CULTIVATION OF THREE *PLEUROTUS* STRAINS ON SOME ORGANIC CARBON-ENRICHED MEDIA

CULTIVAREA A TREI TULPINI DE *PLEUROTUS* PE MEDII ÎMBOGĂȚITE CU SURSE ORGANICE DE CARBON

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Abstract. *An enhanced availability of nutrients in the culture media and the enrichment with carbon sources, nitrogen sources and micro and macro- mineral elements can ensure both qualitative and quantitative development of mycelia cultivated in vitro on solid nutrient media or in submerged cultures. The influence of diverse organic carbon sources incorporated into experimental variations of medium made with aqueous extracts from white grape marc, red grape marc, brewery waste, corn cobs, coffee grounds and fruit waste were investigated in this paper. For each stage of the technological phase in which the biological material is present, Pleurotus spp mycelia can be characterized according to their physiological state when interacting with the culture medium and the environmental parameters provided. Only uniformity, vigour, vitality, phenotypic integrity and homogeneity between samples/replicates can provide evidence of the mycelial quality.*
Key words: lignocellulosic waste, mycelium, optimization, *Pleurotus*, quality

Rezumat. *O dezvoltare calitativă și cantitativă a miceliului cultivat in vitro pe medii nutritive solide sau în culturi submerse poate fi asigurată printr-o accesibilitate sporită a nutrienților din mediul de cultură și îmbogățirea acestuia prin suplimentare cu surse organice de carbon, azot și minerale de tipul micro și macroelementelor. Articolul urmărește influența unor diferite surse organice introduse în variantele experimentale de medii preparate cu extracte apoase provenind din tescovină de struguri albi respectiv struguri roșii, borhot de bere, ciocălăi de porumb, zaț de cafea și coji de fructe. Caracterizarea miceliilor de Pleurotus spp se poate face după starea fiziologică a acestora aflate în interacțiune cu mediul de cultură și condițiile de mediu asigurate, pentru fiecare etapă a fluxului biotehnologic în care materialul biologic se află. Calitatea miceliilor poate fi confirmată numai prin stabilitate, vigoare, vitalitate, integritate fenotipică și omogenitate între probe/replicate.*
Cuvinte cheie: calitate, deșeuri lignocelulozice, micelii, optimizare, *Pleurotus*

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INTRODUCTION

A fertile *Pleurotus* spp. dikaryotic mycelium can be characterized by its physiological state in relation to the culture medium used and the provided environmental conditions (Martínez-Carrera, 1998). The quality indicators observed at this stage of the biotechnological flow are the growth rate, color, appearance, mycelial network texture, hyphal density, growth streak patterns, flat or aerial tendency of development, lack of sectoring, presence/absence of exudate droplets, biological purity and potential fruiting primordia appearance - the latter require an extended amount of time and low temperatures (Bilay *et al*., 2000, Ahmad *et al.,* 2015, *Zăgrean et al.,* 2016). The most commonly used media types for the cultivation of the majority of edible and/or medicinal macromycetes species are usually those based on malt or potato dextrose extracts and enriched with glucose, peptone, fructose, yeast extract, organic sources represented by different lignocellulosic biomasses, vitamins and minerals added in various forms (Tello *et al*., 2016; Shruti *et al.,* 2022).

In addition to the categories of agro-industrial auxiliary materials examined in this paper, numerous scientists have reported their findings on a variety of byproducts, including biomasses from the industrialization of olives, vegetal oil residues, by-products of soybean production and some teas (Gao *et al.,* 2009; Țețu, 2019; Werghemmi *et al.,* 2022).

The nature and composition of the nutrient medium, pH value, influence of temperature and incubation time, presence or absence of light, O_2/CO_2 concentration and the biological purity of the material are the extrinsic factors that have an impact on mycelial development. Intrinsic factors that also play an important role include strain characteristics, age and physiological condition (Zhong and Tang, 2004). Variations in these factors may result in atypical colonies with affected morphological and growth characteristics, not succeeding to comply with the requirements for both this stage of the technological flow and the other ensuing biopreparations, such as intermediate mycelium (inoculum) and seeding spawn (commercial mycelium).

MATERIAL AND METHOD

The biological material used in this paper belongs to the macromycetes collection of RDIVFG Vidra, where all practical laboratory experiments were carried out during January-August 2023.

Six solid nutritional media were investigated and each of them have been enriched with organic carbon sources obtained from agro-industrial biomasses. In this paper, water-based extracts of white and red grape marc, brewery by-product, corn cobs, coffee grounds and semi-composted fruit waste were used. By mechanically blending equal amounts (m/m) of each category of organic materials with distilled water, extracts from each category were produced. Following decantation, separation and filtration, a 50% volume of Malt Extract medium was homogenized with a 50% extract volume from each lignocellulosic category, as follows: V0 (malt extract -

control), V1 (white grape marc), V2 (red grape marc), V3 (beer waste), V4 (corn cobs), V5 (coffee grounds) and V6 (fruit peels). After preparation, the pH of every media has been adjusted to 6.5. The samples were transferred into Erlenmeyer flasks and autoclaved for 25 minutes at 121ºC.

As biological material, mycelia from three *Pleurotus* species were used: *P. ostreatus* with the strain PoM-77, *P. columbinus* strain PcM-98 and *P. eryngii* strain PeM-41. The Petri dishes were inoculated aseptically with a mycelial fragment collected with a cork borer (Ø5 mm) in the centre of each plate. The incubation was realized at 25-26ºC in the absence of light. The three *Pleurotus* strains tested exhibited colonies with white, tightly compact mycelia, displayed a medium-high aerial growth, a few concentric growth patterns and strain-specific morphological and cultural features. The measurements were quantified and statistically examined using one-factor Anova analysis.

RESULTS AND DISCUSSIONS

The investigated *Pleurotus* strains displayed colonies with medium or increased aerial growth, a few concentric growth patterns, with a white, fluffy, densely packed mycelium. The expansion of the colonies from the point of inoculation to the edge of the hyphal network, averaged over 4 perpendicular radii, was used to calculate the mycelial growth rates of the three strains, at 7 days after inoculation.

Table 1 displays the mycelial growth of the PoM-77 strain of *Pleurotus ostreatus*

Table 1

Mycelial growth of strain PoM-77 on solid media

Strain/ variant	Mean value	Martor ratio	Martor differnce	Significance of difference
	mm	*%*	*mm*	
PoM-77/V0	35.44	100	0	Mt
PoM-77/V1	37.94	107.05	2.50	
PoM-77/V2	28.63	80.78	-6.81	000
PoM-77/V3	39.50	111.46	4.06	**
PoM-77/V4	38.06	107.41	2.63	*
PoM-77/V5	35.31	99.65	-0.12	
PoM-77/V6	34.69	97.88	-.075	
		DL 5%	2.50	
		DL 1%	3.40	
		DL 0.1%	4.59	

Pleurotus ostreatus strain PoM-77 exhibited white, fluffy mycelia, with a medium aerial growth and no exudate droplets or sectoring. Growth rate had the highest values on the medium variant V3 with an average of 5.64 mm/day, followed by V4: 5.4 mm/day, V1 with 5.41 mm/day, V0: 5.06 mm/day, V5: 5.04 mm/day, V6: 4.95 mm/day and last, V2: 4.08 mm/day. This strain best results were obtained on the culture medium variant enriched with brewery waste (V3) attributed to the contribution of malt and starch substances present in the by-

products resulting from brewery production (Iwase *et al.,* 2000, Thomas and Rahman, 2006, Pestsov *et al.,* 2021), the composition of this medium variant also had a significant impact on the hyphal density, with a denser gradient compared to the other media. The weakest results were recorded on the V2 variant, with lower values than the V0 control, due to the high concentration of tannins and biomolecules potentially inhibiting mycelial development present in the red grape marc. The addition of lignocellulosic matter and carbohydrates led to an increased hyphal density on V4 medium in addition to the raised speed. Values very close to the control were found for V5 and V6, the medium enriched with fruit waste extract produced colonies with a lower mycelial density, generating a laxer mycelium.

Table 2 shows the mycelial growth of the PcM-98 strain of *Pleurotus columbinus*

Table 2

Mycelial growth of strain PcM-98 on solid media

Strain/ variant	Mean value	Martor ratio	Martor difference	Significance of difference
	mm	*%*	*mm*	
PcM-98/V0	34.31	100	0	Mt
PcM-98/V1	37.88	110.38	3.56	***
PcM-98/V2	35.63	103.83	1.31	
PcM-98/V3	39.06	113.84	4.75	***
PcM-98/V4	37	107.83	2.69	**
PcM-98/V5	35.38	103.10	1.06	
PcM-98/V6	36.25	105.65	1.94	*
		DL 5%	1.81	
		DL 1%	2.47	
		DL 0.1%	3 .33	

The PcM-98 strain exhibited white, silky mycelia with modest aerial development, evident circular growth patterns and no exudate droplets or sectoring. The medium variation V3 had the highest growth rate, with an average of 5.58 mm/day. Both extracts from winemaking residues led to increased values relative to the control (V0: 4.90 mm/day), variant V1 with 5.41 mm/day, and V2 with 5.08 mm/day. The medium enhanced with corn cobs (V4) yielded good results, with an average growth rate of 5.28 mm/day followed 5.17 mm/day for V6 and latter 5.05 mm/day for V5. The fruit waste extract produced a looser mycelium with a flattened, rhizomorphic appearance, limited aerial development and irregular edges, similar to strain PoM-77 on this medium variant.

Table 3 displays the mycelial growth of the PeM-41 strain of *Pleurotus eryngii*

Table 3

Mycelial growth of strain PeM-41 on solid media

Strain/ variant	Mean value	Martor ratio	Martor difference	Significance of difference
	mm	*%*	*mm*	
PeM-41/V0	16	100	0	Mt
PeM-41/V1	25.31	158.20	9.31	***
PeM-41/V2	24.31	151.95	8.31	***
PeM-41/V3	32.63	203.91	16.63	***
PeM-41/V4	19.69	123.05	3.69	*
PeM-41/V5	11.44	71.48	-4.56	00
PeM-41/V6	17.5	109.38	1.50	

DL 5% 2.97
DL 1% 4.04
DL 0.1% 5.46

Pleurotus eryngii is recognized for its moderate growth rate compared to the rapid colonization of other *Pleurotus* genus members, the mycelium of the PeM-41 strain exhibited the lowest growth rates compared to the other two strains observed. The V3 medium variety enriched with brewery waste had the highest mean growth rate of 4.30 mm/day, as both the PoM-77 and PcM-98 strains. Winemaking residues resulted in higher values than the control (V0: 2.28 mm/day), with V1 having 3.61 mm/day and V2 having 3.47 mm/day. The V4 medium produced good results, with an average growth rate of 2.81 mm/day, followed by the V6: 2.5 mm/day and finally the V5: 1.63 mm/day.

The concentration of brewery waste in the composition of the V3 variant was 25% and produced the best results for all 3 strains. In order to optimize this medium variant, different variations were prepared by changing the amount of brewer's borage extract in the composition of the media, respectively: V0 (MEA - Martor) 0%, V1: 25%, V2: 50%, V3: 75% and V4: 100% in relation to 3 values of the index of pH: 5.5, 6, 6.5. The expansion of the mycelia from the point of inoculation to the edge of the hyphal network, averaged over 4 perpendicular radii, was used to calculate the mycelial growth rates of the three strains, at 7 days after inoculation.

Table 4 displays the mycelial growth of strain PoM-77 on brewery-based media

Table 4

Mycelial growth of strain PoM-77 on brewery-based media

Strain/ variant	pH	Mean value	Martor raport	Martor difference	Significance of difference
		mm	*%*	*mm*	
PoM-77/V0	5.5	36.33	101.40	0.50	
	6	34.79	97.40	-1.04	
	6.5	35.83	100	0	Mt
PoM-77/V1	5.5	36.75	102.56	0.92	
	6	37.83	105.58	2	
	6.5	35.83	100	0	
PoM-77/V2	5.5	35.67	99.53	-0.16	
	6	33.42	93.26	-2.41	
	6.5	35.08	97.91	-0.75	
PoM-77/V3	5.5	35.83	100	0	
	6	36.25	101.16	0.42	
	6.5	36	100.47	0.17	
PoM-77/V4	5.5	37.33	104.19	1.50	
	6	36.08	100.70	0.25	
	6.5	35.75	99.70	-0.08	

DL 5%	2.75
DL 1%	3.72
DL 0.1%	4.99

Pleurotus ostreatus strain PoM-77 exhibited white, fluffy mycelia, moderate aerial development and no exudate droplets. The results for this strain did not show statistically significant differences, but from a certain perspective they did maintain the trends of the previous experiment, in which the best results were recorded on the V3 variant (25% brewery waste), in this case we are dealing with slightly higher values on the V1 variation (25% brewery waste), but only by a few millimeters.

Table 5 displays the mycelial growth of strain PcM-98 on brewery-based media

Table 5

Mycelial growth of strain PcM-98 on brewery-based media

Strain/ variant	pH	Mean value	Martor raport	Martor difference	Significance of difference
		mm	*%*	*mm*	
PcM-98/V0	5.5	36	106.40	2.17	
	6	36.33	107.39	2.50	
	6.5	33.83	100	0	Mt
PcM-98/V1	5.5	37.67	111.33	3.83	*
	6	38	112.32	4.17	*
	6.5	36.75	108.62	2.92	
PcM-98/V2	5.5	36.50	107.88	2.67	
	6	36.50	107.88	2.67	
	6.5	37.83	111.82	4	*

PcM-98/V3	5.5	37.17	109.85	3.33	
	6	37.25	110.10	3.42	
	6.5	36.58	108.13	2.75	
PcM-98/V4	5.5	37.17	112.81	4.33	*
	6	37.75	111.58	3.92	*
	6.5	36.83	108.87	3	
		DL 5%		3.73	
		DL 1%		5.02	
		DL 0.1%		6.66	

Pleurotus columbinus PcM-98's mycelia showed pearl-white and fluffy mycelia, with medium aerial development and no exudate drips. This strain's mycelium displayed a statistically significant difference for medium variations in relation to a slightly more acidic pH value. When compared to pH, the addition of varying concentrations of brewery waste had a minimal influence, with statistically significant differences arising between the control and experimental versions.

Table 6 displays the mycelial growth of strain PeM-41 on brewery-based media.

Table 6

Mycelial growth of strain PeM-41 on brewery-based media

Strain/ variant	pH	Mean value	Martor raport	Martor difference	Significance of difference
		mm	*%*	*mm*	
PeM-41/V0	5.5	16.17	118.29	2.5	
	6	13.67	100	0	
	6.5	13.67	100	0	Mt
PeM-41/V1	5.5	21.58	157.93	7.92	***
	6	20.92	153.05	7.25	***
	6.5	18.22	133.34	4.56	**
PeM-41/V2	5.5	17.67	129.27	4	*
	6	17.25	126.22	3.58	*
	6.5	15.50	113.41	1.83	
PeM-41/V3	5.5	22.08	161.59	8.42	***
	6	16.08	117.68	2.42	
	6.5	16.42	120.12	2.75	
PeM-41/V4	5.5	22.00	160.98	8.33	***
	6	17.58	128.66	3.92	*
	6.5	17.75	115.24	2.08	
		DL 5%		3.18	
		DL 1%		4.29	
		DL 0.1%		5.70	

Pleurotus eryngii PeM-41 generated white, fluffy mycelia and yielded highly statistically significant results regarding of the influence of brewery waste concentration and pH value, with a strong affinity for pH values of 6 and 5.5, with the best values recorded on V1/ pH 5.5,6; V3/ pH 5.5, and V4/ pH 5.5.

CONCLUSIONS

Increased nutritional availability in the culture medium, as well as enrichment with carbon sources can assure qualitative and quantitative development of mycelium cultivated *in vitro* on solid media.

For all 3 *Pleurotus* strains, the best yields were obtained on the culture media variant enriched with brewery waste (V3). PcM-98 and PeM-41 exhibited a predilection for a slightly more acidic pH levels.

The influence of organic carbon sources represented by brewer's borage in the composition of solid culture media was statistically significant between the control and experimental variants, but differences between concentration values had lower impact, especially for *Pleurotus ostreatus* strain PoM-77.

Given the potential for organic waste and residue to be converted into valuable products, substantial efforts had been directed into promoting circular bio-economy initiatives. The valorization of organic waste minimizes the demand for nonrenewable resources while simultaneously reducing waste production.

REFERENCES

1. **Ahmad I., Fuad I., Khan Z. K., 2015 -** *Mycelia growth of pink oyster (Pleurotus djmour) mushroom in different culture media & environmental factors*. Agriculture and Food Sciences Research, 2(1), 6-11.
2. **Bilay V. T., Solomko E. F., Buchalo A. S., 2000 -** *Growth of edible and medicinal mushrooms on commercial agar media. Science and cultivation of edible fungi*. Rotterdam: Balkema, 2, 779-782.
3. **Gao X., He X., Ren G., Gou H., Liu K., 2011 -** *Isolation of best tissue from Pleurotus eryngii and screening of optimal medium for mother mycelium culture*. Journal of Southern Agriculture, 42(7), 705-707.
4. **Iwase K., Umezawa Y., Masuda K., 2000 -** *Cultivation of Pleurotus ostreatus with beer spent grains and utilization of carbonized waste substrate as a soil ameliorant*, 819-826.
5. **Pestsov G. V., Glazunova A. V., Atroshchenko Y. M., Sidorov R. A., 2021 -** *Cultivation of mycelium of the edible fungus Pleurotus ostreatus (oyster mushroom) with the use of wet sparging grain, the waste of the brewing industry*. agriRxiv, (2021), 20210468622.
6. **Shruti T. M., Ramamoorthy V., Vellaikumar S., 2022 -** *Effect of different culture media, temperature and pH on the mycelial growth of Pleurotus eryngii (King Oyster Mushroom)*.
7. **Tello I., Montiel E., Romero O., Nav, E., Leon I., 2016 -** *Comparative Mycelial Growth of Pleurotus ostreatus in Culture Media.* Journal of Pure and Applied Microbiology, vol 10(4), p.2563-2568.
8. **Țețu G., 2019 -** *Biotechnological recycling of fruit tree wastes through organic cultivation of mushroom species.* Scientific Bulletin Series F. Biotechnologies, 23.
9. **Thomas K. R., Rahman P., 2006 -** *Brewery wastes. Strategies for sustainability. A review*. Aspects of Applied Biology, 80.
10. **Werghemmi W., Abou Fayssal S., Mazouz H., Hajjaj H., Hajji L., 2022** - *Olive and green tea leaves extract in Pleurotus ostreatus var. florida culture media: Effect on mycelial linear growth rate, diameter and growth induction index.* IOP Conference Series: Earth and Environmental Science (Vol. 1090, No. 1, p. 012020).
11. **Zăgrean V., Sbîrciog G., Buzatu M. A., Mândru I., 2016 -** *Effect of Nutritive Media and pH on Mycelial Growth of some Pleurotus eryngii Strains in vitro*. Bulletin UASVM Horticulture, 73(2), 276-278.
12. **Zhong J.J., Tang Y.J., 2004 -** *Submerged cultivation of medicinal mushrooms for production of valuable bioactive metabolites*. Biomanufacturing, 25-59.