.5	2.00 ± 0.12 ^{ab}	3.83 ±0.28 ^{bcd}	8.88 ± 0.13 ^{ab}	1.15	9
7.0	2.05 ± 0.13^{a}	4.35± 0.56 ^{ab}	9.00 ± 0.00 ^{a}	1.38	8
7.5	1.89 ± 0.08 ^{ab}	4.53 ± 0.33^{a}	9.00 ± 0.00 ^{a}	1.40	8
CD (0.05)	0.188	0.536	0.466		

*Values are mean ± SD of four replication; **Days taken for completion of growth in petri dish; Values followed by similar superscripts are not significantly different at 5%

had sparse growth at 5DAI. All the treatment had fluffy growth with regular margin 7DAI (Table 6, Fig. 4). Radial growth of mycelium (3DAI) indicated minimum growth at pH 4.0 while maximum growth at pH 7.0 (2.05cm). On 5 and 9 DAI, similar trend was noticed with maximum growth at pH 7.0, 7.5 and minimum growth at pH 4.0. The rate of mycelial growth was maximum at pH 7.0 and 7.5 (1.38 and 1.40 cm respectively). In all the other treatments, the rate of mycelial growth ranged 1.02 - 1.21 cm. The minimum days for completion of growth in Petri dish were observed at pH 7 and 7.5 and maximum at pH 4 (Table 7). Adebayo Tayo (2011) observed an alkaline pH of 8.0 was optimum for the mycelial growth, biomass and EPS production in *P. ostreatus.*

Similar results were reported by Yadav and Chandra (2014) whereby different strains of *Pleurotus sp*. grew best in slightly acidic to slightly basic pH. Acidic pH was observed to slow down the mycelium growth. Gorai and Sharma (2018) identified the pH of 6.5 to 7.5 as optimum for mycelial growth of *P.ostreatus, P.sajor caju* and *P.florida* which is in congruence to our obtained results.

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