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STUDIES CONCERNING THE *IN VITRO* CULTIVATION OF SOME INDIGENOUS MACROMYCETE SPECIES

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Abstract

For long time mushrooms presented interest for consumption as food, as traditional medicine or in bioremediation, due to their nutritional, antioxidant, antimicrobial and therapeutic values. The valorisation of indigenous species of mushrooms both for research or practical applications is a goal for researchers all over the world and requires well characterized collections. Despite the importance of macromycetes, few collections are developed in Europe and they contain mainly standard strains. The existence of extremely rich mycoflora in the forests and grasslands of Romania creates favourable conditions for initiating a scientific approach with definite applicability, in order to diversify the cultivated assortment by introducing of some new indigenous mushroom strains and species. For example, several species from spontaneous mycoflora of Romania have nutritional value or could be used as therapeutic agents: Agaricus campestris, Clytocibe geotropa, Boletus edulis, Hericium coraloides, Pleurotus ostreatus, Armillaria mellea, Flammulina velutipes, Coprinus comatus, Laetiporus sulphureus, Ganoderma applanatum, Ganoderma lucidum etc. Exploitation of such fungal species by introducing into controlled culture may diversify the range of mushrooms for domestic market or for obtaining of innovative products. For this reason the aim of our work was the realization of a fungal germplasm collection based on indigenous isolates obtained from natural ecosystems. 40 mushroom varieties belonging to at least 35 species were isolated and in vitro propagated on MEA or PDA media. The possibility of submerged cultivation of mycelium for some species as well as antimicrobial potential was also examined. Using specific biotechnologies the new mushroom strains included in our collection will be tested for genetic variability and for other important characteristics (producing of enzymes, degradation abilities, antimicrobial and/or antitumor properties etc).

Keywords: *fungal germplasm collections, in vitro cultivation, Romanian mycoflora, wild edible medicinal mushrooms*

INTRODUCTION

Edible mushrooms are considered food with a high nutritional value and some of them therapeutic. Spread all over the world, fungi (kingdom Fungi / Mycota) have almost 200 thousand species, subspecies and varieties. It is estimated that there are about 140 000 mushroom species on earth and only 22 000 of these are known (Alves et al., 2012). Until now a number of 2000 mushroom species are reported in Romania (Popescu, 2006). In fungi, besides the composition rich in high in fiber, quality protein, vitamins and minerals, there are biologically active compounds such as: b-glucan, polysaccharides, phenolic compounds, which are involved in antitumor, antiviral, immunomodulatory, hypoglycemia, cholesterol lowering, antioxidant, anti-inflammatory and antimicrobial activities(Kalac et al., 2012.). Mushrooms are not only found to have medicinally properties but also found to have significant antioxidant capacity (Aggarwal et al., 2012). Therefore, mushrooms can be used both as a food ingredient and in pharmaceutical industry. In spontaneous flora of Romania, among of edible mushrooms with therapeutic value are: *Agaricus campestris, Cantharelus cibarius, Pleurotus ostreatus, Armillaria mellea, Flammulina velutipes, Coprinus comatus, Laetiporus sulphureus, Ganoderma lucidum, Ganoderma applanatum, Laetiporus sulphureus* etc. In Asian countries some of these mushrooms are cultivated widely. An example is *Flammulina velutipes*, a fungus species which in China, according to Yang (1986) and Wang (1995) has been cultivated since the 8th century. Experience in the field

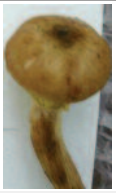
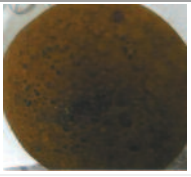
and the existence of extremely rich mycoflora in the forests and grasslands of Romania creates favourable conditions for initiating a scientific approach with definite applicability, in order to diversify the cultivated assortment by introducing of some new indigenous mushroom strains and species and to valorise the large quantities of the existing recyclable agro-forestry materials. Using specific biotechnologies the new mushroom strains obtained and the genetic variability of this precious biological material will be stored / preserved through the establishment of a mycelium strains collection, which can be developed further. In this context, the goal of our work was to obtain the fungal inocula (mycelium and young fruiting bodies) from native mushroom species and initiation of a fungal germplasm collection originated in Romanian mycoflora.

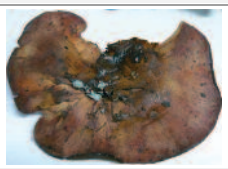
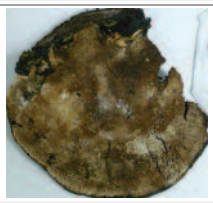
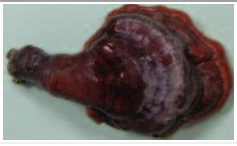
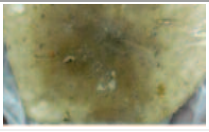
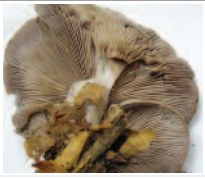
MATERIALS AND METHODS

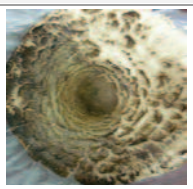
Materials. 18 wild edible and/or medicinal mushroom species were harvested from the different regions of Romania (Sinaia, Chitila, Teleorman, Voinesti and Bucharest) (Table 1) and were authenticated by specialists (Dr. ing. Ioana Tudor and Dr. ing. Paul Covic).

***In vitro* cultivation**.Initially, fruiting bodies of the selected mushrooms were surface washed with sterile distillate water. The mycelia were isolated by aseptically removing a small piece of mycelium from inside. The pieces were transferred in to Petri dishes on 2% malt extract agar or PDA (potato-dextrose-agar) media. The plates were incubated at 25^0C in the dark. The mycelia growing on the medium surface were picked and transferred to PDA or malt extract media. The pure cultures were kept on these media for further use.

Table 1.Wild edible medicinal mushroom species from Romanian mycoflora and their properties

Species	Location	Properties	Morphological aspect
Agaricus campestris	Voinesti forest	Edible Medicinal - enhance the secretion of insulin, treatment of ulcers .	
Armillaria mellea	Fundata forest Chitila forest	Edible Medicinal (Meniere's Syndrome, vertigo, epilepsy, neurasthenia and hypertension), antioxidant, antimicrobial (Jiangsu Journal of TCM. 1980; Donnelly et al, 1985 Obuchi et al., 1990; Kalyoncu et al., 2010)	
Boletus edulis	Fudata forest	Edible Medicinal (antiviral, anti-inflammatory, antimicrobial, antioxidant) (Chang and Miles, 2004; Shu-Yao T, 2007)	
Bovista plumbea	Fundata forest	Edible Medicinal (head affections, diabetes, ovarian cysts, acne) (Jain, 1997)	
Coprinus sp.	USAMV-Bucharest campus	Edible with caution - poisonous if consumed with alcohol Medicinal – anti-tumor (Ohtsukaet al., 1973), anti-fungal (Florianowicz, 2000)	

Fistulina hepatica	Chitila forest	Edible Medicinal- antibacterial, antioxidant (Ribeiro et al., 2007)	
Flammulina velutipes (Enokitake)	Fundata forest	Edible Medicinal- regulation of the immune system, cancer immunotherapy, antioxidant (Bao et al., 2009)	
Ganoderma applanatum	Chitila forest	Medicinal - antibacterial (including *E. coli* and *Staphylococcus aureus),* anti-inflammatory, and anti- tumor, tumor inhibition, immune stimulation, homeostasis, esophageal carcinoma, rheumatic TB and as an anti-viral for HIV, etc. (Acharya et al., 2005).	
Ganoderma lucidum	Teleorman forest	Medicinal –antioxidant, cardiovascular and immune systems, hepatoprotective, anticancer, antibacterial (Clinical Biochemistry and Nutrition, 2007)	
Hericium coralloides	Fundata forest	Edible Medicinal-antibacterial and nematicidal activities, anti-inflammatory properties, anti- tumours, nerve regenerator in Muscular Dystrophy, Parkinson's Disease, Alzheimer's and Dementia (Mori et al., 2008; Mizuno et al, 1992; Mizuno and Takashi, 1995).	
Lepista nebularis (*Clitocybe nebularis*)	Fundata forest	Edible Medicinal- stops leukemia T cells from proliferating, antimicrobial activity (Kim et al, 2008)	
Lepista nuda (*Clitocybe nuda*)	Fundata forest	Edible Medicinal- antioxidant and antimicrobial properties (Dulger et al., 2002), immunologic effects (Lyn et al., 2011)	
Laetiporus sulphureus	Carol Park - Buharest	Edible Medicinal - hemolytic and hemaglutination activities (Mancheño et al., 2005), antimicrobial and antioxidant activities (Turkoglu et al., 2007)	

Macrolepiota procera	Chitila forest	Edible Medicinal-antioxidant properties	
Pleurotus ostreatus	Chitila forest Voinesti forest	Edible Medicinal- cardiovascular, hypertensive, hypercholesterolemia (Wasser, 2002), antioxidant and antimicrobial activities (Vamanu, E., 2013)	
Trametes versicolor	Sinaia forest	Medicinal – in a variety of cancers, including Gastric, Lung, Breast and Colorectal, Hepatoprotective, herpes, Chronic Fatigue Syndrome (Medicinal Mushrooms - A Clinical Guide by Martin Powell)	
Stropharia sp.	Fundata forest	Edible, but undesirable due to mildly spicy taste Medicinal- antitumor (Ohtsuka et al., 1973), neuromodulatory effects (Moldavan et al., 2001)	
Xylaria polymorpha (Dead Man's Fingers)	Fundata forest	Medicinal- cytotoxicty against human cancer cell lines (Shiono et al., 2009), antibacterial activity (Ramesh, 2012).	

RESULTS AND DISCUSSIONS

18 wild mushroom species were harvested from different indigenous regions (Sinaia, Voinesti, Chitila, Fundata, Teleorman forests and Bucharest parks), in order to analyze their ability to grow under *in vitro* conditions and to initiate a germplasm collection for further investigation. Two types of culture media, 2% malt extract agar (MEA) and potato-dextrose-agar (PDA), were chosen for *in vitro* cultivation of the samples. Depending on the species, after 1-3 weeks of incubation the mycelia have been well developed on both types of culture media (fig. 1). For some wild mushroom species, primordia of young fruiting bodies have been emerged from the mycelium grown on the medium surface (e.g. *Trametes versicolor*, fig.1T). In addition, fungal mycelia samples exhibited different colours and morphological characteristics (fig.1). The pure cultures obtained in this way were kept in collection on PDA or MEA slants for further use. Thus, over 40 mushroom varieties belonging to at least 35 mushroom species were isolated and *in vitro* propagated on MEA or PDA media.

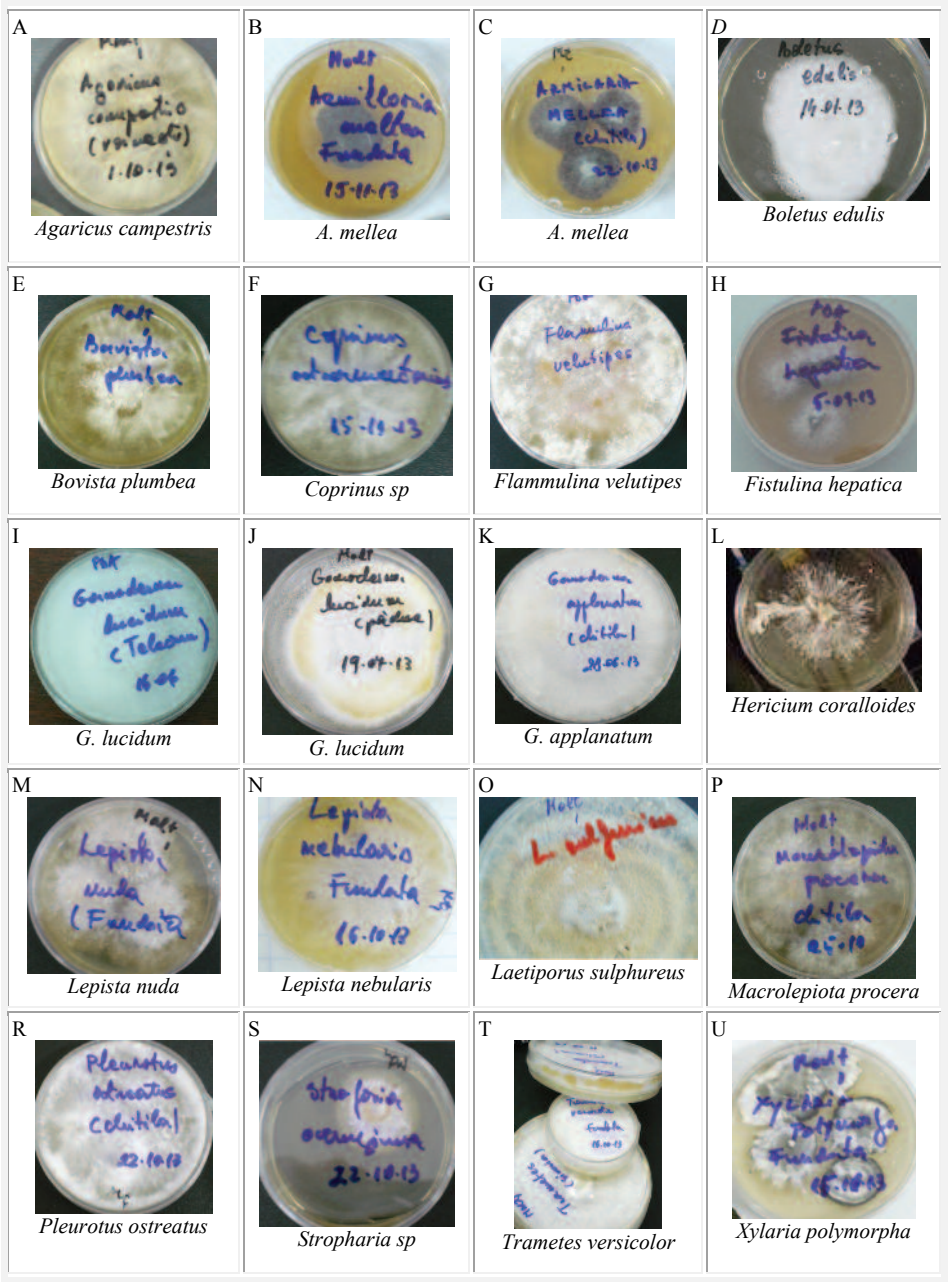


Figure1. Fungal mycelia of various wild edible and/or medicinal mushroom species isolated from indigenous mycoflora

CONCLUSIONS

The i*n vitro* wild edible medicinal mushrooms cultivation (in Petri dishes or other culture vessels) could have many advantages:

- It is much faster than growing mushrooms in a natural way;
- This method may have industrial benefits;
- Mycelium grown on agar is therefore used to inoculate logs (or other substrates) in plantations;

- The methods offer the possibility of cloning or genetic manipulation mycelium.

In addition, wild edible medicinal mushrooms can be a source of nutraceuticals with valuable antioxidant properties, which can positively influence the oxidative stress in cells and related diseases (Ferreira, 2009). Using specific biotechnologies the new mushroom strains included in our collection will be tested for genetic variability and for other important characteristics (producing of enzymes, degradation abilities, antimicrobial and/or antitumor properties etc).

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