Antimicrobial potentialities of mushroom-based selenium biocomposites

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Contemporary biotechnological applications of Se are undoubtedly broad. Elemental selenium and chemically synthesized Se-conjugates are known to possess antimicrobial properties. However, more ecologically safe and beneficial approaches to manufacture the Se-based antibacterial agents are current challenge. In this relation, of especial interest are the selenium-enriched preparations of higher-fungal origin owing to their availability, biocompatibility, and proved biological activity. Wood-decaying higher fungi attract attention as the possible participants of the plant wastes biodestruction processes, as well as the producers of unique complex of biologically active substances. The approach developed in our works recently would allow the bioproduction of submicrostructured elemental selenium-based composites using the edible mushrooms cultures to be put into practice. We demonstrated the occurrence of bacteriostatic and bactericidal effects of the agents under study, and the results favor the supposition on advisability of further research into the selenium bionanocomposites as the agents for agricultural recovery from the bacterial pathogens.

Keywords: antibacterial activity; biocomposites; selenium; mushrooms; culture characteristics; chemical composition

1. Introduction

Wood-decaying higher fungi attract attention as the possible participants of the plant wastes biodestruction processes, as well as the producers of unique complex of biologically active substances. Mushrooms are recognized to be promising ecologically pure raw material for developing the medicinal preparations for care and prophylaxis with wide spectrum of action.

The trace element selenium (Se) is essential nutrition mineral. Selenium deficiencies in the human and animal organism are recognized worldwide to be related to a number of pathologies. However, at higher Se concentrations, harmful consequences occur. The contradictions found in the course of Se studies might be related to poor understanding of controversial mechanisms involved in selenium biochemistry. Therefore, a rich area of selenium explorations could be considered as two fields: Se as dietary component and Se as toxic agent. The possible mechanism of toxicity appears to be production of free radicals, adhesion to proteins and uncontrollable accumulation, induction of apoptosis, growth arrest, and cell death.

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Interest in using mushrooms as a Se carrier increases. The approach developed in our works recently would allow the bioproduction of submicrostructured elemental selenium-based composites using the edible mushrooms cultures to be put into practice. We demonstrated the occurrence of bacteriostatic and bactericidal effects of the agents under study, and the results favor the supposition on advisability of further research into the selenium bionanocomposites as the agents for agricultural recovery from the bacterial pathogens.

Analyzing the selenium content of mushrooms and its utilization, the contribution of mushrooms to the human's selenium demand, selenium content of mycelium cultivated under different conditions, effect of technology (growing) on the selenium content of mycelia, selenium species occurring in mushrooms, bioavailability of selenium in different oxidation states, one could conclude on the significantly positive trends in edible and medicinal mushrooms' implementation in this area.

The importance and possibilities for increasing the Se pool of mushroom culture at submerged cultivation, a fate of organoselenium xenobiotics in macrobasidiomycetes for elaborating upon the "green" techniques of submicrostructured Se-containing biomaterials production and evaluating their antimicrobial potentialities are discussed. The favorable profile of newly synthesized organoselenium compounds including those explored in our research warrants their recognition as a promising option for fortification purposes. Further thorough investigation should be focused on the
mechanism of Se-containing compounds’ toxicity to take that into account when using the various Se sources in biotechnological fields, including the production of ecologically safe antibacterial agents.

2. Fungal component of antimicrobial biocomposites

2.1 Mushrooms, promising ecologically pure multipurpose material

Defining the exact number of fungi on the earth has always been a point of discussion, and several studies have been focused on enumerating the world’s fungal diversity [1]. Wild mushroom harvesting and mushroom cultivation provide a much-needed alternative source of income for rural households. The greatest threat for many mushroom species is that of habitat loss and over-harvesting of wild stocks, thus, by creating awareness of these issues, one enables a more sustainable use of these natural products [2].

Since the wild fungi play an important role to maintain the health of forests besides their medicinal importance and nutritional value in most of the cases, therefore it becomes quite necessary to explore, document and conserve this natural wealth.

Mushrooms are ubiquitous organisms found in almost every ecosystem and play central roles in the recycling of organic matter. A considerable amount of literature has been published on the ecology, physiology, genetics, and biotechnology of mushrooms. Edible mushrooms are readily available at any food market owing to their commercial cultivation [3]. The mushrooms do not merely constitute a highly nutritious source of food. More recently, attention has been focused on a second area of exploitation following the discovery that many of these fungi produce a range of metabolites of intense interest to the pharmaceutical and food (e.g. flavour compounds) [4, 5].

Relatively low levels of commercial cultivation of the mushrooms limit their availability for use as food and medicine [6]. A good alternative to mushrooms’ fruit bodies production is provided in this respect by the submerged fermentation. The process offers several advantages including a fast growth and high biomass productivity [7], compact and controlled environment and shortened production time [8]. This resourceful biotechnological approach in the mushrooms application has been used widely to yield bioactive compounds (polysaccharides, glycoproteins, selected low-molecular substances) in different basidiomycetes [9, 10], as well as mycelial biomass itself. The latter is valuable not only as food and fodder supplement, but also as the intermediate product, seeding material, for obtaining fruiting bodies [11]. Mycelia formed by growing pure cultures under the submerged conditions are high-quality, consistent, safe, predictable and economical mushroom products [12, 13], and a suitable alternative to yield mushroom product fortified with selenium.

2.2 Choice of fungal material with special attention to Ganoderma mushrooms

A part of contemporary researches in mycology is encouraged by strategies for drug discovery as well as for monitoring and managing diseases caused by Ganoderma in woody crops and forest ecosystems [14]. The genus Ganoderma was established by Finnish mycologist Peter Adolf Karsten in 1881 for the Polyporus lucidus W. Curt. In non-edible medicinal species, Ganoderma, which belongs to the polypores (mushrooms that contain pores that hold reproductive spores, rather than gills), is the leader in terms of production [15].

The discovery of Tomophagus cattienensis sp. nov. was reported as earlier as in 2012 by the authors of [16]. Those xylotrophic mushrooms degrade the wood over time and produce a fruiting body (or conk) on the surface of the wood. The extremely important biologically active extracts and compounds from these mushrooms exert the pharmacological effects on tumor cell. Extensive research over the last 10 years has provided evidence of the anticancer activities of both the triterpenoids isolated from Ganoderma [17] and the carbohydrate-enriched crude extracts from these fungi [18]. Ganoderma species exhibit a broad spectrum of antibacterial, antiviral [19], immunostimulatory [18], cytotoxic [20], antifungal [21] activities. It appears that both polysaccharides and triterpenoids are the major antiviral constituents of these mushrooms, polysaccharides playing a more important role for their antibacterial properties. The studies of antibacterial action of Ganoderma extracts does not restricted by G. lucidum [22], other species are also the active players [18, 20, 23].

Ganoderma species are among those fungi that can thrive under hot and humid conditions and are usually found in subtropical and tropical regions [24, 25]. There are numerous Ganoderma species that are native to Vietnam. Most likely that Vietnamese Ganoderma contains not only similar substances to this genus mushrooms isolated elsewhere, but also several unusual or even unique secondary metabolites.

G. lucidum species complex is composed of several species that can be difficult to distinguish from one another. There is nevertheless a strong consensus about the true identity of G. lucidum among contemporary European mycologists, that is G. lucidum is probably restricted to western parts of Europe [14], G. valesiacum Boud. distribution range includes the areas of Siberia along with Europe, China and Japan [26].

Comparative studies of Vietnamese Ganoderma (5 species) and species cultured in Russia were performed within a framework of the joint research project [27]. Those 5 species from Ganoderma genus, including rare G. colossus, G. neo-japonicum, G. cattienensis along with the strains of G. lucidum, G. applanatum intrinsic for tropical area, were explored with the purposes of the subsequent comparative studies with the herbarium strains of G. lucidum, G. applanatum, and G. valesiacum (IBPPM RAS and Irkutsk State University). This research was aimed at elucidating and
systematizing of physiological-biochemical differences of *Ganoderma* species by the properties of the set of secondary metabolites, stress-resistance, and at the development of fundamental basis of species specificity within *Ganoderma* genus and practical recommendations on optimization of the directive cultivation of the selected species.

Morphological features, culture and developmental characteristics of species and chemical components such as secondary metabolites contribute greatly to the traditional identification of *Ganoderma* species [28]. The primary tasks posed for the work were to determine suitable storage and growth conditions of the mushrooms, and to research into their low-molecular composition.

Mycelial cells can degenerate due to lack of nutrients or oxygen, infections (viruses), a change in substrate pH or the accumulation of unfavourable metabolites. [29]. Most fungal cultures can be maintained on agar by subculturing at two to six month intervals [30]. "Agar to agar" is a relatively cheap method of storage, with little time required for the production of new mycelial growth. A disadvantage of this technique is that the mycelium under refrigeration may result in degeneration of the mycelium, since the fungus can still be growing under these low temperature conditions [29]. We used "agar to agar" method for short term storage the cultures strains in laboratory.

The importance of selecting the suitable short term and long term storage of the fungi was to ensure the fungi remained viable throughout this investigation, as well as for future reference and use. It was also desirable to obtain maximum growth of the fungal mycelium in a liquid cultivation medium so that sufficient material was available to pursue further studies on its biological activity.

The conditions of solid-phase cultivation (thermal optima) and liquid-phase submerged cultivation (growth media formulation, temperature, culture duration) were selected by the criteria of highest mycelial biomass accumulation and primordia formation under a laboratory conditions. Optimal growth of mycelia was searched by variation of the environmental parameters, as the nutrient medium composition, carbon and nitrogen sources' kinds and proportion, thermal conditions of culture. Besides, the efficiency of cultivating the seeding mycelia of *Ganoderma* using the wastes of agricultural production characteristic for tropical zone, as well as using the relatively cheap food components, was shown.

Contemporary physicochemical methods (scanning electron microscopy (SEM), gas chromatography coupled with mass spectrometric detection (GC-MS), gas-liquid chromatography) were applied for further characterization of the cultures. The key objective of this research step was to generate the base-line information on *Ganoderma* species of the largest protection areas in Vietnam, and to compare those with the species or strains of distinct geographical sampling.

The comparative analysis by the scanning electron microscopy method was attempted to follow the fruit bodies morphology and mycelia microstructures.

The presence of salt crystals on hyphae in mushroom cultures was reported earlier [e.g., 31]. As a rule, crystals cover the hyphae and were rarely found separated from the cells. Usually it is calcium oxalate crystals that are formed on hyphae. Crystals occurred to appear at some mushrooms' culture under different nutrient media (agar and liquid media, grain, compost, etc.) and represented a relatively stable characteristic of the cultures [31]. The density of crystals on the surface of hyphae, as well as the morphology of crystals may vary with a fungus systematic position.

In the samples of *G. colossus* pileus and mycelium on wort agar, calcium salt microparticles were observed (Fig. 1). The presence of calcium was proved by EDX analysis. Near-cubic shape and smooth surface of the particles allowed classifying of crystal structure as calcite, the most stable crystalline structure of calcium carbonate. Back-scattered electron images of the samples of *G. valesiacum* 120702 mycelium on wort agar showed the presence of small areas inside the samples containing atoms heavier than carbon, oxygen or nitrogen, and formation of calcite particles in the samples can be supposed as well (Fig. 2).

![Fig. 1. Ganoderma colossus SIE1301 calcium salt crystals in: a - pileus tissue; b - mycelium on wort agar (full-length scale bars are 50 μm)](image1.png)

![Fig. 2. Ganoderma valesiacum 120702 calcium salt crystal in the mycelium (scale bar is 10 μm)](image2.png)
The presence of yellow pigment is thought to be among the features suitable for characterizing the differentiation rate of *Ganoderma* species [32]. Using the isolates of *Ganoderma* strains under study as the inoculum, the cultures were tested for their ability to produce yellow extracellular pigment on media plates. Some relevant peculiarities were observed in our studies, being much more profound for *G. colossus*. One could notify that the unfavorable culture conditions facilitate the vegetative mycelium pigmentation. Brown color occurrence could be stimulated by a limited variety of carbohydrates (potato-glucose-based formulation). Relatively low culture temperature is a further contributory factor to *G. colossus* mycelium becoming yellow.

Pigmented mycelia of *Ganoderma* species appear to contain valuable compounds. 1-Octen-3-ol and 3-octanol were found as main volatile flavor compounds in our work, in compliance with the earlier few works dealt with the volatile compounds in *Ganoderma lucidum* [33] and *G. sinense* mycelia [34].

Different liquid crystalline systems are based on natural low-molecular substances, which are fatty acids derivatives. Those could serve as chemical stability enhancers for protect drugs from chemical instability reactions [35], as coatings for microspheres in the process of gastroretentive drug delivery [36], as subjects for broad discussion on biodiesel [37, 38]; Monoglycerides are also among the detected compounds. Further studies should be driven by a wide range of promising biotechnological applications based on the mycelial chemical components of lipid nature.

Thus, by applying common mycological, SEM, and current chromatographic methods, we have studied micromorphological, cultural and selected biochemical characteristics that might provide a routine basis for identifying the most promising strains as biotechnological subjects. *Ganoderma* species were under the comparative study in respect to *Ganoderma* strains from European and Siberian regions' mushroom collections.

### 3. Mineral component of antimicrobial biocomposites

#### 3.1 Selenium, essential mineral

The trace mineral selenium (Se) is an essential element for human and animal nutrition. Selenium deficiencies in the human and animal organism are recognized worldwide to be related to a number of pathologies [39]. However, at higher Se concentrations, harmful consequences occur. It has been hypothesized that the intake of excessive doses of selenium may cause oxidative damage, leading to genomic instability [40]. The contradictions found in the course of Se studies might be related to poor understanding of controversial mechanisms involved in selenium biochemistry. Therefore, a rich area of selenium explorations could be considered as two fields: Se as dietary component and Se as the agent toxic for organisms at different developmental steps, therewith the toxicity being severely dependent on the dose and chemical essence of Se-containing compound. The possible mechanism of toxicity appears to be production of free radicals, adhesion to proteins and uncontrollable accumulation, induction of apoptosis, growth arrest, and cell death.

#### 3.2 Inorganic and organic Se: dramatically different biological effects

Recommended dietary intakes are not currently met by most diets, unless Se-rich foods are included. Therewith one should take into consideration a poor bioavailability of the most common inorganic forms of selenium. Selenium content in a foodstuff critically influence Se bioactivity to humans and animals. Foodstuffs processing and treatments, along with foodstuff-matrix major and minor components, affect Se bioavailability [41]. A great deal of information has been accumulated indicating that dietary form of Se is a major determinant of its efficiency [42], and the chemical form of Se plays a very important, if not a decisive role in its bioavailability [43]. A growing body of evidence indicates dramatically different biological effects of inorganic and organic chemical forms of selenium, which may explain apparent inconsistencies across studies by inadequate assessment of health risk [44]. The human or animal exposure to selenium in different chemical forms leads to not only different, but in some cases opposite nutritional and toxicological consequences [45, 46]. The worse cases are Se compounds having lower concentrations but the highest toxicological activity such as the inorganic forms selenite and selenate. [47-49]. At the same time, it has been shown repeatedly that Se is more bioavailable to animals and humans in organic forms than in inorganic forms [39, 50, 51], and toxicity of inorganic (tetravalent) Se greatly exceeds that of organic Se. When comparing the bioactivity of Se-containing agents, C.S. Hoefig et al. [52] explored a number of different selenocompounds known from nutrition, supplements or pharmacological use, and tested their ability to support and increase selenoprotein P (SEPP) production by human and murine hepatocytes in culture. Upon comparison of the two inorganic Se compounds, sodium selenite Na₂SeO₃ was found to be more toxic, and decreased viability was already observed in the micromolar concentration range. LD₅₀ of 2 μM selenite solution was more than 30 times lower than the corresponding value of selenate.

The investigations aimed at the development of novel synthetic organoselenium compounds and at the discovery of naturally occurring selenium compounds that are more effective and less toxic than inorganic forms of selenium were initiated at the beginning of the 1980s. Important aspects of the modern organoselenium chemistry are the use of organoselenium reagents as catalysts (organocatalysis), green chemistry, bioinspiration, antioxidant activity. The
classical synthetic application of organoselenium reagents are electrophilic, nucleophilic and free radical reagents. Organoselenium compounds find applications in organic synthesis, materials synthesis, ligand chemistry [53-55], antioxidative agents [56-58]. The synthesis and the synthetic applications of some emerging classes of selenium compounds such as hypervalent selenium species and selenoamides, address some biological aspects such as the antimicrobial activity of organoselenium derivatives and the biochemistry of selenoproteins, along with biologically relevant processes as potent therapeutic and chemopreventive agents [59-61] known to be clinically safe and to possess a well-established pharmacology profiles [62].

Today this area of organoselenium research is growing rapidly, and the outcomes of these investigations are highly promising. Exciting studies performed in vitro with respect to cellular responses showed that the dose and form of selenium compounds are critical experimental parameters. Inorganic (at doses up to 10 μM) and organic selenium compounds (at doses equal to or greater than 10 μM) elicit distinctly different cellular responses [40]. One should take into account that toxicity of Se-containing compound is rather a compound-specific property, and is not related directly to the biosynthesis of enzymatically active selenoproteins. Thus, secretion of hepatically derived SEPP, the central selenoprotein in blood controlling Se transport and distribution, is crucial to convert nutritional sources into serum Se, supporting Se status and selenoprotein biosynthesis in other tissues. The selenocompounds different in chemical nature, although showing a positive effect on SEPP production in the cell culture medium, failed to support cell viability in vitro. Sodium selenite, methylseleninic acid, L-selenocystine, and selenodiglutathione caused the increased SEPP concentrations in murine and two human liver cell lines, but induced cell death in micromolar concentrations, whereas the less effective with SEPP selenomethionine (SeMet) or synthetic preparation ebselen were not toxic within the concentration range tested [52].

4. Mushrooms as a Se carrier

4.1 Selenized mycelia

Interest in using mushrooms as a Se carrier increases. Analyzing the selenium content of mushrooms and its utilization, the contribution of mushrooms to the human's selenium demand, selenium content of mycelium cultivated under different conditions, effect of technology (growing) on the selenium content of mycelia, selenium species occurring in mushrooms, bioavailability of selenium in different oxidation states, one could conclude on the significantly positive trends in edible and medicinal mushrooms' implementation in this area.

Mushrooms and yeasts have attracted a number of researchers in food and pharmaceuticals. Mushroom-based foods enriched with selenocompounds could be a convenient source of Se to balance the deficiency. Therewith the safety and efficacy factors favor the organic forms of Se. The addition of selenium to the diet through dietary supplements or fortified food/feed becomes increasingly common owing to the frequently suboptimal level of this microelement in standard nutrition in many countries [63]. One of the basic questions arising in relation to Se-fortified food is on the threshold quantities of selenium causing unexplored harmful consequences of using common food. The content of selenium in food of not-fungal-origin, i.e., plants and animals, depends critically on the selenium content in environment. Thus, the selenium concentration in such nutrient products is highly variable [64]. The bioavailability of selenium from fish can be modified by the presence of various contaminants, including arsenic and mercury [65].

Much attention should be paid to the biotechnological processes with the implements of selenium-enrichment of mushroom growing mycelium and post-harvest treatment thereafter, because of the ambiguous influence of elemental selenium upon human and animals. Fortification of edible mushroom cultures with the selenium-containing compounds has proven to be an effective and cost saving strategy for the prevention of Se deficiency.

Satisfaction of the human selenium requirements can be considerably contributed from mushrooms, since the selenium enriched mushroom mycelia are valuable functional foods. It is obvious that risks and benefits of Se intakes should be quantified and balanced. The mushrooms are commonly used food product and dietary supplement convenient to apply in the selenium-fortified form. Possibilities for increasing selenium content in foods by increasing selenium content of mushrooms fruit bodies and vegetative mycelia are explored from the end of 20th century up to now. Mycelia of many tested mushroom species at submerged growing are satisfactory Se-sources due to the fact that Se-concentrations absorbed from the sodium selenite-enriched medium could achieve tens percent of its content in the medium (e.g., 62.5% for P. ostreatus) [66]. The mycelial selenium content could be several times higher than in fruiting bodies.

In order to optimize fortification process and yields, selenium enrichment in the cultivation substrate can be an approach to increase the Se concentration in fruiting bodies of mushrooms. Popular mushrooms with high commercial values and thus cultivated world wide, e.g. Agaricus bisporus, Lentinula edodes, Pleurotus spp., Flammulina velutipes, Hypsizigus marmoreus appeared to contain nutritionally significant but yet insufficient amounts of Se [67]. Most of edible mushroom species examined are selenium-poor (< 1 microg Se/g dry weight) [68]. These values can be considerably, even by order of magnitude, increased in mushrooms picked in polluted areas. Moreover, some species have accumulating and even hyperaccumulating ability for Se [69]. So, the solution is artificial growing of basidiomycetes. A particularly rich source of selenium could be obtained from selenium-enriched mushrooms that are
cultivated on a solid media fortified with selenium (as inorganic salt or selenized-yeast). Addition of Se oxyanions in the form of selenite or selenate to the substrate for growing basidiomycetes results in an increase of the Se content in their fruiting bodies [70, 71].

Growth-compost irrigated with sodium selenite solution appeared to cause the increase in the selenium level in button mushroom, *Agaricus bisporus* by tens times compared to the control mushroom irrigated solely with water [72].

The enrichment of *Lentinula edodes* (shiiitake mushroom) fruit bodies with Se could be performed by adding the sodium selenite to the cold-shock water used to induce primordial formation in artificial logs [73]. As a result, shiitake absorbs and accumulates Se in carpophores, therewith the color, moisture, and the protein content of mushroom being unaffected. Thus, selenium-enriched fruit bodies are industrially cultivated as functional food or medicinal food in China and Southeast Asia and could provide an efficient way in delivering functional organic Se. However, the composition of selenium substances, as well as the distribution of the main bioactive components, remain still unknown [74].

Several data testify to the greater efficiency of selenized mushroom compared to yeasts. Thus, the high level of SeCys in several Se-enriched mushrooms [72] could provide greater biological benefits than Se-enriched yeast which contains mainly SeMet, since the SeCys residue functions as an active-site amino acid in selenoenzymes, e.g. glutathione peroxidase and thioredoxin reductase [75], and provides antioxidant properties in non-enzymatic selenoproteins [76]. Indeed, the effect of dietary supplementation with Se-enriched *Agaricus bisporus* on different selenoproteins expression in rats showed that the selenized button mushroom can positively increase GPx-1 and GPx-2 gene expression and GPx-1 enzyme activity in rat colon [77]. The extracts from *Lentinula edodes* mycelia rich in the organic forms of selenium possess putative cancer-preventive properties more effective than those of selenized-yeast [78].

### 4.2 Speciation of selenium in Se-enriched mycelial cultures

An increasingly growing database on chemical forms of selenium in mushrooms indicates that the seleno-compounds identified in carpophore include selenocysteine, selenocystine, selenomethionine, Se-methylseleno-L-cysteine, inorganic selenium [79] and several unidentified seleno-compounds; their proportions vary widely [68]. For mycelia at submerged cultivation, the increased mycelial concentration of the above selenoamino acids is feasible by optimizing the concentration of sodium selenite in the liquid medium, e.g. of Se-methylseleno-L-cysteine from essentially non-detectable levels to 120 μg/g dry weight [80], the much higher levels of SeCys (detected as (SeCys)₃) compared to SeMet and MeSeCys were found for *Agaricus bisporus* grown on compost enriched with inorganic Se [72]. Study by Jadwiga Turlo et al. [78] indicate that the shiitake mushroom *Lentinula edodes* cultivated in media enriched with sodium selenite is capable of incorporating selenium into the proteins, mainly in the form of selenomethionine. The amount of selenomethionine in the cultivated biomass increased in proportion to the selenium content of the mycelium, but the percentage of selenium accumulated as the selenoamino acid decreased. For greater selenium concentrations, the selenocysteine content of the mycelial biomass does not depend directly on the concentration of selenium in the medium and was essentially unchanged. Thus, a fate of a considerable portion of the introduced selenium is unknown. A sample of *Hericium erinaceus* (lion's mane mushroom) belonging to Agaricomycetes was examined in the study [81] after moderate selenium enrichment from inorganic selenium source. It was revealed that nearly 50% of the selenium was in organic forms: selenomethionine and Se-methylselenocysteine. Three Se-adenosyl-compounds were identified: Se-methyl-5-selenoadenosine, Se-methyl-5-selenoadenosine-Se-oxide and the previously unreported Se-dimethyl-5-selenonium-adenosine.

Evaluation of the efficacy of supplementation of several medicinal mushrooms with inorganic selenium salts (Na₂SeO₃ and Na₂SeO₄) attracted the attention of many researchers in 21st century. Submerged cultivated mycelium of *Lentinula edodes* accumulated selenium from the cultivation medium very effectively. Selenium was well bioavailable from the mycelial preparations in *in vitro* and *in vivo* tests, for which different preparations of selenated mycelium were compared [82]. The speciation of selenium in Se-enriched mycelial cultures testified to the fact that the main, inorganic, part of Se in the tested mycelium was in the zero (elemental selenium) and IV oxidation states. The response of *Phanerochaete chrysosporium* to the aforementioned selenium oxyanions showed a high sensitivity to selenium, particularly selenite. The latter inhibited the fungal growth and substrate consumption at a level of 10 mg/l in the growth medium, whereas Se(VI) of selenite did not have such a strong influence on the mushroom [83]. *P. chrysosporium*, too, was found to be a selenium-reducing organism, capable of synthesizing elemental selenium from selenite but not from selenate. Studies with Ganoderma lucidum, Agrocybe aerugina, and *Hericium erinaceus* showed that the growth of Ganoderma lucidum fruit bodies was observed with up to 0.8 mM Se accompanied by the highest total Se content, macroscopic changes in the fruiting bodies of the examined mushrooms, and color changes of fruiting bodies [84]. In a work by Ivan N. Milovanović [85], such biotechnologically important mushrooms fortified with inorganic Se as *Ganoderma lucidum* (Leyss.:Fr.) Karst., *Pleurotus ostreatus* (Jacq.: Fr.) Kumm., *Pleurotus eryngii* (DC.:Fr.) Quél. var. eryngii, *Pleurotus pulmonarius* (Fr.) Quél., *Flammulina velutipes* (Curt.: Fr.) Sing., *Ganoderma applanatum* (Pers.:Wallr.) Pat., *Lenzites betulinus* (L.:Fr.) Fr., *Trametes hirsuta* (Wulf.:Fr.) Pil. were explored in respect to their morpho-physiological characteristics, as well as biological activities. During cultivation on selenite-enriched medium, the appearance of mycelium of brick-red color with significant morphological and ultrastructural changes in
comparison with the control was observed. Hyphal density was lower, the cell wall was thick with more expressed extracellular matrix, septa were abundant, and branch frequency and occurrence of clamp-connections were rare.

Cytological analysis demonstrated that the majority of selenium was accumulated in cell membrane and vacuoles, while changes taking place in a cell wall were insignificant [85].

The latter study along with a relatively large number of others are focused on the effect of inorganic selenium salts' concentrations on mycelium morphological and ultrastructural features. At a relatively high selenium concentration in Lentinula edodes liquid nutrient medium, the excess selenium is eliminated via its reduction to elemental Se [86]. As for another very popular cultivated mushroom, Pleurotus ostreatus, the studies also showed that higher selenium concentrations caused firstly Se accumulation in P. ostreatus mycelium, and during the suppression growth phase, selenium was reduced to amorphous Se in zero oxidation state and this gave the mycelium and medium a reddish color [87, 88]. Hyphal morphology of P. ostreatus was dependent on Se concentration in the liquid medium. Electron-dense spots, visible in both the control and Se-enriched samples, were described [89] as proteinaceous bodies, since lipid bodies would be extracted during preparation for transmission electron microscopy. In the presence of Se, the number of these bodies increased, and changes in their shape, color, and size were slight. It was shown that the reduction of the ionic Se and production of amorphous Se(0) are really occurring round the bodies [89]. Selenium stress exerts a significant effect on ultra-architectural features of the fungal hyphae and spores of mushroom cultures, e.g., Ganoderma lucidum [90]. A number of works deals with the Se distribution among different cellular compartments, and, in particular, polysaccharide structures contained in fungal cell walls. Se-enriched submerged mycelia of Pleurotus ostreatus were explored in respect to the incorporation of selenium from the growth medium to mushroom [91]. A polysaccharide-containing fraction of mycelia was treated alternatively with Tris-HCl or with chitinase. Better solubility and increased contribution of low molecular mass compounds were observed in chitinase extracts (UV detection), confirming the degradation of polysaccharides by the enzyme. The results obtained suggest selenium binding to chitin-containing polysaccharide structures in fungal cell walls [91]. Selenite influenced the pellet morphology of Phanerochaete chrysosporium by reducing the size of the fungal pellets and inducing their compaction and smoothness [83]. Analysis of P. chrysosporium mycelia with transmission electron microscopy, electron energy loss spectroscopy, and a 3D reconstruction showed that elemental selenium was produced intracellularly as nanoparticles.

5. Se-containing antibacterial agents of fungal origin

Almost all of the published works dealt with the Se-fortified fungal cultures are concerned with selenium exclusively in the form of inorganic substances, sodium selenite Na$_2$SeO$_3$ or selenate Na$_2$SeO$_4$. The source of selenium should reasonably be the organic substance 1,5-diphenyl-3-selenopentanedianide-1,5 (synonyms - diacetonphenylselenide, bis(benzoylmethyl)selenide, preparation DAPS-25) [92, 93], since its low toxicity at physiological concentrations in combination with high efficiency (compared to, e.g., selenites) has been proved earlier for various living organisms. It is the source of selenium we use in our work.

The growth parameters and the dynamics of lectin activity of Lentinula edodes (shiitake mushroom) on liquid media enriched with selenium in the organic form have been studied. Diacetoxyphenylselenide exerts the appreciably positive effect upon the vital processes of L. edodes [94]. The role of spatial and electron structure, hydrophobic properties and concentration of organoselenium compounds on their interaction with fungal metabolites - extracellular lectins of shiitake mushroom has been considered. Along with DAPS-25, several other compounds of the 1,5-di(4-R-phenyl)-3-selenopentanedianides-1,5 series interaction with the basidiomycete L. edodes lectins were explored involving both computations and experiment [95].

Appreciable effect of the selenium-containing preparation DAPS-25 on the vital activity of L. edodes exhibited as the change in mushroom growth parameters and lectin activity on various organic and mineral, agar and liquid media has been expanded in our studies toward the elemental selenium accumulation at the definite DAPS-25 concentration in culture liquid. For the first time, the intensive red pigmentation of mycelium caused by the elemental selenium accumulation resulted from the organoselenium compound destruction by the mushroom L. edodes has been revealed.

The biotransformation of 1,5-diphenyl-3-selenopentanedianide-1,5 at the growth of shiitake mushroom under the liquid-phase and solid-phase culture conditions has been studied. At the initial DAPS-25 concentration equal to or higher than 1·10$^{-4}$ mol/l in the synthetic liquid medium, a red color of L. edodes mycelium develops, the intensity and initiation time of which being related to this Se-additive concentration. The semi-quantitative data obtained by physicochemical methods on comparative selenium level and acetophenone production in the samples allow us to conclude on the L. edodes submerged culture capability of sorbing and/or destructing a xenobiotic of organoselenium nature [96].

Starting from the results of qualitative reaction, the data of X-ray fluorescence, X-ray phase and GC-MS analyses, we should conclude on the L. edodes submerged culture capability of destructing the organoselenium xenobiotic to occur red modification of elemental selenium and to evolve acetophenone. The process of elemental selenium elimination was followed by its precipitation onto gyphae [97].

Within the framework of more recent studies, the growth parameters of more than twenty strains of xylotrophic basidiomycetes belonging to 8 genera, 13 species on liquid media enriched with selenium in organic form were studied.
The effect of 1,5-diphenyl-3-selenopentanediene-1,5 within a wide concentration range \((1\cdot10^{-4} - 1\cdot10^{-8}\ \text{mol/l})\) on the mycelial growth was observed. The phenomenon of mycelium growth stimulation was assumed to be related to the selenium antioxidant properties, due to which the negative consequences of exhausting the nutrient components of medium were smoothed over.

DAPS-25 concentrations exceeding \(5\cdot10^{-5}\ \text{mol/l}\) inhibited considerably the mycelium growth. At the initial DAPS-25 concentrations in the synthetic medium not lower than \(5\cdot10^{-5}\ \text{mol/l}\), we observed a red pigmentation of mycelium caused by the elemental Se in line with the results we obtained earlier for the shiitake mushroom [97]. The color intensity and time of appearance were dependent on the selenium additive's concentration, on the given fungal species, and its growth external conditions. The culture liquids of the fungal species under study were successfully tested for their reducing and stabilizing properties toward organic selenide and elemental selenium, respectively.

The results of studying the effect of biologically obtained selenium nanocomposites on the bacterium *Clavibacter michiganensis* ssp. *sepedonicus* \((\text{Cms})\) have been obtained [98]. *Cms*, a Gram-positive bacterium, causes ring rot, which is one of the most dangerous potato diseases. The effective alongside ecologically safe methods for combating *Cms* are lacking. As the agents feasible for use in this purpose, we examined the selenium biocomposites obtained from the submerged cultures of mushrooms. For exploring the biocomposites effect on *Cms*, several microbiological techniques were implemented. The results showed that all the biocomposites under study lowered the absorption values of bacterial suspensions, in comparison with the reference specimen, that testified to the bacteriostatic effect of the agents under question. The suppression action of biocomposites in respect to *Cms* was also revealed. Thus, we demonstrated the occurrence of bacteriostatic and bactericidal effects of the agents under study, and the results favor the supposition on advisability of further research into the selenium bionanocomposites as the agents for agricultural recovery from the bacterial pathogens [98].

Recently the impact of Se-containing biocomposites based on *Ganoderma* mushroom isolates grown in the presence of oxopropyl-4-hydroxycoumarins, on the bacterial phytopathogens has been examined [99]. The effect of selenium biocomposites obtained by using the submerged cultures of macrobasidiomycetes *Ganoderma* *applantium*, *G. cattienensis*, *G. colossus* *G. lucidum*, *G. neojaponicum*, *G. valesiacum*, on the phytopathogenic bacteria *Clavibacter michiganensis* ssp. *sepedonicus* \((\text{Cms})\), *Micrococcus luteus*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Pseudomonas fluorescens*, *Pseudomonas viridiflava*, *Xanthomonas campestris* has been studied. These bacterial strains used in our work were kindly provided by the Collection of Rhizosphere Microorganisms of IBPPM RAS (http://collection.ibppm.ru).

Oxopropyl-4-hydroxycromones have been used as the components of fungal nutrient media. By means of such methods as the colony-forming units count, the agar well diffusion method, and the bacterial suspension turbidity measurement, the bacteriostatic and bactericidal activity of the Se-containing substances of fungal origin in respect to phytopathogenic bacteria has been elucidated. The composites produced from the extracellular metabolites of *G. cattienensis* SIE1302 with 4-hydroxy-3-(3-oxo-1,3-diphenylpropyl)-chromen-2-one \((\text{S(45)})\), and of *G. lucidum* SIE1303 with 4-hydroxy-3-(3-oxo-1-(3-nitrophenyl)-3-phenylpropyl)-chromen-2-one \((\text{S(NO2)})\) possessed the most profound antibacterial action against *Cms*. The composites produced from the isolates of *G. valesiacum* 120702 with S(NO\(_2\)) showed the maximal antibacterial activity vs. *Xanthomonas campestris* B-610. High antimicrobial effect of *G. lucidum* 1315 with S(NO\(_2\)), and of *G. colossus* SIE1301 against *Xanthomonas campestris* B-610 and *Pseudomonas fluorescens* EL-2.1, respectively, has been revealed. The pioneering information on the biological activity of coimarin series compounds in their application for producing the substances of fungal origin has been provided [99].

**6. Conclusive remarks**

The novel aspects concerned with the essential nutrient and antioxidant Se properties discovered in the 2nd half of XX century and more recently, have changed the views on selenocompounds. Different chemical forms of selenium possess excellent biochemical properties and have been implicated for use in biotechnology.

The favorable profile of newly synthesized organoselenium compounds including those explored in our research warrants their recognition as a promising option for fortification purposes. Further thorough investigation should be focused on the mechanism of Se-containing compounds' toxicity to take that into account when using the various Se sources in biotechnological fields, including the production of ecologically safe antibacterial agents.

As for the future perspectives of research in this area the following should be noted. Mushrooms are recognized to be promising ecologically pure raw material for developing the medicinal preparations for care and prophylaxis with wide spectrum of action. Contemporary biomedical applications of selenium are undoubtedly broad. In this relation, of especial interest are the selenium-enriched preparations of higher-fungal origin owing to their availability, biocompatibility, and the proved biological activity.

The development of elemental selenium should be based on natural edible and medicinal products, e.g., mushroom isolates, and considered to be appropriate "green" method. As being originated from the biotransformed organoselenium compound, the selenium submicroparticles incorporated in or separated from the submerged mycelium possess the benefit of their non-toxic source and provide the potential multipurpose use [27, 100].
The approach developed in perspective research should allow the bioproduction of submicrostructured elemental selenium using the edible mushrooms cultures to be put into practice.

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