MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF THE MYCELIAL ISOLATES BELONGING TO SOME VALUABLE EDIBLE/MEDICINAL MACROMYCETES COLLECTED FROM THE SPONTANEOUS MYCOBIOTA

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Abstract

A number of 18 wild mycelial isolates were obtained from sporocarps of edible/medicinal macromycetes collected from the spontaneous mycobiota of natural habitats in various areas/regions of Romania – the counties of Argeș, Covasna, Giurgiu, Ilfov, Ialomița, Prahova, and Bucharest. The isolates belong to different species of basidiomycetes (9) and ascomycetes (1) with significant nutritional, gastronomic, and/or therapeutic value. The mycelia were evaluated in vitro for morphological and cultural characteristics on three types of different agar media – malt extract (MEA), potato extract (PDA), and compost extract (MECA). The growth and morphology of the colonies highlighted differences between isolates belonging to different species, these characteristics being a useful support in assessing taxonomic affiliation and in maintaining the identity of pure mycelium cultures.

Isolates of the species Pleurotus ostreatus (7) were subsequently checked for growth characteristics in a comparative test on MEA and PDA media and on the granular substrate specific to that used for producing seeding mycelium (spawn). The malt extract agar medium (MEA) promoted higher growth of the mycelia of wild Pleurotus ostreatus strains than the potato dextrose agar medium (PDA). The isolates Po1Br, Po4V, Po6M and Po7B recorded the highest growth rates on both agar media and the granular wheat grain substrate.

Key words**: **macromycetes, mycelium, wild isolates, Pleurotus ostreatus

INTRODUCTION

Edible/medicinal macrofungi play a special role in the bioeconomy of terrestrial ecosystems, as well as in human nutrition and health. They are involved in numerous productive activities based on eco-efficient biotechnologies applied in various fields, such as agriculture, forestry, food, medicine, etc. Due to their remarkable nutritional, gastronomic, and therapeutic qualities, mushrooms are considered functional superfoods and nutraceuticals (Valverde et al., 2015; Pohleven et al., 2016; Chang and Buswell, 2021).

The extent of their distribution and their extraordinary diversity make fungi a resource that needs to be increasingly known, preserved, and used by us as well as by future generations. The danger of losing the genetic diversity of fungi, as in the general case of plants, extends its effects not only to the destruction of ecosystems but also to the chances of identifying new valuable sources of genes useful in the improvement of cultivated species.

Specimens collected from related wild strains and local varieties constitute elements of agrobiodiversity that can most likely bring genetic diversity to a new level, necessary to support innovation in breeding programs (Savoie et al., 2013; Cristea and Murariu, 2018; Kabacia and Muchane, 2023). From this perspective, macromycetes that grow in various forest, meadow, and pasture ecosystems, etc., constitute an important genetic resource

that must be scientifically studied, evaluated, and preserved in order to be readily available for the development of the edible/medicinal mushroom industry or for other uses.

Romania benefits from a very rich, diverse, and still healthy spontaneous mycobiota, present in all regions within forest ecosystems, pastures, meadows, plains, etc. Macromycetes – over 1600 species, of which 400 are edible – are generously represented here by valuable genera and species (Sălăgeanu and Sălăgeanu, 1985; Șesan and Tănase, 2004). The isolation, preservation, and evaluation of their mycelia for growth and fruiting capacity represent the first steps towards their introduction into cultivation and/or use in other biotechnological applications. Several in vitro studies regarding the isolation and characterization of some species of lignicolous macromycetes collected from our forests have highlighted a great diversity of morphological characteristics and fungal colony growth (Bălăieș and Tănase, 2012; Petre and Tănase, 2013; Popa et al., 2014). In this paper, we aimed to evaluate the morphological and cultural characteristics of the mycelial isolates belonging to some valuable edible/medicinal macromycetes collected from the spontaneous mycobiota in several regions of Romania.

MATERIALS AND METHODS

Specimens of mushrooms belonging to several species of edible/medicinal macromycetes were collected from the spontaneous mycobiota of natural habitats (forests, meadows, and parks) located in various areas/regions of Romania – the counties of Argeș, Covasna, Giurgiu, Ilfov, Ialomița, Prahova, and from Bucharest. In the initial stage of our research, we focused primarily on cultivable saprotrophic macromycete species, especially lignicolous ones that have significant value from a nutritional, culinary, and/or therapeutic perspective or with other biotechnological applications. They were used for the clonal obtaining of mycelial isolates through the method of transferring plectenchymatic tissue onto agarized nutrient media.

Table 1 presents 18 different isolates obtained from basidiocarps belonging to 9 species of basidiomycetes and 1 species of ascomycete fungi (*Morchella esculenta*).

Mycelia were grown onto 90 mm Petri dishes (triplicates), using three types of agar media with a pH value of 6.5: malts extract agar (MEA), potato dextrose agar (PDA), malt extract + compost extract (1/1) agar (MECA). Incubation was carried out in darkness at 24-26ºC for 10-20 days. Observations and measurements were made regarding the morphology and growth of the colonies.

For the study of *Pleurotus ostreatus* isolates, the mycelium growth onto MEA and PDA media, expressed in mm and mm/day as appropriate, was determined after 7/10/14 days of incubation by measuring the diameter of the mycelial colonies along two perpendicular axes and calculating the average of these for three repetitions/experimental variant.

The mycelia were then checked for growth on a granular substrate made from boiled wheat grains, to which 6% CaSO4 and 2% CaCO3 were added. The granular substrate thus obtained was distributed into 250 ml prismatic glass flasks for measuring the mycelium growth capacity. They were then sterilized at 123°C for 100 minutes. After cooling and handling, the flasks with the sterilized substrate were inoculated with the mycelia of wild *Pleurotus ostreatus* isolates, after which they were transferred for incubation at 24-26°C in the dark and maintained for 16-18 days. The growth in the 250 ml bottles was measured after 14 days of incubation.

RESULTS AND DISCUSSIONS

Table 1 presents 18 different isolates obtained from basidiocarps belonging to 9 species of basidiomycetes and 1 species of ascomycete fungi (*Morchella esculenta*).

The main cultural and morphological characteristics of the wild isolates grown on

solidified nutrient media are presented in Table 2.

The morphological and cultural characteristics of the 18 wild isolates obtained from spontaneous mycobiota were highlighted in vitro by cultivating them on three different agar media: MEA, PDA, and MECA. The growth and colony morphology revealed differences between isolates belonging to different species, these characteristics being a useful support in assessing taxonomic affiliation and in maintaining the identity of pure mycelium cultures.

In this regard, the clear differences between the appearance of colonies of isolates belonging to the species *Armillaria mellea* and *Laetiporus sulphureus* or *Polyporus squamosus* and *Trametes versicolor* can be observed when grown on the same type of medium (MEA/PDA). The fact that the genotype determines morpho-cultural characteristics is also evidenced by the differences that appear in isolates of different origins belonging to the same species, as in the case of the 7 wild strains of *Pleurotus ostreatus* grown on PDA medium or the 3 strains of *Agaricus campestris* grown on MECA medium (table 2). The culture medium is, in turn, a factor that strongly influences the growth of mycelia and the appearance of their colonies (Mushimiyimana et al., 2016; Mykchaylova et al., 2019). Even a single medium produced by different companies can cause significant differences in growth rate and colony morphology (Psurtseva et al., 2023). One can observe how much the mycelium of the same *Morchella esculenta* isolate differs when grown on two different nutrient media – PDA and MECA (table 2).

Evaluation of the growth of wild *Pleurotus ostreatus* isolates on agar nutrient media

The 7 wild isolates of *Pleurotus ostreatus* were tested for growth characteristics in a comparative trial on MEA and PDA media and, subsequently, on the granular substrate specific to that used for spawn production.

Table 3 presents the results of the mycelial growth measurements, averages for three replicates per strain, after ten days of incubation.

The Po1Br strain had the highest mycelial growth, with a value of 39.08 mm, followed by Po5C with 38.58 mm and Po7B with 38.50 mm, all on MEA medium, values that are statistically very significant. The lowest mycelial growth capacity values were recorded for isolate Po3M, with 17.92 mm/PDA and 19.42 mm/MEA, followed by Po2T with 24.42 mm/PDA, values that are statistically very significantly negative.

The nature of the culture medium had a strong influence on the growth of the mycelium of the wild P. ostreatus isolates studied. In Table 4 it can be seen how the malt extract medium (MEA) favored greater growth of the experimental mycelia compared to the potato extract medium (PDA). The better growth of the mycelia on the malt extract medium (MEA) can be explained by its additional content of nutrients and beneficial bioactive compounds, approximately 6% protein, amino acids, vitamins and antioxidants. Similar results regarding the better growth of *Pleurotus ostreatus* mycelia on MEA medium compared to PDA have also been reported by others (Phadke et al., 2020; Roy and Chakraborty, 2023).

From Table 5, it appears that the Po1Br strain ensured the fastest mycelial growth on both media tested, with an average value of 37.62 mm, surpassing the Po7B control by a very significant difference of 3.46 mm. The Po3M and Po2T strains had the smallest growths, 18.67 mm and 28.46 mm, with very significant negative differences of 15.49 mm and 5.70 mm, respectively, compared to the Po7B control.

Evaluation of the growth of wild *Pleurotus ostreatus* isolates on granular substrate.

Isolates of the species *Pleurotus ostreatus* were subsequently checked for growth characteristics on the granular substrate specific to that used for producing mycelium for seeding (mushroom spawn production).

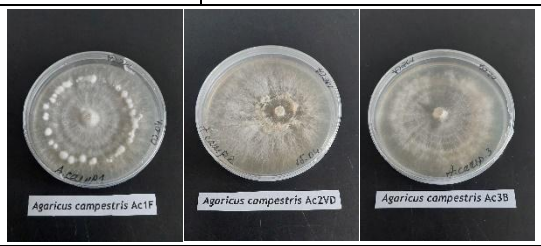
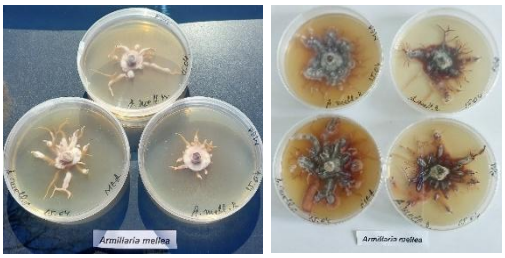
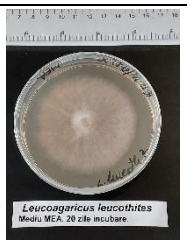
Figure 1. Growth of mycelium of wild *Pleurotus ostreatus* isolates on granular substrate
Isolates Po1Br, Po2T, Po3M, Po4V, Po5C, Po6M, Po7B
Incubation for 10 days at 24-26°C

Table 6 shows that the Po1Br strain stood out with the highest mycelial growth on granular substrate, measuring 120.33 mm, exceeding the control Po7B by a significant difference of 3.66 mm. It was followed, in order, by isolates Po6M with 116.33 mm, Po4V with 114.67 mm, and Po5C with 114.00 mm, with growth values slightly lower than the control. As on agar media, the lowest mycelial growth values were recorded for the wild strain Po3M with 99.67 mm, followed by Po2T with 101.00 mm, both showing very significant negative differences compared to the control. These results highlight the major influence of the genotype, specifically the strain, on mycelial growth on different nutrient substrates.

Table 1 Wild-type mycelial isolates obtained from collected macromycete specimens

No	Order – Family - Species	Isolate	Collection site	Date of collection	Date of isolation
1	Agaricales→Agaricaceae ***Agaricus arvensis***	Aar1V	*Meadow* – Vidra Ilfov County	12.10.2024	14.10.2024
2	Agaricales→Agaricaceae ***Agaricus campestris***	Ac1F	*Pasture* – Fierbinți Ialomița County	15.09.2024	16.09.2024
3		Ac2VD	*Meadow* - Valea Doftanei Prahova County	21.09.2024	23.09.2024
4		Ac3B	*Park* - ASAS Sector 1, Bucharest	10.10.2024	11.10.2024
5	Agaricales→Physalacriaceae ***Armillaria mellea***	Arm1BM	*Forest* – Boroșneu Mare Covasna County	23.08.2024	24.08.2024
6	Polyporales→Laetiporaceae ***Laetiporus sulphureus***	Ls3VD	*Forest* - Valea Doftanei Prahova County	21.09.2024	24.09.2024
7	Agaricales→Agaricaceae ***Leucoagaricus leucothites***	Llt2B	*Park* - ASAS Sector 1, Bucharest	10.10.2024	11.10.2024
8	*Agaricales→Tricholomataceae* ***Lepista personata***	Lps1M	*Forest* - Mogoșoaia Ilfov County	11.10.2024	14.10.2024
9	Pezizales→Morchellaceae ***Morchella esculenta***	Mes1M	*Forest* - Mogoșoaia Ilfov County	08.04.2025	08.04.2025
10	Agaricales→Pleurotacea ***Pleurotus ostreatus***	Po1Br	*Park* - Pajura Sector 1, Bucharest	19.09.2023	20.09.2023
11		Po2T	*Forest* - Tigveni Argeș County	25.05.2024	28.05.2024
12		Po3M	*Forest* - Mogoșoaia Ilfov County	04.11.2024	06.11.2024
13		Po4V	*Forest* – Vidra Ilfov County	14.11.2024	15.11.2024
14		Po5C	*Forest* - Comana Giurgiu County	16.11.2024	18.11.2024
15		Po6M	*Forest* - Mogoșoaia Ilfov County	19.11.2024	25.11.2024
16		Po7B	*Park* - Dr. Romniceanu Sector 5, Bucharest	26.11.2024	28.11.2024
17	Polyporales→Polyporaceae ***Polyporus squamosus***	Psq2M	*Forest* - Mogoșoaia Ilfov County	07.11.2024	09.11.2024
18	Polyporales→Polyporaceae ***Trametes versicolor***	Tv1VD	*Forest* - Valea Doftanei Prahova County	21.09.2024	24.09.2024

Table 2 Cultural and morphological characteristics of wild isolates on agar media

Agaricus arvensis Aar1V		White-cream mycelium, felted-powdery, medium hyphal density and low aerial development, sectorized - with areas appressed to the medium and very slow growth; marginal areas with more vigorous growth. MECA medium - more favorable
Agaricus campestris Ac1F Ac2VD Ac3B		Light cream-white, felt-like mycelium, radial growth, medium density, medium aerial development; concentric striations more evident in Ac1F and Ac3B. Numerous primordia (teleomorphic) in a perfect circle in Ac1F. Favorable medium - MECA
Armillaria mellea Arm1BM	 medii MEA și PDA	Young white mycelium, compact on the surface of the medium; long and thick rhizomorphs in the medium, covered with a sleeve of fine hyphae. Over time, from the inoculum towards the fringed edges of the colony, the mycelium becomes leathery, with a thick gray-brown crust. The tips of the rhizomorphs are lighter in color and accompanied by a fine mat of short, whitish hyphae. Favorable media – MEA, PDA
Laetiporus sulphureus Ls3VD		Whitish mycelial colony with beige shades, felted-powdery type, medium hyphal density. Growth streaks - concentric bands of different densities and low aerial development. Colonies with a fairly uniform appearance, with regular circular edges. Favorable media - MEA and PDA
Leucoagaricus leucothites Llt2B		Young colony - white mycelium with fine hyphae, a uniform felt-like mat with radial expansion and regular edges; medium density and aerial development. Small compact white nodules - fruiting primordia (teleomorph) more numerous near the inoculum. MEA medium – more favorable.
Lepista personata Lps1M		Whitish mycelial colony with very fine hyphae forming a felt-like ± loose mat and with moderate aerial development. The mycelium grows quickly, covering the entire surface of the medium in 8-10 days. Over time, the colony's color takes on a light violet hue. Small white nodules appear early, especially nearby the inoculum plug, representing future fruiting primordia (teleomorph). MECA medium - more favorable.
Morchella esculenta Mes1M		Colonies with different appearances on the examined culture media. The mycelium grown on PDA medium is light creamy-white, velvety-powdery, very compact, with medium aerial development; the colony is round with well-defined edges, shows concentric growth striations, and has slow radial growth. On MECA medium – faster growth, the mycelial colony is light creamy-white, also perfectly circular with well-defined edges, but very uniform and with a looser hyphal mat. The color of the media remained unchanged.

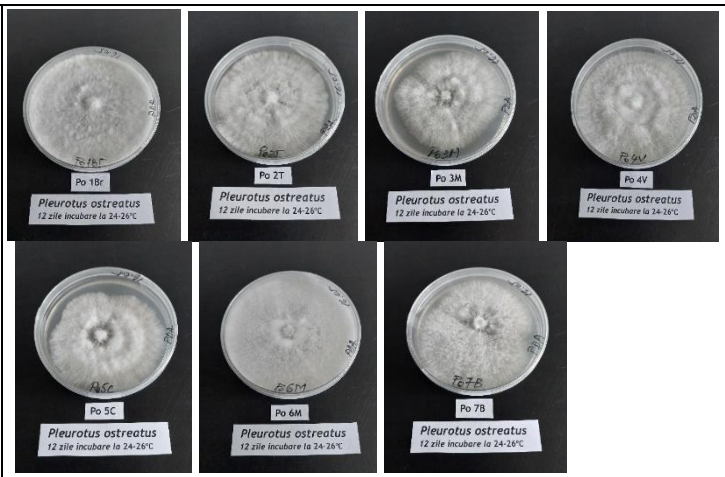
Pleurotus ostreatus Po1Br Po2T Po3M Po4V Po5C Po6M Po7B		Po2T, Po3M, Po4V, Po5C: vigorous, white mycelia, fluffy-slightly woolly type, dense hyphal network, ± uniform, medium-large aerial growth, concentric striations. Po1Br, Po6M, Po7B: white colonies with a very uniform appearance, fluffy-cottony type with a compact hyphal network, no obvious striations, ± medium aerial growth. All grow quickly, covering the medium surface in 10-14 days of incubation. Sometimes, small droplets of yellowish exudate appear. Tendency to form fruiting body primordia (teleomorph stage). They did not change the color and consistency of the media they were grown on. Favorable media - MEA and PDA.
Polyporus squamosus Psq2M		The colony has a light cream-white mycelium, felty ± uniform, with radial growth; hyphal density and aerial development increase towards the edges of the colony; with age, the color takes on ochre-brown shades, the center of the colony becomes more adherent to the medium, flattened with crusty edges; no exudate droplets. Favorable media - MEA and PDA.
Trametes versicolor Tv1VD		The young mycelial colony is white, uniform, finely felted-powdery, compact with medium aerial development and rapid growth. Over time, around the inoculum fragment, the mycelium becomes adherent powdery, with darker beige-gray shades and thick brown crusts. The color of the culture medium remains unchanged. MEA medium – more favorable.

Table 3 Growth of the mycelium of wild *Pleurotus ostreatus* isolates on two agar media 10 days of incubation at 24-26℃

No.	Strain	Nutrient medium *	Mycelium growth		Difference (± mm)	Significance
			mm	%		
1	Po1Br	MEA	39.08	131.00	9.25	xxx
2	Po1Br	PDA	36.17	121.25	6.34	xxx
3	Po2T	MEA	32.50	108.95	2.67	ns
4	Po2T	PDA	24.42	81.86	-5.41	ooo
5	Po3M	MEA	19.42	61.10	-10.41	ooo
6	Po3M	PDA	17.92	60.07	-11.91	ooo
7	Po4V	MEA	37.25	124.87	7.42	xxx
8	Po4V	PDA	32.50	108.95	2.67	ns
9	Po5C	MEA	38.58	129.33	8.75	xxx
10	Po5C	PDA	28.92	96.94	-0.91	ns
11	Po6M	MEA	35.00	117.33	5.17	xx
12	Po6M	PDA	37.00	124.03	7.17	xxx
13	Po7B	MEA	38.50	129.06	8.67	xxx
14	Po7B (mt)	PDA	29.83	100.00	-	-
	DL 5%	=			2.90	
	DL 1%	=			3.92	
	DL 0.1%	=			5.23	

* PDA (potato dextrose agar ; MEA (malt extract agar)

Table 4 The influence of the culture medium on the mycelial growth of wild isolates of *Pleurotus ostreatus* on two agar media
10 days of incubation at 24-26℃

No	Nutrient medium	Mycelium growth		Difference (± mm)	Significance
		mm	%		
1	MEA	34.33	116.25	4.8	xxx
2	PDA (Mt)	29.53	100.00	100.00	-
	DL 5%	=		1.09	
	DL 1%	=		1.47	
	DL 0.1%	=		1.96	

Table 5 The influence of the strain on the mycelial growth of wild *Pleurotus ostreatus* isolates on two agar media
10 days of incubation at 24-26℃

No.	**Strain**	**Mycelium growth**		**Difference (± mm)**	**Significance**
		mm	**%**		
1	Po1Br	37.62	110.12	3.46	xxx
2	Po2T	28.46	83.31	-5.70	ooo
3	Po3M	18.67	54.65	-15.49	ooo
4	Po4V	34.87	102.87	0.81	ns
5	Po5C	33.75	98.87	-0.41	ns
6	Po6M	36.00	105.38	1.84	ns
7	Po7B (mt)	34.16	100.00	-	-
	DL 5%	=		2.04	
	DL 1%	=		2.75	
	DL 0.1%	=		3.67	

Table 6 The influence of the strain on the mycelial growth of wild *Pleurotus ostreatus* isolates on granular substrate
14 days of incubation at 24-26℃

No	**Strain**	**Mycelium growth**		**Difference (± mm)**	**Significance**
		mm	**%**		
1	Po1Br	120.33	103.14	3.66	x
2	Po2T	101.00	86.57	-15.67	ooo
3	Po3M	99.67	85.43	-17.00	ooo
4	Po4V	114.67	98.28	-1.66	ns
5	Po5C	114.00	97.71	-2.67	ns
6	Po6M	116.33	99.71	-0.34	ns
7	Po7B (mt)	116.67	100.00	-	-
	DL 5%	=		3.05	
	DL 1%	=		4.28	
	DL 0.1%	=		6.05	

CONCLUSIONS

The mushroom specimens collected from the spontaneous mycobiota in different regions of Romania were morpho-taxonomically identified according to classical criteria as belonging to a number of 10 species of wild edible/medicinal macromycetes and were used for the clonal production of a number of 18 viable mycelial isolates.

Pure cultures of these wild isolates were introduced for preservation into the laboratory/ICDLF Vidra strain collection, enriching it with valuable sources of genetic variability.

Through in vitro evaluation studies, the main morphophysiological and cultural characteristics of the mycelium of these wild-type isolates on laboratory nutrient media were successfully established. These characteristics are a useful

complementary tool for establishing taxonomic affiliation, with the certainty of systematic classification at the species/genus level ultimately being based on molecular genetics techniques, which we will use in the next stage.

Knowledge of the morphological and growth characteristics of mycelia is a valuable aid in ensuring the control of the identity, purity, and stability of pure cultures during their preservation in mushroom strain collections, in the process of producing mycelium for seeding (spawn) and in biotechnological research activities.

The mycelia of wild *Pleurotus ostreatus* isolates proved to be viable, robust, and with special morpho-physiological and cultural characteristics, which recommend them as valuable genetic material to be preserved and used in future breeding-selection programs for the development of the cultivated mushroom assortment in our country.

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