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Asian Journal of Plant Science and Research, 2013, 3(1):163-169

ISSN : 2249-7412
CODEN (USA): AJPSKY

Comparative studies on growth parameters and physio-chemical analysis of *Pleurotus ostreatus and Pleurotus florida*

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ABSTRACT

The present investigation clearly indicated that the Potato Dextrose broth has produced more number of spores followed by maize grains. The results of the study carried out on carbon requirement of P.ostreatus and P. florida showed that dextrose was the best carbon source closely followed by fructose, sucrose and galactose. Of all the tested nitrogen sources, the best mycelia yield was recorded in the medium that contained casein followed by urea, malt extract, peptone, yeast extract and ammonium chloride. Amino acids arginine stimulated the best yield followed by glutamic acid and cysteine. Of all the carbon/nitrogen ratios used ratio 5:3 was best for growth of Pleurotus ostreatus and Pleurotus florida. The growth was significantly enhanced in the medium suggests that all the macro-elements used are required for the growth of this fungus although the level of utilization varies. Magnesium is the best utilizable macro-element, followed by calcium, potassium and sodium. On Potato Dextrose medium the optimum temperature was 25^0C - 30^0C, the optimum pH was 5.5 and the best growth was observed in the inoculants of NaCl concentration 0.5% and 2.5%.

Key words: *Pleurotus ostreatus* , *Pleurotus florida* , Potato Dextrose broth, fungi.

INTRODUCTION

Higher fungi have been used by mankind for millennia**.** *Pleurotus* species have high medicinal value. Compounds extracted from these mushrooms exhibit activity against various chronic diseases including hypertension, hypercholesterolemia [1-3]. The medicinal beneficial effects of *Pleurotus* species were discovered independently in different countries. The awareness of their medicinal properties came not only from Asia but from the folklore of central Europe, South America and Africa [2]. Oyster mushrooms (*Pleurotus* species) are excellently edible and nutritious, rank among one of the most widely cultivated mushrooms in the world [4]. Species of *Pleurotus* are found to possess significant antioxidant, anti-inflammatory and antitumor activities [5-6]. The methanol extract of fruiting bodies of *Pleurotus florida* was found to possess ˙OH radical scavenging and lipid peroxidation inhibiting activities [5]. The extract also showed significant reducing power and radical scavenging property as evident from FRAP assay [7]. Methanol extract of the fruiting bodies of *Pleurotus florida* [5]. *Pleurotus pulmonarius* [6] occurring in South India showed profound antitumor activity against the Ehrlich's ascites carcinoma (EAC) cell line induced solid tumor model in mice. The National Cancer Institute (NCI), United States has recently intensified its emphasis upon natural products such as plants, marine organisms and selected class of microorganisms as sources for new drug discovery. So due to many advantages, the selected fungi *Pleurotus ostreatus* and *Pleurotus florida* are taken into study to develop standard protocol for cheap growth and development *in in vitro* cultivations.

MATERIALS AND METHODS

Studies on different media to observe the growth of *Pleurotus* isolates

Determination of growth

Growth pattern of the *Pleurotus* isolates was studied in Potato Dextrose broth and other commonly available natural agricultural wastes. The isolated fungal strain was inoculated into the media and the culture was incubated at 20^0C. At every 24hrs interval, growth was noticed. The growth of the culture in broth is noticed by taking the spore counts and dry weights at successive time intervals**.**

Procedure

The ingredients were weighed and dissolved in distilled water in a conical flask. The pH of the medium was adjusted to 5.5 by adding either acid or alkali. Then the conical flask containing medium was autoclaved at 15 lbs pressure for 15 minutes at 121^OC. Antibiotic (Streptomycin) was added to the medium after cooling and mixed gently. Then the medium was inoculated with the loop of *Pleurotus* isolates culture. Then the flasks were kept at room temperature for growth observation.

Methods for observation of growth (Spore counting method)

Growth of Pleurotus isolates on different media was observed by spore count method using Haemocytometer.

Effect of carbon compounds on the growth of *Pleurotus* isolates

The Czapek's-Dox medium was prepared and dissolved in distilled water in a conical flask. The pH of the medium was adjusted to 5.5 by adding either acid or alkali. Then the conical flask containing medium was autoclaved at 15 lbs pressure for 15 minutes at 121^OC. Antibiotic (Streptomycin) was added to the medium after cooling and mixed gently. Then the medium was inoculated with the loop of *Pleurotus* isolates. Then the flasks were kept at room temperature for growth observation.The liquid medium was supplemented separately with 1% carbon of each carbon compound. The medium without any carbon source served as the control [8].

Utilization of nitrogen sources by *Pleurotus* isolates

The ingredients of Czapek-Dox medium were weighed and dissolved in distilled water in a conical flask. The pH of the medium was adjusted to 5.5 by adding either acid or alkali. Then the conical flask containing medium was autoclaved at 15 lbs pressure for 15 minutes at 121^OC. Antibiotic (Streptomycin) was added to the medium after cooling and mixed gently. Then the medium was inoculated with the loop of *Pleurotus* isolates.Complex nitrogen sources (peptone, urea, yeast extract, malt extract and casein hydrolysate) were added separately at the rate of 2 g/1000 cm^3. The basal medium without nitrogen source served as the control.Then the flasks were kept at room temperature for growth observation.

Effect of different carbon/nitrogen ratios

The basal medium used was the same as for the nitrogen sources but without glucose. The best carbon and nitrogen sources in the last two experiments, i.e. glucose and yeast extract (0.1 g of each), were supplemented in the 1000 cm^3 of basal medium; this formed a ratio 1:1. Other ratios were also prepared to form different concentrations [9].

Utilization of macro-nutrients by *Pleurotus* isolates

The ingredients of basal medium were weighed and dissolved in distilled water in a conical flask**.**.The pH of the medium was adjusted to 5.5 by adding either acid or alkali. Then the conical flask containing medium was autoclaved at 15 lbs pressure for 15 minutes at 121^OC**.** Antibiotic (Streptomycin) was added to the medium after cooling and mixed gently. Then the medium was inoculated with the loop of *Pleurotus* isolates. Each macro-element to be used, one having all the macro-nutrients and the other having none. Then the flasks were kept at room temperature for growth observation.

Effect of trace elements on the growth of *Pleurotus* isolates

The ingredients of basal medium were weighed and dissolved in distilled water in a conical flask. The pH of the medium was adjusted to 5.5 by adding either acid or alkali. Then the conical flask containing medium was autoclaved at 15 lbs pressure for 15 minutes at 121^OC. Antibiotic (Streptomycin) was added to the medium after cooling and mixed gently. Then the medium was inoculated with the loop of *Pleurotus* isolates.Five trace elements (copper, iron, manganese, cobalt and zinc in their sulphate form), were added separately to the basal medium at the rate of 10 mg/1000 cm^3. Two sets of controls were also prepared. The first consisted of all the trace elements while the second set contained basal medium without any micro-element.Then the flasks were kept at room temperature for growth observation.

Calculating the dry weight of mycelium

The dry weights of the mycelia were calculated by the following procedure. After incubation, the cultures were filtered at an interval of seven days, through a pre-weighed filter paper to separate the mycelium. The mycelium was dried at 80^0C and weighed.

Physico-chemical parameters:

Effect of temperature on *Pleurotus* isolates

100ml of potato dextrose broth was prepared in different conical flasks of 250ml volume. Set of flasks were incubated under static condition at different temperatures (25^0C, 28^0C, 30^0C, 35^0C, 37^0C, 40^0 C, 45^0C, 55^0C) for 4 days. The flasks were kept in autoclave for sterilization. Flasks were inoculated with *Pleurotus* isolates culture. Flasks were incubated for 7 days and the growth of *Pleurotus* isolates was compared with the control [10].

Growth observation of *Pleurotus* isolates at different pH levels

The ingredients of the Potato Dextrose broth were weighed and dissolved in distilled water.100ml of medium were distributed in each conical flask. The pH of the each medium was adjusted as 4, 5,6,7,8 and 9.Then the conical flasks with different pH level as mentioned above were autoclaved at 15 lbs pressure for 20 minutes at 121^OC.The flasks were allowed to cool. Each flask was inoculated with loop full of *Pleurotus* isolates culture aseptically.

Growth observation of *Pleurotus* isolates at different salt concentrations

100ml of potato dextrose broth was prepared in different conical flasks of 250ml volume. 0%, 0.5%, 5%, 7.5% and 10% of NaCl was added to all the flasks. The flasks were kept in autoclave for sterilization. Flasks were inoculated with *Pleurotus* isolates culture. Flasks were incubated for 3-5 days and the growth of *Pleurotus* isolates was compared with the control

RESULTS AND DISCUSSION

Studies on the growth of *Pleurotus* isolates

Growing fungi on laboratory media is a costly and tedious process. Keeping this in view, the present study was carried out to determine the performance of growth of screened *Pleurotus* isolates on different media. So that the media that produces maximum growth can be selected for the commercial production as the sources used other than Potato Dextrose broth were naturally available cheaper sources. From the observations, it was clearly evident that the Potato Dextrose broth has produced more number of spores followed by maize grains and least number of spores was produced in Oil palm empty fruit bunches powder. In all the media used, *Pleurotus ostreatus* produced more number of spores than *Pleurotus florida* (Table 1).

Table.1: Growth of *Pleurotus* isolates on different media

S.No	Medium	Spore count per ml	
		Pleurotus Ostreatus	***Pleurotus florida***
1	Potato Dextrose broth	$6.93X10^3$	$5.32X10^3$
2	Maize grains	$3.32X10^3$	$3.13X10^3$
3	Rice Straw	$2.84X10^3$	$2.54X10^3$
4	Rice husk	$1.29X10^3$	$0.932X10^3$
5	Oil palm empty fruit bunches	$0.912X10^3$	$0.72X10^3$

Effect of carbon compounds on the growth of *Pleurotus* isolates

The results of the study carried out on carbon requirement of *P.ostreatus* and *P. florida* showed that dextrose was the best carbon source. This was closely followed by fructose, sucrose and galactose. The utilization of dextrose by tropical edible mushroom has not been reported while glucose utilization has been reported [8-9, 11-13]. Dextrose, which is an isomer of glucose, can be transformed to glucose during metabolism [14]. The result of this present findings are contradictory to the observation of [15] who suggested that mannitol and fructose are the most commonly utilizable sugars after glucose. The utilizable carbon sources used during this study have significant effect on the growth of the fungi (Table.2).

Utilization of nitrogen sources by *Pleurotus* isolates

Of all the tested nitrogen sources, the best mycelia yield was recorded in the medium that contained casein. This was followed by urea, malt extract, peptone, yeast extract and ammonium chloride. Amino acids generally support good growth of this macro fungus with arginine stimulating the best yield followed in order by glutamic acid and cysteine. All other inorganic nitrogen sources showed poor growth. Sodium nitrate and calcium nitrate supported growth which is only slightly higher than that of the control. Nitrate ions have been implicated in the inhibitory effect of

some basidiomycetes [15-16] which may be difficult to transport across the fungal membrane where it can promote growth (Table.3).

Table.2: Growth of *Pleurotus* isolates on different carbon compounds

S.No	Carbon Source	Dry weight (g)		Final pH of the filtrate	
		P.ostreatus	*P.florida*	*P.ostreatus*	*P.florida*
Hexoses					
1	Dextrose	1.86	1.84	6.2	6.2
2	Fructose	1.64	1.52	5.9	5.8
3	Galactose	1.21	1.02	5.4	5.4
4	Mannitol	0.94	0.89	5.2	5.1
Pentoses					
5	Xylose	0.86	0.76	5.0	4.9
6	Arabinose	0.78	0.71	4.9	4.9
7	Ribose	0.73	0.7	4.9	4.8
Disaccharides					
8	Sucrose	1.45	1.34	6.0	5.9
9	Lactose	0.41	0.34	4.7	4.7
10	Maltose	0.5	0.48	4.5	4.5
11	Blank	0.6	0.58	4.6	4.6

Table.3: Growth of *Pleurotus* isolates on different nitrogen sources

S.No	Nitrogen Source	Dry weight (g)		Final pH of the filtrate	
		P.ostreatus	*P.florida*	*P.ostreatus*	*P.florida*
Inorganic					
1	Ammonium Chloride	0.94	0.89	5.1	5.1
2	Calcium nitrate	0.5	0.48	4.5	4.5
3	Sodium nitrate	0.41	0.34	4.7	4.7
Organic					
4	Urea	1.86	1.84	5.6	5.6
5	Yeast Extract	1.3	1.2	6.0	6.0
6	Peptone	1.2	1.1	6.0	6.0
7	Casein	2.1	1.8	5.4	5.4
8	Malt extract	1.45	1.34	5.3	5.2
Amino acids					
9	Arginine	1.43	1.41	5.2	5.2
10	Methionine	0.82	0.78	5.9	5.9
11	Glutamic acid	1.41	1.42	4.7	4.7
12	Cysteine	0.88	0.88	5.9	5.9
14	Blank	0.4	0.4	6.2	6.2

Effect of different carbon/nitrogen ratios

All the carbon/nitrogen ratios used in this study promoted growth significantly. The fungus grew best on a medium with ratio 5:3 followed by 1:4, 1:2 and 2:1, while the least growth was obtained with the ratio 3:4. These ratios were different from that obtained by [17] for *Psathyerella atroumbunata*. This shows that *Pleurotus* isolates had specific growth requirements. This fungi was able to utilize substrates that contain carbon and nitrogen sources within a tolerable limit [18] (Table.4).

Table.4: Growth of *Pleurotus* isolates at different carbon/nitrogen ratios

S.No	Carbon:Nitrogen ratio	Dry weight (g)		Final pH of the filtrate	
		P.ostreatus	*P.florida*	*P.ostreatus*	*P.florida*
1	01:01	0.71	0.69	5.5	5.5
2	01:02	1.74	1.7	5.8	5.8
3	01:03	0.7	0.7	5.5	5.5
4	01:04	1.72	1.6	5.8	5.8
5	02:01	1.2	1.0	5.6	5.6
6	02:03	0.85	0.8	5.6	5.6
7	03:04	0.67	0.65	5.6	5.6
8	04:02	0.89	0.85	5.6	5.6
9	05:02	1.0	0.8	5.6	5.6
10	05:03	1.1	1.09	5.4	5.4
11	00:00	0.4	0.3	6.1	6.1

Utilization of macro and trace nutrients by *Pleurotus* isolates

Growth of *Pleurotus ostreatus* and *Pleurotus florida* were significantly enhanced in the medium that contained all the macro-elements. This suggests that all the macro-elements used are required for the growth of this fungus although the level of utilization varies. Complete medium without Na stimulated the best growth followed in order by medium that lacks potassium, magnesium and calcium. This result indicates that magnesium is the best utilizable macro-element, followed by calcium, potassium and sodium. Similar observations were made by [9, 11] on *Volvariella esculenta* and *Psathyerella atroumbunata*. [15] Attributed the importance of calcium in fungal growth to its enzyme activity while magnesium is importance in ATP metabolism. Among the trace elements, the highest mycelia yield was obtained on Co-free medium followed in order by media without manganese and copper, while there was no growth in medium without iron and zinc. This result implies that *Pleurotus ostreatus* and *Pleurotus florida* can grow effectively in the absence of manganese and cobalt. Inability of this fungus to utilize these two trace elements for growth may be related to their toxicity to fungal cells. A similar toxic effect was reported by [8] for *Volvariella volvacea*. The absence growth recorded in iron and zinc free media implies that they were needed for growth. Both iron and zinc are needed for fungal enzyme activities and for intermediary metabolism respectively [15]. Poor mycelial yield obtained on basal medium (control 2) suggests that *Pleurotus ostreatus* and *Pleurotus florida* requires some trace elements for their growth [15-16]. It was clear from these studies that growth of *Pleurotus ostreatus* and *Pleurotus florida* were enhanced by dextrose and casein. These could be incorporated into the medium in a ratio of 5:3. Calcium, magnesium and potassium also stimulated good growth, while iron and zinc (in a very low concentration) are needed for propagation of *Pleurotus ostreatus* and *Pleurotus florida*. All these can be supplemented in the growth media to produce high mycelia yield needed for spawning and fruit body production of this edible mushroom (Table.5).

Table.5: Growth of *Pleurotus* isolates by utilizing micro and trace nutrients

S.No	Nitrogen Source	Dry weight (g)		Final pH of the filtrate	
		P.ostreatus	***P.florida***	***P.ostreatus***	***P.florida***
Macro-elements					
1	Complete medium (control 1)	1.4	1.34	6.2	6.2
2	Complete medium without sodium	2.29	2.24	5.2	5.2
3	Complete medium without magnesium	0.81	0.68	4.8	4.8
4	Complete medium without potassium	1.24	1.15	5.6	5.6
5	Complete medium without calcium	0.38	0.32	4.6	4.6
6	Basal medium only (control 2)	0.52	0.49	4.5	4.5
Trace-elements					
7	Complete medium (control 1)	0.98	0.98	6.1	6.1
8	Complete medium without copper	0.13	0.12	4.2	4.2
9	Complete medium without iron	No Growth	No Growth	4.3	4.3
10	Complete medium without manganese	0.87	0.84	5.1	5.1
11	Complete medium without cobalt	0.45	0.41	5.5	5.5
12	Complete medium without zinc	No Growth	No Growth	4.8	4.8
13	Basal medium only (control 2)	0.35	0.32	4.7	4.7

Studies on the effect of Physico-chemical parameters (Temperature, P^H and Salt concentration) on the growth of *Pleurotus* isolates

After inoculation of *Pleurotus ostreatus* and *Pleurotus florida* in the Potato Dextrose broth separately in conical flasks and incubated at different temperatures (25-55^{0}C) for 7 days, maximum growth was observed at temperatures of 25^{0}C for *Pleurotus ostreatus* and 30^{0}C for *Pleurotus florida* whereas minimal growth was observed till temperatures 35^{0}C and 37^{0}C by both the organisms and there was no growth above temperatures of 40^{0}C. This reveals revels that the optimum temperature was 25^{0}C - 30^{0}C.

Observations on Potato Dextrose broth with different pH (2.5- 9.5) suggest that there was no growth at pH 2.5, and 3.5. The maximum growth was observed at pH 5.5 and the moderate growth was observed at pH 6.5, 7.0, 7.5 and 8.5 whereas minimal growth at pH 4.5 and no growth was observed in all the inoculants at pH 9.5. These results indicate that the optimum pH for growth of fungi at these conditions was 5.5 and moderate growth in between 6.5 to 8.5. Similar trend was noticed by both the organisms.

Growth medium with different salt concentrations (0%, 0.5%, 5%, 7.5% and 10%) were inoculated with *Pleurotus ostreatus* and *Pleurotus florida* separately and incubated for 7 days. The growth was observed in all the inoculants of NaCl concentration 0%, 0.5% and 2.5% whereas no growth was observed in 5%, 7.5% and 10% of NaCl concentration containing inoculants. Maximal growth was noticed in the inoculants with salt concentration. Similar

trend was noticed by both the organisms. This gives the information that the growth was possible for our desired fungi was upto 2.5% Sodium chloride (NaCl) concentration only (Table.6). Similar growth pattern was observed by [19-21] while growing *Pleurotus* species on different nutrient media.

Table.6: Effect of Physico-chemical parameters (Temperature, P^H and Salt concentration) on thre growth of *Pleurotus* isolates

Physico-chemical parameter	Varied value	*Pleurotus ostreatus*	*Pleurotus florida*
Temperature(°C)	25	+++	++
	28	++	++
	30	++	+++
	35	+	+
	37	+	+
	40	-	-
	45	-	-
	55	-	-
pH	2.5	-	-
	3.5	-	-
	4.5	+	+
	5.5	+++	+++
	6.5	++	++
	7	+	+
	7.5	+	+
	8.5	+	+
	9.5	-	-
Salt concentration (%)	0	+++	+++
	0.5	++	++
	2.5	+	+
	5	-	-
	7.5	-	-
	10	-	-

CONCLUSION

The present investigation clearly indicated that the two species *P.ostreatus* and *P. florida* showed more or less similar characters in its growth behaviour and physiochemical characters. The Potato Dextrose broth is the best medium suggested for the culture of these species. Of all the tested nitrogen sources, the best mycelia yield was recorded in the medium that contained casein. Amino acids arginine stimulated the best yield. Of all the carbon/nitrogen ratios used ratio 5:3 was best for growth of *Pleurotus ostreatus* and *Pleurotus florida*. For best yield and production of the above species all the above suggested parameters are used because of the medicinal values of these species. Much of the bioactive compound can be isolated and used for the betterment of mankind from these medicinally important stains.

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