THE INFLUENCE OF pH AND THE SOURCE OF NITROGEN ON THE MYCELIAL GROWTH OF THE PLEUROTUS OSTREATUS MUSHROOMS

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Abstract

Pleurotus ostreatus mushrooms (popularly called phage trout or oyster mushroom) are edible flora mushrooms under natural conditions, created spontaneous by beech wood waste, from which he also received the popular name of "Trout phage". Cultivation in artificial conditions required a long work of research and selection carried out in different countries around the world to adapt to the growing conditions in artificially climate, with making a substrate of culture optimal to obtain yields bigger per unit area, in terms of profitability.

Pleurotus ostreatus mycelium development requires a rich material in polysaccharides and lignin, sufficiently low in essential minerals and without having been previously attacked by bacteria (decomposed). Compared with mineral sources, organic nitrogen sources have a particular influence on mycelial growth. Thus, the addition of barley flour or malt fangs is favorable.

In contrast to the genus *Agaricus* mushroom, at *Pleurotus* species the nutrient medium does not require a fence part (fermentation) of the transformed microorganisms or partially hydrolysed by physico-chemical processes.

Pleurotus ostreatus mycelium growth is carried out at pH values between 5 and 6.5 the limits being from 4.2 to 7.5. A highly acidic pH (pH 4) stops the growth of mycelium. Increasing the pH of 4 to 6.5 has a positive influence on the growth of mycelium, unlike higher values, ie pH neutral or slightly alkaline (pH 7-8), when the mycelium growth is again partially inhibited.

With regard to sources of nitrogen, it has been found that the addition of albumin, in the form of soybean meal, reduces the mycelium growth and delay the production of mushrooms.

Key words: *Pleurotus ostreatus*, mushrooms, mycelium, pH, nitrogen source

Latin and greek roots: *Pleurotus* comes from the greek “pleuro” which means formed laterally or in a sideways position, referring to the lateral position of the stem relative to the cap. The species ephitet *ostreatus* refers to its oyster shell-like appearance and color.

General description: Cap convex at first, expanding to broadly convex, eventually flat and even upturned in age; 5-20cm (+) in diameter. White to yellow to grayish yellow to tan, rarely with pinkish tones, to lilac gray to gray-brown. Cap margin smooth to undulating like an oyster shell. Color varies according to the strain, lighting and temperature conditions. Stems are typically eccentrically attached to the cap. Flesh generally thin. Some strains form clusters; others forms individuals.

Microscopic features: Spores white to slightly lilac to lilac gray, 7.5-9.5x3-4µ. Clamp connections present. Context monomitic.

Mycelial characteristics: Whitish, longitudinally radial, soon becoming cottony, and in age forming a thick, tenacious mycelial mat. Aged mycelium often secrets yellowish to orangish droplets of a metabolite, a toxin to nematodes. This metabolite deserves greater study.

Fragnance signature: Sweet, rich, pleasant, distinctly anise, and almost almond-like.

MATERIAL AND METHOD

Isolation of *Pleurotus ostreatus* mycelia.

Materials: Fresh mushrooms, inoculation needle, sterilized agar medium, Petri plates, laminar airflow, Bunsen burner and sterilized PDA slants.

Method: Was taked a fresh mushroom for tissue culture, cleaned the surface with 70% aqueous ethyl alcohol, transferred a small piece with the help of inoculation needle under laminar airflow. Both hands and bottles was cleaned with alcohol and inserted into the laminar air cabinet. The needle was holded with two fingers in an 45º angle and was flamed to disinfect (until the needle turns red). While the needle cools down (15-20

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seconds) using other fingers the mushroom were teared lengthwise . With the needle was cuted a small piece (2 mm x 2 mm) of fleshy tissue from inside the mushroom and was placed in the middle of the PDA surface. The PDA plates was labeled and incubated at 24ºC for 7 days and observed the growth.

For determination the influence of the nitrogen source on the *Pleurotus ostreatus* mushroom mycelium gowth, we used a semisolid agar medium using the following formula/recipes: sterilized PDA medium (diced potato 200g/L, dextrose 20g/L and agar 15g/L) used as control, PDA medium with 15 g/L soybean meal (PDAS) and PDA medium with yeast (PDAY) at different pH values: 4, 4.5, 5, 5.5, 6, 6.5, 7 and 7.5. The results of experiences were recorded during 7 days of mycelium growing in mm/day.

RESULTS AND DISCUSSIONS

It was analyzed the unilateral influence of agar recipe on the *Pleurotus ostreatus* mycelium growth expressed in mm/day. *Table 1* presents the obtained values. Analyzing the data in the table, it can be said that PDAY recipe, recorded positive differences compared to the experience average, the PDA recipe. At PDAS recipe we recorded negative differences from the average.

Table 1

Unilateral influence of agar recipe on the *Pleurotus ostreatus* mycelium growth

Recipe	Mycelium growth -mm/day-		Difference ±D	Signification of difference
	Obtained values	%		
PDA	4.11	100.0	0.00	Mt.
PDAS	3.34	81.3	-0.77	0
PDAY	4.68	113.9	0.57	*
DL (p 5%)			0.49	
DL (p 1%)			0.80	
DL (p 0.1%)			1.50	

It was analyzed the unilateral influence of agar recipe pH on the *Pleurotus ostreatus* mycelium growth expressed in mm/day. Table 2 presents the obtained values. Analyzing the data in the table, it can be said that pH 4, 4.5 and 5, recorded very significant positive differences compared to the experience average. At pH 6 to 7.5 we recorded very significant negative differences from the average.

Table 2

Unilateral influence of agar recipe pH on the *Pleurotus ostreatus* mycelium growth

Recipe pH	Mycelium growth -mm/day-		Difference ±D	Signification of difference
	Obtained values	%		
	4.04	100.0	0.00	Mt.
4	4.73	117.1	0.69	***
4.5	7.63	188.8	3.59	***
5	4.71	116.5	0.67	***
5.5	3.88	95.9	-0.17	-
6	3.47	85.7	-0.58	000
6.5	3.26	80.5	-0.79	000
7	2.88	71.2	-1.17	000
7.5	1.79	44.2	-2.25	000
DL (p 5%)			0.22	
DL (p 1%)			0.29	
DL (p 0.1%)			0.38	

Synthesis using comparisons by Duncan test for the agar recipe pH is presented in table no. 3. We can see that on the first place is located the pH 4.5 recipe with 7.63 mm/day of growth, followed by the pH 4 and 5 recipes. On the last place was located the pH 7.5 recipe with 1.79 mm/day.

Table 3

Synthesis using comparisons by Duncan test for the agar recipe pH on the *Pleurotus ostreatus* mycelium growth

Recipe pH	Mycelium growth mm/day	Significance*
4.5	7.63	A
4	4.73	B
5	4.71	B
5.5	3.88	C
6	3.47	D
6.5	3.26	D
7	2.88	E
7.5	1.79	F

DS 0.22-0.25

* Values marked with different letters are significant

In combining experimental factors, pH value and recipe, on mycelia growth we recorded significant values (table 4).

The influence of combined factors, pH on the growth recipe of mycelium, at pH 4.5 on PDAY recipe was recorded the highest values 8.93 mm/day of growth, followed by pH 4.5 on PDA recipe with 8.17 mm/day of growth. On the last place was located pH 7.5 on PDAS recipe with 1.37 mm/day of growth.

Table 4

Combining experimental factors, pH and recipe, on mycelia growth of *Pleurotus ostreatus* mushroom

pH	Recipe	Mycelia growth –mm/day-		Difference ±D	Signification of difference
		Obtained values	%		
		4.11	100.0	0.00	Mt.
4	PDA	4.73	115.2	0.63	**
4.5	PDA	8.17	198.8	4.06	***
5	PDA	4.67	113.6	0.56	**
5.5	PDA	3.57	86.8	-0.54	00
6	PDA	3.53	86.0	-0.58	00
6.5	PDA	3.33	81.1	-0.77	000
7	PDA	2.93	71.4	-1.18	000
7.5	PDA	1.93	47.1	-2.18	000
		3.34	100.0	0.00	Mt.
4	PDAS	3.83	114.7	0.49	*
4.5	PDAS	5.80	173.6	2.46	***
5	PDAS	3.97	118.7	0.63	**
5.5	PDAS	3.47	103.7	0.12	-
6	PDAS	3.03	90.8	-0.31	-
6.5	PDAS	2.83	84.8	-0.51	00
7	PDAS	2.43	72.8	-0.91	000
7.5	PDAS	1.37	40.9	-1.98	000
		4.68	100.0	0.00	Mt.
4	PDAY	5.63	120.4	0.95	***
4.5	PDAY	8.93	190.4	4.25	***
5	PDAY	5.50	117.5	0.82	***
5.5	PDAY	4.60	98.3	-0.08	-
6	PDAY	3.83	81.9	-0.85	000
6.5	PDAY	3.60	76.9	-1.08	000
7	PDAY	3.27	69.8	-1.41	000
7.5	PDAY	2.07	44.2	-2.61	000
DL (p 5%)				0.38	
DL (p 1%)				0.50	
DL (p 0.1%)				0.66	

In combining experimental factors, recipe and pH value, on mycelia growth we recorded significant values (table 5).

The influence of combined factors, the recipe and pH value on mycelium growth, at pH 4.5 on PDAY recipe was recorded the highest values 8.93 mm/day of growth, followed by pH 4.5 on PDA recipe with 8.17 mm/day of growth, taked as control. On the last place was located pH 7.5 on PDAS recipe with 1.37 mm/day of growth.

Table 5

Combining experimental factors, pH and recipe, on mycelia growth of *Pleurotus ostreatus* mushroom

Recipe	pH	Mycelia growth –mm/day-		Difference ±D	Signification of difference
		Obtained values	%		
PDA	4	4.73	100.0	0.00	Mt.
PDAS	4	3.83	81.0	-0.90	0
PDAY	4	5.63	119.0	0.90	*
PDA	4.5	8.17	100.0	0.00	Mt.
PDAS	4.5	5.80	71.0	-2.37	000
PDAY	4.5	8.93	109.4	0.77	*
PDA	5	4.67	100.0	0.00	Mt.
PDAS	5	3.97	85.0	-0.70	0
PDAY	5	5.50	117.9	0.83	*
PDA	5.5	3.57	100.0	0.00	Mt.
PDAS	5.5	3.47	97.2	-0.10	-
PDAY	5.5	4.60	129.0	1.03	**
PDA	6	3.53	100.0	0.00	Mt.
PDAS	6	3.03	85.8	-0.50	-
PDAY	6	3.83	108.5	0.30	-
PDA	6.5	3.33	100.0	0.00	Mt.
PDAS	6.5	2.83	85.0	-0.50	-
PDAY	6.5	3.60	108.0	0.27	-
PDA	7	2.93	100.0	0.00	Mt.
PDAS	7	2.43	83.0	-0.50	-
PDAY	7	3.27	111.4	0.33	-
PDA	7.5	1.93	100.0	0.00	Mt.
PDAS	7.5	1.37	70.7	-0.57	-
PDAY	7.5	2.07	106.9	0.13	-
DL (p 5%)				0.59	
DL (p 1%)				0.90	
DL (p 0.1%)				1.50	

CONCLUSIONS

Pleurotus ostreatus mycelium growth is carried out at pH values between 5 and 6.5 the limits being from 4.2 to 7.5. A highly acidic pH (pH 4) stops the growth of mycelium. Increasing the pH of 4 to 6.5 has a positive influence on the growth of mycelium, unlike higher values, ie pH neutral or slightly alkaline (pH 7-8), when the mycelium growth is again partially inhibited.

With regard to sources of nitrogen, it has been found that the addition of albumin, in the form of soybean meal, reduces the mycelium growth and delay the production of mushrooms.

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