SUMMARY OF THE DOCTORATE THESIS

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„CHARACTERIZATION OF SOME MACROMYCETES SPECIES USEFUL FOR BIOTECHNOLOGY”

Keywords: macromycetes, submerged cultures, fungal biomass, fructification bodies, ethanolic extracts, antimicrobial activity, polyphenols, flavones, ligninolytic enzymes, biodegradation, genetic variability, molecular markers.

This doctoral thesis includes the research carried out during the period 2015-2018 at the Faculty of Biotechnologies of USAMV Bucharest in the laboratories of Genetics, Molecular Biology and Applied Microbiology.

Mushrooms are well known worldwide for their nutritional and therapeutic values due to their metabolic products. Among the many molecules synthesized by macrofungi are bioactive compounds such as polysaccharides, terpenoids, lectins, as well as a wide variety of proteins with biological actions of interest, many of which have valuable biotechnological potential that can be harnessed in medicine, in industry or in the bioremediation of the environment.

In the present PhD theses were aimed at biotechnological exploitation of some commercial mushroom species or of our spontaneous flora with nutritional and / or medicinal value, which would lead to the diversification of the mushroom range both for the domestic market and for the products offered for export, for obtaining nutritional and therapeutic nutritional supplements or for their use in biodegradation. It has been considered that the edible / wild mushrooms studied can be used in the food or pharmaceutical industry or for the preservation and regeneration of the environment as a result of the results obtained. In this respect, we aimed at biotechnological evaluation of ten species of commercial macromycetes or of our spontaneous flora with nutritional and / or medicinal value, some of them less known and exploited in our country. These species are: *Flammulina velutipes*, *Lentinus edodes*, *Pleurotus*

eryngii strain 2600, *P.ostreatus* var. Florida, *Trametes versicolor*, *Hericium coralloides*, *Ganoderma lucidum*, *Ganoderma applanatum*, *Agaricus campestris*, *Laetiporus sulphureus*.

In order to achieve the proposed goal, the following research objectives were addressed:

- *In vitro* production of fungal material - the source of inoculum - from fungal species considered to be biotechnologically useful;
- Evaluation of the growth potential of the mushrooms under submerged conditions and of the culture conditions for the production of fungal biomass;
- Evaluation of antimicrobial activity of ethanol extracts prepared from fungal biomass obtained *in vitro* under submerged conditions;
- Biochemical analysis of fungal extracts obtained from mushroom fruit bodies;
- *In vitro* investigation of the biotechnological potential of some macromycetes species for their use in the biodegradation of organic pollutants (dyes and aromatic hydrocarbons);
- Molecular characterization of some strains of macromycetes of biotechnological interest.

The thesis is structured in two parts: the first part, including 4 chapters, addresses bibliographic studies on the current state of knowledge of the issues addressed in the paper; the second part, which totals 8 chapters, presents the personal contribution to the achievement of the proposed objectives.

The bibliographic studies have addressed a number of issues that will be presented below.

The first chapter addresses the economic aspects of cultivation of edible and medicinal mushrooms worldwide and nationally, highlighting the social and environmental impact of cultivation technologies.

In Chapter II, the nutritional and medicinal properties of other major macromycetes species are highlighted, highlighting the main bioactive compounds of biotechnology interest.

Considering that the experimental part has approached a series of molecular biology techniques for the characterization of species of interest, **chapter III** describes the molecular molecular techniques used to identify macromycetes species.

Chapter IV addresses the potential industrial and biotechnological applications of enzymes isolated from macromycetes (lignolytic enzymes), describing their mode of action.

The personal contribution is detailed in 6 experimental chapters, correlated with the main objectives of the thesis, preceded by a chapter presenting the motivation and context of the research and finalizing with a chapter of general conclusions of these researches. The six

experimental chapters, after a brief introduction to the topic of interest, present a subchapter describing the methodology used, followed by results and discussions and finalizing with a series of partial conclusions of the researches.

Thus, **chapter V** describes the context of the experiments, mentions the motivation and the general objectives, the main stages of the research activities, the locations of the experiments and equipment in those locations.

In **Chapter VI**, the objective was to obtain in vitro the fungal material as the source of inoculum from the fungal species considered to be biotechnologically useful. It was concluded that the solid culture media tested, PDA and MEA, respectively, were optimal for obtaining the fungal inoculum for all the studied macromycete species. The exception were the mushrooms species of *Agaricus campestris* and *Hericium coralloides* for which the MEA culture medium was more conductive to mycelian growth. Biological material obtained on solid synthetic media was used as the source of inoculum for subsequent experiments.

Chapter VII approached the evaluation of the growth potential of the mushrooms under submerged conditions and optimal conditions for cultivation for the production of fungal biomass, namely the chemical composition of the culture media and the influence of pH evolution on the production of biomass. Compared to solid media, liquid cultures have the advantage of obtaining a high amount of mycelian biomass within a short period of time in a restricted space. Of the 10 types of mushrooms studied, *Ganoderma* species exhibited the greatest biomass production potential under submerged conditions in all 6 variants of tested media, and *Lentinus edodes* have the smallest potential. Measuring pH variations over the mycelial growth period was found to have decreased gradually (in the case of cultures on ME and PD media) or increased gradually (in the case of PMP culture), the test species having the capacity to turn the pH to the environment depending on extracellularized metabolites. The most drastic decrease from baseline pH was recorded in *Laetiporus sulphureus* in ME and PD culture media (from a baseline pH of 5.4 and 5.0 at pH 2.8 respectively). Generally, it has been found that acidification of the culture medium (at pH 3.27) resulted in a reduced amount of biomass. High-glucose environments (2%) have a favorable influence on the production of biomass. Thus, on the MCM medium the maximum biomass yield was obtained for *L. sulphureus, P. ostreatus* Florida and *F. velutipes,* and the PD medium was favorable for obtaining the maximum biomass for the species: *L. edodes, G. applanatum, T. versicolor* and *H. coralloides*.

In **Chapter VIII** the antimicrobial activity of some ethanolic extracts prepared from fungal biomass *in vitro* obtained under submersed, filtered and dry conditions was evaluated. The results showed that the ethanolic extracts of *L. sulphureus*, *G. applanatum*, *F. velutipes*, *H. coralloides*, *T. versicolor*, and *A. campestris* had significant inhibitory activities in particular on *B. subtilis* subsp. *spizizenii*. None of the tested fungal extracts had antimicrobial activity in interaction with *C. albicans* ATCC 10231 and *C. parapsilosis* CBS604 pathogenic yeasts.

Chapter IX: approached the biochemical analysis of the hydroalcoholic extracts obtained from mushroom fruit bodies. The total content of polyphenols and flavones in fungal extracts was evaluated by spectrophotometric methods, correlated with thin layer chromatography (TLC), as well as the content of ligninolytic enzymes. The highest content in phenols was recorded for the extract of *A. campestris* and *P. ostreatus* var. Florida. The TLC analysis for flavones revelad that all the fungal extracts presented flurosecences spots specific to C-glucozidic flavones orientin and vitexin. Regarding the enzymatic activity, the species that recorded an increased value for manganese peroxidase (MnP) were *P. ostreatus* var. Florida, followed by *A. campestris*, *G. applanatum* and *L. sulphureus*; Lignin peroxidase (LiP) had an increased activity in all fungal isolates tested, with the highest values for *A. campestris*, *P. ostreatus* var. Florida, *T. versicolor* and *F. velutipes*. The highest lacase activity recorded *P. ostreatus* var. Florida.

Chapter X describes the *in vitro* investigation of the biotechnological potential of some fungus species to discolour certain dyestuffs used in the textile industry, and the potential of *P. ostreatus* strains to degrade crude oil from the culture medium was evaluated. Of all the samples tested, only *T. versicolor*, *P. ostreatus* var. Florida and *G. applanatum* showed bleaching ability on all Bemacid type dyes. Regarding the Congo Red (CR) dye, no extract from the tested ones had a fading action on it. The strains of *Pleurotus ostreatus* adapted differently to the presence of the prelol in the culture medium and showed different degrees of development. In all experimental variants it was noted that mycelium developed on petroleum-treated media showed a dark brown color compared to the white-gray color typical of the fungus, which means that *P. ostreatus* has the ability to remove the pollutant by accumulating it in the fructification bodies.

Chapter XI addresses genetic variability in the case of commercial strains of *Pleurotus* sp. originating from different geographical areas and their genetic link with indigenous *Pleurotus ostreatus* isolates. Molecular analyzes with β-tubulin markers have revealed the existence of a

polymorphism at both intra- and interspecific level. All varieties of European commercial mushrooms were similar and comparable to the natural isolate in Romania. Significant differences were observed between European and US strains. The overall conclusion was that genetic diversity among macromycetes populations is closely related to their geographical distribution.

In the last chapter, **chapter XII** are presented the conclusions that result from the results obtained from the experimental analyzes performed.

The novelty and originality of the thesis consist of the following aspects: analysis of a large and varied number of isolated mushroom species with nutritional / medicinal properties; submerged cultivation and the establishment of optimal cultural environments to achieve maximum yield of fungal biomass in some wild species such as *L. sulphureus, Ganoderma* spp, *F. velutipes, L. edodes, T. versicolor, H. coralloides,* less studied species. Optimal submerged cultivation environments have been established for studied macromycetes species, which, optimized, could produce large amounts of biomass and bioactive compounds of high value in biotechnological processes.