THE MAIN DIRECTIONS OF RESEARCH IN THE FIELD OF MEDICINAL MUSHROOMS IN THE LAST THIRTY YEARS AT THE JAGIELLONIAN UNIVERSITY MEDICAL COLLEGE

MUSZYŃSKA Bożena[1], SUŁKOWSKA-ZIAJA Katarzyna[1], KAŁA Katarzyna[1], LAZUR Jan[1]*, KRAKOWSKA Agata[2]

[1]Jagiellonian University Medical College, Faculty of Pharmacy, Department of Pharmaceutical Botany, Medyczna 9 Str., 30– 688 Kraków

[2]Jagiellonian University Medical College, Faculty of Pharmacy, Department of Analytical and Inorganic Chemistry, Medyczna 9 Str., 30– 688 Kraków

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Abstract

Since prof. Stanisław Kohlmünzer became head of the department, the fruiting bodies of mushrooms were the main subject of research in the Department of Pharmaceutical Botany of the Medical Academy in Kraków (today: Jagiellonian University Medical College). At that time, the main interest was focused on the study of substances responsible for the toxic properties of mushrooms.

Thirty years ago, in the early days of medicinal mushroom research by the team of the Department of Pharmaceutical Botany of the Jagiellonian University Medical University, the focus was on polysaccharides and their immunostimulatory effects.

Currently, scientific research by the same team is focused on medicinal mushrooms and their biologically active compounds, cosmetic properties, and potential for bioremediation.

A major achievement in the field of mushroom biotechnology has been the extraction of submerged mycelial cultures as a standardized and enriched source of mycelia rich in bioactive compounds.

The future of this research team is related to the pharmacokinetics of selected biologically active compounds.

Keywords: culinary/medicinal mushrooms, polypore mushrooms, immunostimulatory activity, anti-inflammatory activity, antidepressant activity

Corresponding author: janlazur@gmail.com

Since prof. Kohlmünzer became head of the department, the fruiting bodies of mushrooms were the main subject of research in the Department of Pharmaceutical Botany of the Medical Academy in Cracow (today: Jagiellonian University Medical College). At that time, the main interest was focused on the study of substances responsible for the toxic properties of mushrooms.

From 1989 to 1999, fruiting bodies and mycelium from solid and liquid cultures of *Calocera viscosa* (Pers.: Fr.) Fr, an inedible mushroom species belonging to the division Basidiomycota, common in coniferous forests of southern Poland, were studied. The aim of the study was to determine the optimal conditions for the growth of the mycelium of *Calocera viscosa*. The analysis of fruiting bodies and mycelium of this species focused mainly on β,β-carotene.

Thirty years ago, when the team of the Department of Pharmaceutical Botany of the Jagiellonian University Medical College started researching medicinal mushrooms, the focus of the research was on polysaccharides and their immunostimulatory effect.

Since then, the fruiting bodies of medicinal mushrooms, their biologically active compounds, and biotechnological methods for obtaining mycelium with health–promoting and cosmetic properties, as well as with the potential for bioremediation, have been the target of scientific research conducted by the same team.

The large number of mushroom species studied (fruiting bodies and mycelia from *in vitro* cultures) comes from the division Basidiomycota, for example.: *Agaricus spp., Armilaria mellea, Auricularia polytricha, Boletus spp., Cantharellus cibarius, Daedaleopsis confragosa, Gloeophyllum sepiarium, Fomitopsis spp., Ganoderma spp., Hericium spp., Imleria badia, Lactarius spp., Leccinum spp., Lentinula edodes, Leatiporus sulphureus, Macrolepiota procera, Pleurotus spp., Flamulina velutipes, Phellinus spp., Polyporus betulinus, Suillus spp., Trametes versicolor and Ascomycota ones: Cordyceps militaris.*

Mycelia from in vitro cultures of mushrooms selected for the study were used for cultivation. The fruiting bodies were harvested when they reached the stage of maturity characteristic of each species. Only the homogeneous fruiting bodies of the first flush that had a typical appearance were used, and the fragments that were not intended for consumption were not studied further. After selection, the fruiting bodies were frozen, lyophilized and ground to be used for chemical analyzes.

Dietary and pro–health and medicinal properties

An important object of research was the effect of processing edible mushroom fruiting bodies on the content of selected biologically active compounds and the supplementation of mycelia with active substances to obtain material with better dietary and therapeutic properties.

In order to conduct further experiments consisting in estimating the actual amounts of biologically active substances available to the human body, a device called Gastroel-2014 was constructed in 2014. The method used ensures the highest possible correlation between the *in vitro* and *in vivo* systems and provides the best possible imitation of the digestive process and bioavailability of substances from fruiting bodies and mycelia in the human body (Polish patent No. P.417238).

In the field of biotechnology, the aim of the studies was to obtain submerged mycelial cultures as a standardized and fortified source of mycelium rich in bioactive ingredients [1]. The research concerned the methodology of conducting *in vitro* cultures, optimization of composition of the culture media and the development of proprietary bioreactors with the air-lift system [2] (Fig.1.).

Fig.1. Air-lift system for mushroom biomass production

Another direction of research relates to the possibility of obtaining biologically active metabolites from *in vitro* cultures of higher fungi.

The groups of biologically active substances studied were indole compounds, phenolic acids, amino acids, sterols, carotenoids, statins, fatty acids, polysaccharides, vitamins, bioelements and a compound specific for selected mushrooms species such as cordycepin.

An important direction of research is the assessment of biological activity, including anti-inflammatory, antioxidant, anti-aging, cytotoxic, procognitive, antidepressant of extracts obtained from fruiting bodies and mycelia of the studied species [3]. Primary and secondary metabolites contained in mushrooms have beneficial effects on immune system functions. Natural products containing bioactive compounds with the above effect can be used to reduce oxidative damage and inflammation in the human body. Chemical compounds in mushrooms also affect the proliferation and differentiation of the lymphocyte population, as well as the migration and adhesion of these cells. They can also inhibit the synthesis of inflammatory mediators such as cytokines, interleukins, prostaglandins, or nitric oxide by inhibiting pro–inflammatory signaling pathways related to the NF-κB nuclear receptor. In the studies, a series of experiments were conducted to determine the potential anti-inflammatory properties of mushroom extracts from *in vitro* cultures of the species *I. badia, C. cibarius, L. edodes,* and *Agaricus bisporus* in cell models. The new idea was to combine the immunoregulatory properties of zinc and α-linolenic acid with the beneficial properties of mushroom extracts [4,5]. The anti-inflammatory effect of biomass extracts was investigated for the first time. It was studied the simultaneous expression of COX -2, cPGES, and GSTM1 proteins, as well as the activity of NF-κB and PPARγ transcription factors in RAW 264.7 macrophages. An arboreal mushroom *Fomitopsis officinalis* extracts also showed antioxidant and antiproliferative effects in A549 lung cancer cell line, DU 145 prostate cancer cell line and A375 melanoma cell line. The obtained results confirm the medicinal qualities of F. officinalis and indicate its potential new applications. Strong antioxidant activity was found for *Pleurotus spp.* and their enriched mycelium [6,7]. The next studies of Fomitopsis species concerned *F. betulina.* Chemical analysis of fruiting bodies and mycelium from *in vitro* cultures revealed the presence of phenolic acids (syringic acid, gallic acid, p-hydroxybenzoic acid and 3,4-dihydro-phenylacetic acid), indole compounds (L-tryptophan,5-hydroxy-L-tryptophan,5-methyl- tryptamine), sterols (ergosterol, ergosterol peroxide, hexestrol, cholecalciferol) and triterpenes (betulinic acid, betulin). The extract from mycelium showed significant cytotoxic effect on prostate cancer cells, while the extract from fruiting bodies showed moderate effect on viability of melanoma and prostate cancer cells. Incubation of lung epithelial cells with the biomass extract significantly reduced the levels of COX -2 compared with lipopolysaccharide activated A549 cells. The results of the comparison of the metabolite composition and activity of mycelium and fruiting bodies suggest that these cultures could be proposed as a potential biotechnological source of selected biologically active compounds [8].

Importantly, attempts have been made to obtain mycelium under biotechnological conditions, which can be a good source of bioelements, as well as phenolic acids (by adding L-phenylalanine to the mushroom media – plant precursor for the synthesis of phenolic acids). Zinc and selenium compounds, which are the bioelements with the highest immunomodulatory and anti-inflammatory activity, were also used to enrich the media [1]. It has been shown that mycelia from in vitro cultures of edible mushrooms can be successfully enriched with phenolic acids with antioxidant activity by adding L-phenylalanine to liquid media. Of the three species studied, L. edodes showed the best biotechnological potential for enriching the mycelium with phenolic acids. The largest amounts of phenolic acids from this species were also extracted into artificial digestive juices. The addition of bioelements resulted in increased enrichment of zinc and selenium in the mycelium, followed by increased extraction for artificial digestive juices. *A. bisporus* proved to be the richest source of zinc and selenium [1]. From the conducted experiment, it can be concluded that appropriately modified mycelium can be a source of supplementation of substances important for health in the future, including bioelements such as zinc and selenium, as well as phenolic acids. Just a few grams of dried mycelium obtained after appropriate modifications can meet an adult's daily requirement of zinc and selenium. Several studies have proved that enriched mycelium cultures could be a kind of functional food or a component of food supplements in the future [1,4].

Cosmetology

The cosmetic industry also became interested in the biological properties of mushrooms, including extracts from fruiting bodies or biomass from mycelial cultures to produce cosmetic preparations [9].

Studies of cosmetic value demonstrated the properties of biomass from mycelial cultures of *G. applanatum, L. sulphureus,* and *T. versicolor.* The aim of the study was to analyze the content of substances with cosmetological properties (phenolic acids, sterols, indole compounds, and kojic acid) in the extracts obtained from the mycelial cultures. In addition, the influence of the extracts on the inhibition of tyrosinase and hyaluronidase was determined and the values of sun protection factor (SPF) were calculated. The results showed that the mycelial cultures of the studied species can be used as an alternative source of substances used in cosmetics [10].

The latest research on arboreal species concerns the bioactivity and mycochemical profile of extracts from mycelial cultures of *Ganoderma spp. (G. adspersum, G. applanatum, G. carnosum, G. lucidum, G. pfeifferi, G. resinaceum*). The research also focused on antioxidant activity, cytotoxic activity, and inhibition of selected enzymes (tyrosinase, acetylcholinesterase). The evaluation of antioxidant activity, cytotoxic activity, and inhibition of selected enzymes (tyrosinase, acetylcholinesterase), as well as the chemical analysis of the extracts from the mycelial cultures of the mentioned species, suggest that the analyzed mycelial cultures are promising candidates for cosmetic ingredients.

Method of preservation

The study of the fruiting bodies of edible mushrooms consisted in the determination of the content of specific compounds of health-promoting importance in the mushroom material (e.g., indole and phenolic compounds or bioelements), but also in the study of their potential bioavailability. The aim of the few studies was to evaluate the content and the degree of extraction of important compounds relevant to health depending on the studied mushroom species as well as the selected storage or drying method, which is of fundamental importance for consumer nutrition [11–13]. The material selected for the experiments was fresh, frozen, and dried using three methods: freeze drying, hot air drying in a food dryer, and drying in the sun. Various drying methods were found to have a significant effect on the potential bioavailable bioelements to the human body. Drying by freeze-drying and in the sun proved to be more beneficial than drying in a dryer, considering the content of bioelements and their amounts extracted into artificial digestive juices. Importantly, the fresh material was a better source of bioavailable bioelements than the frozen material [12]. Several experiments have shown that edible mushrooms, both those from commercial cultures and those from the natural environment, can be a good source of medicinal compounds for the human body, which can be directly reflected in their dietary and therapeutic potential [11–13].

Veterinary research

Due to the widespread use of mushroom fruiting bodies in feed for farm animals and the need to develop a feed additive with immune-stimulating, prebiotic, anti–inflammatory, and selenium–supplementing effects to reduce mortality in farm animals, a collaboration was initiated with the National Research Institute of Animal Production and the National Veterinary Institute in Puławy. The result of these experiments is the preparation of the mycelium of L. edodes enriched with organic selenium compounds (IV). This supplement, named Selentin (currently under international patent application P.432076), is expected to have an immunostimulatory effect and compensate for selenium deficiency in farm animals (calves). In order to obtain the quantities of the preparation needed for the experiment, the methodology of biotechnological production of selenium-enriched mycelium of L. edodes in 10 L overhead biofermenters with an airlift system was developed [2] (Fig.1).

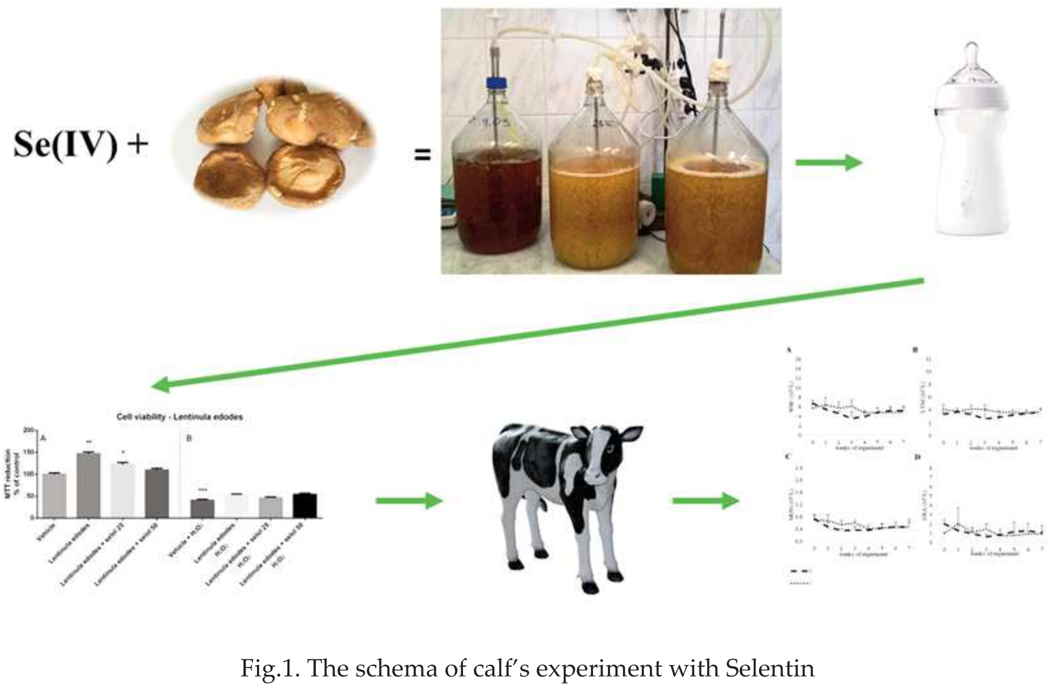

Fig.1. The schema of calf's experiment with Selentin

Bioremediations

White rot fungi are a group of mushrooms which primary role is degradation of dead wood to simple carbon constituents which leads to maintain proper carbon cycle in natural ecosystems. Degradation of dead wood is possible due to synthesis and secretion to the environment enzymes called lignin modyfing enzmes (LMEs). By their nature, LMEs such as laccase and peroxidases: lignin peroxidase, manganese peroxidase are non-substrate–specific and by this way they can catalyze reactions of oxidation not only of lignin but also a wide range of organic substrates. [14]

The conducted research in the field of biotechnology of higher fungi also focused on the assessment of mycelium from *in vitro* cultures of selected species of mushrooms in terms of bioremediation of the natural environment from xenobiotics – substances that are foreign to animal life like commonly used drugs, plant constituents or pollutants. The studies on the biodegradation of selected drugs, in addition to the quantitative analysis of the remaining compounds in the medium by HPLC methods, were accompanied by the analysis of the degradation products by the MS /MS method, which made it possible to propose the biodegradation pathways of the tested substances and predict their possible biological/toxic effects.

The first work of Muszyńska's team on biodegradation of drugs by *in vitro* cultures of edible mushrooms was conducted on example of testosterone and 17α – ethinylestradiol addition to L. edodes *in vitro* cultures. The results from this study confirmed almost 100% efficacy in biodegradation of above–mentioned compounds in *in vitro* conditions. Analysis of degradation products was performed which showed degradation pathways of testosterone and 17α – ethinylestradiol and confirmed that products of degradations have no biological effects. [15] Mechanism of biodegradation of piroxicam was proposed for the first time after incubation in *in vitro* cultures of *L. edodes*. What is more, it was confirmed that biodegradation efficacy in case of piroxicam is not related with concentration of this compound in the culture media. [16] Further studies on efficiency of biodegradation by in vitro cultures of selected edible mushrooms as well as mechanism of this process was carried out in case of azole antifungals, cephalosporins and sulfonamides. [17–19]

Bioremediation of xenobiotics or heavy metals from the environment by fungi is not only related to the enzymatic activity of fungi, but also to their ability to accumulate substances or elements from the soil. Thus, mushrooms like I. badia can be used for biomonitoring the presence of radioactive elements such as cesium–137 in soils after the Chernobyl reactor explosion, which is possible due to badion – a natural dye that colors the cups of the fruiting bodies of *I. badia* and which contains cesium in the chromogenic system in a molecule. [20] The studies on bioaccumulation of selected heavy metals such as cadmium and lead were conducted using in vitro cultures of *L. sulphureus*, *I. badia*, and *A. bisporus.* The results showed that the highest efficiency was observed in the bioaccumulation of cadmium ions in the biomass of *L. sulphureus*, while the *in vitro* cultures of *A. bisporus* were the most efficient in the removal of lead ions. The highest ability to accumulate cadmium and lead ions in mycelium per dry weight was observed in *L. sulphureus*. [21] In addition to the natural ability of edible mushrooms to accumulate heavy metals, Muszyńska's team demonstrated that they are not released in large quantities into artificial gastric and intestinal juices and that the permissible limits for human consumption of heavy metals are not exceeded. Thus, the consumption of fruiting bodies of edible mushrooms, even if they come from locations polluted, for example, with pollutants from cars, is safe for humans.[22]

Conclusions

From more than thirty years the main interest of mushroom team from Department of Pharmaceutical Botany JU MC are dietary /medicinal/cosmetology/veterinary and another important for environment properties of mushrooms. The research showed that fruiting bodies of medicinal mushrooms and their mycelia from *in vitro* cultures are great source of important biological substances with anti–inflammatory, antioxidant, anti–aging, cytotoxic, procognitive, antidepressant, hypoglycemic and probiotic activity. So, the mushroom material under study can be used as functional foods and for preparation of dietary supplements and cosmetics.

What next? The future of this team studies relates to pharmacokinetic research of mushroom selected biologically active compounds.

Resumo

Ekde la tempo de profesoro Stanisław Kohlmünzer la fruktkorpoj de fungoj estis la ĉefa esplortemo en la Departamento de Farmacia Botaniko de la Medicina Akademio en Krakovo (hodiaŭ: Medicina Kolegio de Jagelona Universitato). En tiu periodo oni interesiĝis pri esplorado de substancoj respondecaj por la toksaj proprecoj de fungoj.

Antaŭ tridek jaroj, en la komenco de esplorado de kuracaj fungoj per la membroj de teamo de la Departamento de Farmacia Botaniko de la Jagelona Universitato Medicina Kolegio, la ĉefaj esploroj koncernis la polisakaridoj nkaj iliajn imunostimulajn efikojn.

Nuntempe, la scienca laboro de la sama teamo okupiĝas pri ekzamenado de kuracaj fungoj kaj iliaj biologie aktivaj kemiaj kombinaĵoj, kosmetikaj trajtoj, kaj eblecoj por bioripurigo.

Grava atingo en la kampo de fungobioteknologio estis la eltiro de subakvigitaj miceliaj kulturoj kiel normigita kaj riĉigita fonto de micelio riĉa je bioaktivaj kemiaj komponaĵoj.

La estonteco de ĉi tiu esplorteamo rilatas al la farmakokinetiko de elektitaj biologie aktivaj kemiaj komponaĵoj.

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