

## Conservation of biotechnological important species diversity and genetic resources of rare and endangered fungi of Ukraine

Nina A. Bisko<sup>1</sup>

Margarita L. Lomberg

Oksana B. Mykchaylova

Nadiya Y. Mytropolska

*M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 2 Tereshchenkivska Str., Kyiv 01004, Ukraine*

**Abstract:** The article is devoted to the IBK Mushroom Culture Collection as an essential basis of preservation of genofund of macrofungi in pure culture and fundamental research on biology and biotechnology of edible and medicinal mushrooms in Ukraine. Isolation methods of macromycetes pure cultures are described and nutrient agar media to preserve the vegetative mycelium in a viable state are listed. The verification methods using the DNA nucleotide sequences are described, cultural morphological features of the strains on agar nutrient media and microstructures of vegetative mycelium by optical and scanning electron microscope are characterized. Production of biologically active substances - melanins, polysaccharides, lipids, cytokinins, antimicrobial activity, and the ability of heavy metal sorption by numerous macromycetes was studied. A list of some important for biotechnology edible and medicinal mushroom species maintained in the IBK Collection is provided. A special attention paid in the IBK Collection to cultures of rare fungi species, particularly of those listed in the Red Data Book of Ukraine. Criteria of their identification and verification are briefly discussed. Microscopic features of vegetative mycelia and morphological characters of the colonies of 10 rare for Ukraine fungi species are given. The main parameters of nutrient media, plant biostimulants and cultivation conditions for certain types of macromycetes are characterized. The strains of the IBK Collection, potential producers of biologically active substances, were selected and their cultivation conditions, composition of nutrient media and methods for stimulating the synthesis of biologically active compounds have been established. High-yielding edible and medicinal mushrooms strains for fruit bodies' production on the elective plant substrates were demonstrated.

**Key Words:** *IBK mushroom culture collection, microstructures, cultivation, cytokinins, melanins, polysaccharides, lipids*

### INTRODUCTION

Mushrooms have considerable economic significance as objects of mushroom growing industry and a source of pharmacological substances with oncostatic, immunomodulating, radioprotective, antiviral and other properties, dietary supplements, enzymes, antibiotics, etc. Regarding conservation of mycobiota outside natural habitats of mushrooms, *ex situ* culture collections are crucial in maintaining the gene pool of macrofungi in pure culture. The IBK Mushroom Culture Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine is the largest official Culture Collection of macromycetes in Ukraine and one of the biggest in number of species and strains in Europe. It was founded in 1966 on the basis of the Department of Mycology, firstly for the purpose of investigations in submerged culture. For the moment the Collection contains 1250 strains belonging to 236 species of 104 genera of Basidiomycota and Ascomycota fungi, including rare and biotechnological important species with medicinal properties [Bisko et al., 2016a]. There are some unique dicaryotic strains of mushrooms from various taxonomic and environmental groups of a wide geographical origin, which are producers of biologically active substances, biomass and fruit bodies in the IBK collection. In 2001, the Cabinet of Ministers of Ukraine defined the IBK Mushroom Culture Collection as the National Heritage of Ukraine (Decree № 1709, adopted on 19 December, 2001). The IBK Mushroom Culture Collection is registered in the WDCM international database. For the development of new methods of cultivation, based on selected strains from the IBK Culture Collection, the staff of the Department of Mycology of the M.G. Kholodny Institute of Botany was twice awarded the State Prize of Ukraine in Science and Technique (1989, 2005) and the Award of the National Academy of Sciences of Ukraine, Academy of Sciences of Belarus and the Academy of Sciences of Moldova (2009).

### MATERIAL AND METHODS

*Isolation and identification.* The majority of cultures were isolated from the natural material in Ukraine, Russia, Belarus, Czech Republic, Israel and USA. Some cultures were obtained from other collections, institutions and mycologists. All mycological material before the isolation was identified by mycologists - experts in taxonomy of relevant groups of fungi. A verification of mushroom species and strains of the IBK Mushroom Culture Collection using molecular-genetic and cultural-morphological methods were done. For some strains a complete determination of the nucleotide sequences of the internal transcribed spacers (ITS1, ITS2), 5.8S gene of rDNA, as well as the partial determination of 18S and 28S sequences surrounding the ITS, was performed [Lomberg et al. 2003; Mykchaylova et al. 2017b]. On the basis of received sequences the investigated strains added into NCBI GenBank. To confirm the taxonomic affiliation of the strains we studied their cultural and morphological characteristics. Microstructures of vegetative mycelium by optical and scanning electron microscopy were investigated [Buchalo 1988; Buchalo et al. 2009]. The production of biologically active compounds – polysaccharides, melanins, lipids, cytokinins, investigation of antimicrobial activity and the sorption of heavy metal by some species of macromycetes were studied by standard methods [Al-Maali et al. 2014; Bisko et al. 2012; Dyakov et al. 2011; Poyedinok et al. 2008; Vedenicheva et al. 2016].

*Preservation methods.* For isolation and preservation of cultures the wort, malt, compost, potato-dextrose and other agar media, including added herbal extracts, are used [Buchalo 1988; Buchalo et al. 2011]. Isolation of macromycetes is performed in the field during expedition trips and in laboratory. The storage of pure cultures of Ascomycetes and Basidiomycetes are determined by peculiarities of morphology, ecological and biological properties of the fungi developing in culture mainly as asporous vegetative mycelium. Isolation of pure cultures from fruit bodies tissue or basidio - and ascospores are made using conventional and modified methods [Buchalo et al. 2011]. Each culture was deposited to the Collection under certain number and information is kept in data base of the IBK Collection. Cultures are preserved in refrigerators at  $4\pm 1^{\circ}\text{C}$ . The Catalogue of the IBK Collection are revised and published every 5 years.

## RESULTS AND DISCUSSION

The major purpose of the IBK Collection is to preserve the gene pool of edible and medicinal macrofungi in

pure culture. Unlike other macromycetes collections, in the IBK Collection contains a large number of strains commonly cultivated worldwide by producers of edible mushrooms as well as dietary and pharmacological substances. They belong to the genera *Pleurotus* (Fr.) P. Kumm., *Agaricus* L., *Lentinus* Fr., *Oudemansiella* Speg., *Flammulina* P. Karst., *Hericium* Pers., *Piptoporus* Karst., *Omphalotus* Fayod, *Schizophyllum* Fr., *Ganoderma* P. Karst., *Trametes* Fr., *Laetiporus* Murrill, *Lycoperdon* Pers., *Coprinus* Pers., *Macrolepiota* Singer, etc. and are represented in the collection by a wide diversity of strains. A large number of strains of edible fungi are sustained in the IBK collection, such as *Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes*, *Flammulina velutipes*, others, as well as provide the essential basis for biotechnological potential [Babitskaya et al., 2002, 2003, 2007; Beregovaya et al., 2012; Bisko et al., 2012, 2018a, b; Buchalo et al., 2011; Buchalo, Mitropolskaya, 2002; Dyakov et al., 2011; Ivanova et al., 2014; Krupodorova et al., 2012; Lomberg et al., 2000; Lomberh et al., 2002; Moldavan et al., 2000; Nizhenkovska et al., 2015; Truchonovech et al., 2012; Vasser et al., 2016]. These species are widely cultivated throughout the world. Most of them, except for *L. edodes*, grow in nature in Ukraine. Thus, Culture Collection contains numerous strains of mushrooms from various taxonomic and environmental groups of a broad geographical origin, are widely used in fundamental and applied science.

The strains of macromycetes used in different biotechnologies (edible, producers of biologically active substances, including pharmacological ones) are preserved and the most important fungi species and strains quantity presented in the Table 1 [Bisko et al., 2016a, 2018a, b]. The research data on morphological and cultural characteristics of the strains contribute to reliable continuous preservation of the strains under cultural conditions for the purposes of *ex-situ* conservation, research, and sustainable use of genetic resources.

Mushroom Culture Collection is an important resource for development of mushroom growing in Ukraine and biotechnologies of dietary treatment and prevention supply, food supplements, pharmaceutical and biologically active substances. Cultures of 123 species with known pharmacological properties used in international folk and traditional medicine are represented in the Collection. Besides cultivated species described above, a special attention is also paid to cultures of rare species of fungi, particularly of those listed in the Red Data Book of Ukraine [Bisko et al., 2016b, 2018a, b;

**Table 1.** Important edible and nonedible mushrooms with medicinal properties preserved at the IBK Mushroom Culture Collection (Ukraine).

Species	Common name	Number of strains
* <i>Agaricus bisporus</i> (J.E.Lange) Imbach	Button Mushroom	53
* <i>A. bitorquus</i> (Quél.) Sacc.	Summer Mushroom	7
* <i>A. blazei</i> Murrill	Almond Mushroom	2
* <i>Auricularia auricula-judae</i> (Bull.) Quel.	Juda's Ear, Black ear	7
* <i>Coprinus comatus</i> (O.F.Müll.) Pers.	Shaggy Mane	13
* <i>Cyclocybe aegerita</i> (V.Brig.) Vizzini	Black Poplar, Pioppino Mushroom	15
* <i>Fistulina hepatica</i> (Schaeff.) With.	Beefsteak fungus, Beefsteak Polypore	10
* <i>Flammulina velutipes</i> (Curtis) Singer	Velvet Foot Collybia, Enoki	42
<i>Fomes fomentarius</i> (L.) Fr.	Tinder Conk Mushroom	12
<i>Fomitopsis betulina</i> (Bull.) B.K.Cui, M.L.Han & Y.C.Dai	Birch Bracket Mushroom	26
<i>Fomitopsis officinalis</i> (Vill.) Bondartsev & Singer	Agaricon	3
<i>Ganoderma applanatum</i> (Pers.) Pat.	Artist's Fungus	21
<i>Ganoderma lucidum</i> (Curtis) P.Karst.	Reishi	39
* <i>Grifola frondosa</i> (Dicks.) Gray	Maitake	31
* <i>Hericium coralloides</i> (Scop.) Pers.	Comb Tooth, Coral Fungus	4
* <i>Hericium erinaceus</i> (Bull.) Pers.	Monkey Head, Lion's Mane	20
* <i>Hypsizygus marmoreus</i> (Peck) H.E.Bigelow	Bunashimeji	14
<i>Inonotus obliquus</i> (Fr.) Pilát	Chaga Mushroom	7
<i>Irpex lacteus</i> (Fr.) Fr.	Milk-white Toothed Polypore	27
* <i>Laetiporus sulphureus</i> (Bull.) Murrill	Sulphur Polypore, Chicken-of-the-woods	37
* <i>Lentinula edodes</i> (Berk.) Pegler	Shiitake	68
* <i>Lepista nuda</i> (Bull.) Cooke	Blewitt	9
* <i>Morchella esculenta</i> (L.) Pers.	Yellow Morel, Common Morel	13
* <i>Phallus impudicus</i> L.	Stinkhorn Mushroom	12
* <i>Pholiota mutabilis</i> (Schaeff.) P.Kumm.	Changeable Agaric	7
* <i>Pholiota nameko</i> (T.Itô) S.Ito & S.Imai	Nameko	4
* <i>Pleurotus citrinopileatus</i> Singer	Golden Oyster, Yellow Oyster	2
* <i>Pleurotus djamor</i> (Rumph. ex Fr.) Boedijn	Pink Oyster	3
* <i>Pleurotus eryngii</i> (DC.) Quél.	King Oyster	34
* <i>Pleurotus nebrodensis</i> (Inzenga) Quél.	White Elf	4
* <i>Pleurotus ostreatus</i> (Jacq.) P. Kumm.	Winter Oyster, Pearl Oyster	173
* <i>Pleurotus pulmonarius</i> (Fr.) Quél.	Indian Oyster, Lung Oyster	17
* <i>Sparassis crispa</i> (Wulfen) Fr.	Cauliflower Mushroom	11
* <i>Stropharia rugosoannulata</i> Farl. ex Murrill	Wine-red Stropharia, Garden Giant	7
<i>Trametes versicolor</i> (L.) Lloyd	Turkey Tail	18
<i>Schizophyllum commune</i> Fr.	Split Gill Fungus	24

Note: \* - edible mushroom.

Mykchaylova et al., 2017a, b; Red Data Book, 2009]. There are 11 mushroom species: *Agaricus bresadolanus*, *Clathrus archeri*, *Fomitopsis officinalis*, *Grifola frondosa*, *Gyromitra slonevskii*, *Hericium coralloides*, *Leucoagaricus barssii* (Zeller) Vellinga, *Morchella steppicola*, *M. crassipes*, *Polyporus umbellatus*, and *Sparassis crispa* in the IBK Collection. For species identification of basidiomycetes in vegetative stage of their development, specific criteria for identification and verification of macromycetes of certain taxonomic and

ecological groups in culture are required. The creation of macromycetes identification system in the vegetative stage requires the accumulation of a large amount of experimental data. The search for the identification and verification criteria of individual taxonomic and ecological groups of macromycetes in a pure culture needs the study of the cultural and morphological properties using optical and scanning electron microscopy. Peculiarities of mycelium growth and formation of fruiting bodies in culture are investigated within special program developed by the Collection staff. This program includes research on cultural-morphological characteristics using

scanning electron microscopy, cultivation conditions, physiological and biochemical characteristics of strains that can be used to determine species identity of the culture. It has been established that during cultivation period on reference agar nutrient medium, taxonomically significant characters at species level are the following: ability to form teleomorph stage; presence and type of asexual sporulation; presence, shape and location on the mycelium of clamp connections, chlamydo-spores; formation of crystals inlays and abnormal structures on hyphae; type of filamentous colony and its radial growth rate; nature of the colored test reactions in presence of certain enzymes, etc. As noted above the most reliable criteria for identification of fungi in the culture is their fruit bodies' formation. But, unfortunately, not all fungi form the teleomorph stage in culture and may be easily determined. The results of our observations on the species from the Red Data Book of Ukraine are presented in Table 2. The basic morphological characteristics of some strains are given.

Among considered species, only *H. coralloides* strains were fast enough to form fruit bodies on different agar media, also with some plant additives such as oak sawdust, pine, coriander broth, etc. [Bisko et al., 2016b] (Fig. 1). Other investigated species did not form a generative stage in culture, except *S. crispa* and *F. officinalis* cultures, which started fruiting after a long cultivation term (45–60 days) but only on the selected media [Mykchaylova et al., 2017a, b].

For mushroom species from the Red Data Book of Ukraine, verification is carried out using molecular genetics research methods. On the basis of the conducted molecular analysis of the ITS sequence, the investigated

species of mushrooms were introduced into the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>). Thus, we have started the creation of the first in the state of the genetic bank of vulnerable species of macromycetes included in the Red Data Book of Ukraine. For each species, based on the study of their biology, individual cultivating parameters, optimal conditions of conservation in culture are selected. This work is currently conducting.

Research of growth characteristics and the search for optimal substrates is an important factor in ensuring the vitality of the culture of mushrooms. The main studied medium parameters were: sources of nitrogen and carbon, the ratio of nitrogen to carbon, pH of nutrient media, critical and optimal temperatures and concentration of biostimulants. These parameters have been experimentally selected for many mushrooms cultures from our collection, including rare and biotechnological valuable, such as: mushrooms of genus *Agaricus*, *Fomitopsis*, *Ganoderma*, *Hericium*, *Morchella*, *Pleurotus*, *Sparassis*, *Trametes*, *Verpa* and many others. Most of tested strains from our collection of mushrooms preferred glucose as a source of carbon, but there were exceptions. Thus, some strains of *Ganoderma lucidum*, *G. applanatum*, *Morchella crassipes* grew best on medium with lactose or sucrose. Peptone was the most preferred source of nitrogen for most part of tested mushrooms culture, but some strains of *Morchella conica*, *M. esculenta* and other preferred ammonium phosphate as a source of nitrogen.

The ability to synthesize the polysaccharides by some biotechnologically perspective mushrooms strains from IBK collection was studied. It has been shown that



**Figure 1.** *Hericium coralloides* IBK 2332: 1. fruit bodies formation after 20–30 days cultivation on tube, 2. Petri dish, 3. Erlenmeyer flask.

**Table 2.** Macromycetes species from the Red Book of Ukraine, stored in the IBK Collection with mycelial characteristics of some strains.

Species	IBK number	Morphological features
<i>Agaricus bresadolanus</i> Bohus	104	Mycelial colony white, dense, downy, branching mycelial cords are usual, golden drops of exudates are present; reverse not changed. Crystals and anastomoses are present on hyphae.
<i>Clathrus archeri</i> (Berk.) Dring	2405	Mycelial colony slow growing, not dense, cottony-woolly with silky concentric zones, whitish to brownish; reverse colorless. Clamp connections and anastomoses are present on hyphae. Thin hyphae with irregular ampulatory swelling near the septa.
<i>Fomitopsis officinalis</i> (Vill.) Bondartsev & Singer	5004	Mycelial colony slow growing, the morphology depended on the composition of nutrient medium, were mainly two types: dense, opaque, cottony, less velvet, white with a lot of short air hyphae, reverse not changed; or white but not dense, woolly or floury. Thin-walled, moderately branched, regularly sewed, unpainted generative hyphae, diameter 1.2-3.5 µm. Regular unilateral two types clamp connections: without a slit and seldom medallion-type, some anastomoses and mycelial cords as well as blastoconidia after prolonged cultivation (more than 30 days). The incrustation of hyphae in the form of thin villi.
<i>Grifola frondosa</i> (Dicks.) Gray	332	Mycelial colony white, later light tawny-brown tones longitudinally linear, eventually thickly, cottony, non-rhizomorphic. The mycelium grows out unevenly. At maturity, the dense mycelial mat can be peeled directly off the agar media. Mycelia have developed strong yellowish to orangish-brown mottled zones, with drops of yellowish metabolite. In the vegetative mycelium numerous medallion clamp connections are present, anastomoses between hyphae and clamps occurred. In the younger part of mycelial colony branched thin (< 1 µm width) hyphae (dichohyphidia) are formed. Apical and intercellular chlamydospores form on hyphae, which usually have no clamp connections.
<i>Gyromitra slonevskii</i> V.P. Heluta	1932	Mycelial colony slow growing, not dense, cottony-woolly, becoming grayish or light brown soon; reverse is brown; lacunose, anomalous hyphae, and anastomoses are present.
<i>Hericium coralloides</i> (Scop.) Pers.	2332	Mycelial colony is felt or cottony, dense, white; reverse is yellow with age, easily formed the stage of a teleomorph. Numerous medallion-type clamp connections are present. Intercellar and terminal chlamydospores and anastomoses between hyphae are usual. Abundant crystals of different shape, mycelial rings and conidial sporulations are present.
<i>Morchella crassipes</i> (Vent.) Pers.	2209	Mycelial colony is woolly, more or less dense, white, becoming grayish or light brown soon; reverse is brown. Microsclerotia are yellow-brown, more abundant on oatmeal malt yeast enriched agar media. Lacunose hyphae are present in the zone of sclerotia formation.
<i>Morchella steppicola</i> Zerova	2215	Mycelial colony is woolly, dense, cream or light brown; reverse is brown. Microsclerotia solitary on agar media are usual, more abundant on potato dextrose agar media. Lacunose, anomalous hyphae, and anastomoses are present. Conidial sporulation of <i>Constantinella terrestris</i> type occurs.
<i>Polyporus umbellatus</i> (Pers.) Fr.	2571	Mycelial colony is white, shiny, eventually not dense, cottony or silky depending from nutrient media. The mycelium grows out unevenly. Mycelia developed zonal circles due to different density, yellowish drops of exudates are present; reverse becoming yellow with age. In the vegetative mycelium the clamp connections are present; at low temperatures observed the sclerotia.
<i>Sparassis crispa</i> (Wulfen) Fr.	304	Mycelial colony slow growing, the morphology depended on the media composition, two types: dense, white, reverse not changed; and white, not dense, sparse, transparent, with less short air hyphae, reverse colorless. The hyphae with regular one-sided gapless clamp connections, numerous secretory cells on the surface, anastomoses, filamentous strands and films.



good producers of polysaccharides are some strains of *Ganoderma lucidum*, *Lentinula edodes*, *Lentinus tigrinus*, *Crinipellis schevczenkovi*, *Neolentinus lepideus*. Together with colleagues from the Institute of microbiology of National Academy of Sciences of Belarus we studied various factors affecting the yield of polysaccharides in these strains. Among the factors studied were aeration, temperature, pH of medium, sources of nitrogen and carbon, their concentration and ratio. The obtained results allowed developing recommendations on submerged cultivation of the most perspective strains *G. lucidum*, *C. schevczenkovi*, *L. edodes* - producers of polysaccharides from IBK collection [Bisko et al., 2012, Babitskaya et al., 2003, 2007]. Also, we investigated the effects of various biostimulants on the synthesis of polysaccharides. It was proved that zinc citrate, obtained by the method of aquanotechnology, stimulated the synthesis of exopolysaccharides by *Trametes versicolor*. In addition, we studied the sorption of heavy metal preparations, numerous factors that influence on the lipogenesis, selected strains - highly effective producers of melanins, studied of antimicrobial activity of mushrooms [Al-Maali, 2018; Al-Maali et al., 2014a, b, 2016a, b]. A new concept of using low-intensity artificial light in biotechnology of cultivation of edible and medicinal mushrooms has been developed. It has been found for the first time that short-term low-intensity irradiation in the visible part of the spectrum has stimulated growth and biosynthetic activity of macromycetes. The study involved pure strains of *A. bisporus*, *F. velutipes*, *H. erinaceus*, *L. edodes*, *P. ostreatus*, *G. lucidum* [Poyedinok et al., 2008, 2015a, b, c, 2018].

Last years our research was conducted on the search of phytohormones in mushrooms. We screened the mushrooms cultures from IBK collection for the presence of cytokinins in the biomass. First it was found that mycelium of 13 species of mushrooms (*M. esculenta*, *F. officinalis*, *S. crispa*, *G. frondosa*, *P. nebrodensis*, *L. edodes*, *H. coralloides*, *H. erinaceus*, *T. versicolor*, *C. aegerita*, *G. lucidum*, *P. ostreatus*, *F. velutipes*) contains different concentrations of cytokinins, which are polyfunctional phytohormones and involved in growth and development regulation [Vedenicheva et al., 2016, 2018a, b].

Also in recent years, a work on expanding of cultivated edible and medicinal mushroom range new for Ukraine was continued. We have successfully collaborated with a number of institutions, industrial

enterprises and mushroom farms. Our partners were “Esmash” (Kyiv), “Zhovtneve” (Dnipro), “Mushroom Doctor” (Melitopil). The effective technologies for *P. eryngii*, *H. erinaceus*, *G. lucidum*, *C. aegerita*, *F. velutipes* fruit bodies production were developed (Fig. 2).



**Figure 2.** Development of new edible and medicinal mushroom species cultivation under industrial conditions of Ukraine: 1. *Cyclocybe aegerita* IBK 960, 2. *Flammulina velutipes* IBK 1860, 3. *Hericium erinaceus* IBK 991, 4. *Hericium coralloides* IBK 2332, 5. *Ganoderma lucidum* IBK 1889, 6. *Pleurotus eryngii* IBK 1991.

Many substrate combinations and environment conditions were tested. The selective substrates and media were found, as well as productive promising mushroom strains. Biotechnology of some others perspective mushroom species is under construction.

The fulfilled studies on cultural properties of the species, including growth of vegetative mycelium and development of fruit bodies, are required to important biotechnology introductions as well as to enable successful reintroduction of rare or declining species of fungi into natural habitats. Thus, the Collection staff has developed methods of passages, choosing

selective nutrient media for some species with higher requirements for nutrients and microscopic visual system control cultures in absence of extraneous microflora which allows maintaining the viability of cultures in the Collection, including enzymatic and physiological activity, over decades.

The IBK Mushroom Culture Collection provides consulting on methodological issues and identification of cultures, exchanges of culture collections around the world. The IBK Collection is a major scientific and information basis for fundamental and applied research, training of master, bachelor and PhD theses in biology and biotechnology of macromycetes in research institutions and universities of Ukraine, Belarus, Russia, Moldova, Latvia and other countries. For the last 50 years, the species and strains of IBK Collection were used as the research objects for 25 PhD and 8 Dr. Sci. theses, as well as the basis for publication of 12 monographs, numerous articles in national and international journals. Due to the importance of preserving the biodiversity of fungi, the further work of the collection is seen in the expansion of the taxonomic and ecological diversity of macromycetes with an emphasis on rare and threatened species, as well as species with useful resource properties - edible, medicinal, and producers of biologically active substances.

## REFERENCES

- Al-Maali G.A. (2018) The influence on different compounds of trace elements on the biomass and synthesis of exopolysaccharides of mycelium *Trametes versicolor* (Polyporaceae, Polyporales). *Biosystems Diversity*, 25(4): 289-296.
- Al-Maali G.A., Bisko N., Mustafin K. et al. (2014a) The influence of the metals citrates, obtained using aquanano technologies, on the biomass production of medicinal mushroom *Ganoderma lucidum* (Curtis) P.Karst. *Int. J Engineering Research and Applications*, 4(9): 1-4.
- Al-Maali G.A., Bisko N., Mustafin K. et al. (2014b) The influence of the manganese citrates, obtained using aquanano technologies, on the biomass production of medicinal mushroom *Trametes versicolor* (L.) Lloyd. *Int J Engineering Research and Applications*, 4(9): 22-25.
- Al-Maali G.A., Bisko N., Ostapchuk A.M. (2016a) The effect of zinc citrate and zinc sulfate on the growth and biomass composition of medicinal mushroom *Ganoderma lucidum*. *Mikologiya i fitopatologiya*, 50(5): 313-317.
- Al-Maali G.A., Bisko N., Ostapchuk A.M. (2016b) The effect of citrate and sulfate of different metals on the biomass composition of medicinal mushroom *Trametes versicolor* (L.) Lloyd. *Chornomors'k. Bot. Zh.* 12(1): 64-71.
- Babitskaya V.G., Scherba V.V., Ikonnikova N.V. et al. (2002) Melanin complex from *Inonotus obliquus* (Pers.:Fr.) Pilat. (Aphyllphoromycetidae). *Intern J Med Mushr*; 4(2): 139-146.
- Babitskaya V.G., Bisko N.A., Scherba V.V., Mitropolskaya N.Yu. (2003) Some biologically active substances from medicinal mushroom *Ganoderma lucidum* (Curt.: Fr.) P.Karst. (Aphyllphoromycetidae). *Intern J Med Mushr*; 5(3): 301-305.
- Babitskaya V.G., Bisko N.A., Scherba V.V., Mitropolskaya N.Yu. (2007) Study of melanin complex from medicinal mushroom *Phellinus robustus* (P. Karst.) Bourd. et Galz. (Aphyllphoromycetidae). *Int J Med Mushr*; 9(3-4): 177-184.
- Bereqovaya T.V., Bilay T.B., Wasser S.P. et al. (2012) Makromiseti: lekarstvenniye svoystva i biologicheskiye osobennosti. Kiev: Veles, 285 pp. (In Russian)
- Bisko N.A., Buchalo A.S., Wasser S.P. (1983) Visshiy syedobniye basidiomiseti poverkhnostnoy i qlubinnoy culture. Eds I.A. Dudki, Kiev: Nauk dumka, 312 pp. (In Russian)
- Bisko N.A., Babitskaya V.L., Buchalo A.S. et al. (2012) Biologicheskiye svoystva lekarstvennikh makromisetov v kulture. Sbornik nauchnikh trudov, 2, Kiev: Alterpress, 459 pp. (In Russian)
- Bisko N.A., Lomberg M.L., Mytropolska N.Yu., Mykchaylova O.B. (2016a) The IBK Mushroom Culture Collection. Kyiv: M.G. Kholodny Institute of Botany, Alterpres, 120 pp.
- Bisko N.A., Lomberg M.L., Mykchaylova O.B. et al. (2016b). Ex situ conservation of rare species of macromycetes in the IBK culture collection of mushrooms. Rare plants and fungi of Ukraine and adjacent areas: implementing conservation strategies. Kyiv: Palyvoda (in Ukrainian).
- Bisko N.A., Mytropolska N.Y., Mykchaylova O.B. et al. (2018a) The rare and biotechnologically important mushroom species in the IBK collection. Development of natural sciences in countries of the European Union taking into account the challenges of XXI century collective monograph, Lublin,

- Ukr Bot J*, 75(4): 338-347.
- Buchalo A.S. (1988) Visshiy syedobniye basidiomiseti v chistoy kulture. Kiev: Nauk dumka, 144 pp.
- Buchalo A.S., Babitskaya V.L., Bisko N.A. et al. (2011) Biologicheskiye svoystva lekarstvennikh makromisetov v kulture. Sbornik nauchnih trudov, 1, Kiev: Alterpres, 212 pp. (In Russian)
- Buchalo A.S., Mitropolskaya N.Yu. (2002) Studies on medicinal mushrooms at the National Ukrainian Culture Collection. *Intern J Med Mushr*; 4(3): 245-254.
- Buchalo A.S., Mykchaylova O.B., Lomberg M., Wasser S.P. (2009) Microstructures of vegetative mycelium of macromycetes in pure cultures. Kiev: Alterpress, 224 pp.
- Buchalo A.S., Wasser S., Mykchaylova O.B., Lomberg M. (2011) Taxonomical significance of microstructures in pure cultures of macromycetes. Proc. 7th Inter. Conf. on Mushroom Biology and Mushroom Products (ICMBMP7): 50-57.
- Dyakov M.Yu., Kamzolnikina O.V., Shter O.V. et al. (2011) Morphological characteristics of natural strains of certain species of basidiomycetes and biological analysis of antimicrobial activity under submerged cultural conditions. *Microbiology*, 80(2): 274-285.
- Ivanova T.S., Bisko N.A., Krupodorova T.A., Barshteyn V.Yu. (2014) Breadcrumb as a new substrate for *Trametes versicolor* and *Schizophyllum commune* submerged cultivation. *Korean J Microbiol Biotechnol*, 42(1): 67-72. <http://dx.doi.org/10.4014/kjmb.1309.09004>
- Krupodorova T.A., Barshteyn V.Yu., Bisko N.A., Ivanova T.S. (2012) Some macronutrient content in mycelia and culture broth of medicinal mushrooms cultivated on amaranth flour. *Intern J Med Mushr*, 14(3): 285-293.
- Lomberg M., Buchalo A., Solomko E. et al. (2000) Investigation of mycelium growth and fruit body development of different strains of the beech mushroom Shimeji (*Hypsizygus marmoreus* (Bull.:Fr.) Singer). Science and cultivation of edible fungi. Proceed. of the 15th Int. Congr. of the Science and Cultivation of Edible Fungi, Maastricht, Netherlands, 15/19.05, Balkena, 2: 763-770.
- Lomberg M., Solomko E.F., Buchalo A.S., Kirshhoff B. (2002) Studies of medicinal mushrooms in submerged culture. 4th Int. Confer. "Mushroom Biology and Mushroom Products", Cuernavaca, Mexico, 367-378.
- Lomberg M., Renker C., Buchalo A.S. et al. (2003) Micromorphological and Molecular Biological Study of Culinary-Medicinal Mushroom *Hypsizygus marmoreus* (Peck) Bigel. (Agaricomycetidae). *Intern J Med Mushr*; 5(3): 307-312.
- Moldavan M.G., Solomko E.F., Grodzinskaya A.A. et al. (2000) Neurotropic effect of extracts from the hallucinogenic mushroom *Psilocybe cubensis* (Earle) Sing. (Agaricomycetidae) in vitro studies. *Int J Med Mushr*, 2: 329-338.
- Mykchaylova O.B., Bisko N.A., Sukhomlyn M.M. et al. (2017a) Biological peculiarities of a rare medicinal mushroom *Fomitopsis officinalis* (Fomitopsidaceae, Polyporales) on agar media and plant substrates. *Regul Mech Biosyst*, 8(4): 469-475. (in Ukrainian)
- Mykchaylova O.B., Gryganskyi A.P., Lomberg M.L., Bisko N.A. (2017b) The study of morphological and cultural properties of *Sparassis crispa* (Sparassidaceae, Polyporales). *Ukr J Ecology*, 7(4): 550-558, doi: 10.15421/2017\_159. (in Ukrainian)
- Nizhenkovska I.V., Pidchenko V.T., Bychkova N.G. et al. (2015) Influence of *Ganoderma lucidum* (Curt.: Fr.) P. Karst. on T-cell mediated immunity in normal and immunosuppressed mice line CBA/Ca. *Ces Slov Farm*, 64: 139-143.
- Poyedinok N.L., Buchalo A.S., Sherba V.V. et al. (2008) Light regulation of growth and biosynthetic activity of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetidae) in pure culture. *Int J Med Mushr*, 10(4): 368-378.
- Poyedinok N.L., Mykchaylova O., Tugay T. et al. (2015a) Effect of light wavelengths and coherence on growth, enzymes activity and melanin production of liquid cultured *Inonotus obliquus* (Ach.: Pers) Pilat. *Appl Biochem Biotechnol*, 176(2): 333-343.
- Poyedinok N.L., Mykchaylova O., Negriyko A.M. et al. (2015b) Induction of antimicrobial activity of some macromycetes by low intensity light. *Biotechnologia Acta*, 8(1): 63-70.
- Poyedinok N.L., Mykchaylova O., Negriyko A.M. (2015c) Effects of light wavelengths and coherence on basidiospores germination. *J Microbiol Biotech Food Sci*, 4(4): 352-357.
- Poyedinok N.L., Mykchaylova O., Sergiichuk N., Negriyko A. (2018) Realization of macromycete photoinduced growth activity: influence of cultivation ways and the concentration of carbon and nitrogen. *Inov Biosyst Bioeng*, 2(3): 196-202.
- Red Data Book of Ukraine. Plant Kingdom (2009) Ed.



- Ya.P. Didukh. Kyiv: Hlobalkonsal'tynh, 912 pp. (in Ukrainian)
- Trukhonovech V.V., Bisko N.A., Poyedinok N.L. et al. (2012) Rost i plodonosheniye bazidialnogo griba *Hericum erinaceus* (Bull.: Fr.) na rastitelnykh substratakh. Trudi BQTU. *Lesnoye khozyaystvo*, pp. 113-127. (In Russian)
- Vedenicheva N.P., Al-Maali G.A., Mytropolska N.Y. et al. (2016) Endogenous cytokinins in medicinal Basidiomycetes mycelial biomass. *Biotechnologia Acta*, 9(1): 55-63.
- Vedenicheva N.P., Al-Maali G.A., Mykchaylova O.B. et al. (2018a) Endogenous cytokinins dynamics in mycelial biomass of Basidiomycetes at different stages of cultivation. *Int J Biochem Physiol*, 3(2): 100-122.
- Vedenicheva N.P., Al-Maali G.A., Bisko N.A. et al. (2018b) Comparative analysis of cytokinins in mycelial biomass of medicinal mushrooms. *Intern J Med Mush*, 20(9): 837-847.
- Wasser S.P., Boyko S.M., Vonq K.KH. et al. (2016) Makromiseti: lekarstvenniye svoystva i biologicheskiye osobennosti. 2, Kiev: Nash format, 261 pp. (In Russian)
- World Data Centre for Microorganisms (WDCM) international database ([http://www.wfcc.info/ccinfo/index.php/collection/by\\_id/1152](http://www.wfcc.info/ccinfo/index.php/collection/by_id/1152)).

**Ukraynanın nadir və təhlükə altında olan göbələklərinin biotexnoloji əhəmiyyətli növ müxtəlifliyi və genetik resurslarının saxlanması**

**Nina A. Bisko**

**Marqarita L. Lomberg**

**Oksana B. Mykchaylova**

**Nadiya Y. Mytropolska**

*M.Q. Xolodnov adına Botanika İnstitutu, Ukrayna Milli Elmlər Akademiyası, 2 Tereşçenkivsska küçəsi, Kiyev 01004, Ukrayna*

Məqalə papaqlı göbələklərin genofondunun təmiz kulturada saxlanması mühüm əsası kimi IBK Makromiset Kultura Kolleksiyasına və Ukraynada yeməli və tibbi əhəmiyyətli göbələklərin biologiyası və biotexnologiyası üzrə fundamental tədqiqatlara həsr edilib. Makromisetlərin təmiz kulturalarının ayrılması üsulları təsvir edilir və vegetativ miseliləri canlı şəraitdə saxlamaq üçün aqarlı qida mühitlər sadalanır. DNT nukleotid ardıcılıqlarına əsasən yoxlama üsulları təsvir olunur, aqarlı qidalı mühitlərdə ştammların kultural morfoloji xüsusiyyətləri, optik

və skan elektron mikroskopu vasitəsilə vegetativ miselilərin mikrostrukturları səciyyələndirilir. Çoxsaylı makromisetlər tərəfindən bioloji aktiv maddələrin - melaninlər, polisaxaridlər, lipidlər, sitokininlərin istehsalı, antimikrob aktivliyi, ağır metal udulması qabiliyyəti öyrənilmişdir. IBK Kolleksiyasında saxlanılan biotexnologiyada istifadə üçün vacib olan bəzi yeməli və tibbi əhəmiyyətli göbələk növlərinin siyahısı təqdim edilir. IBK Kolleksiyasında saxlanılan nadir göbələk növlərinin kulturalarına, xüsusilə bunlardan Ukraynanın Qırmızı Kitabına daxil olanlara diqqət yetirilir. Onların təyin edilməsi və yoxlanması meyarları qısa şəkildə müzakirə olunur. Ukrayna üçün 10 nadir makromiset növünün vegetativ miselisinin mikroskopik əlamətləri və koloniyalarının morfoloji xüsusiyyətləri verilir. Qida mühitin əsas parametrləri, bitki biostimulyatoru və makromisetlərin müəyyən növləri üçün becərmə şəraiti xarakterizə edilir. IBK kolleksiyasında saxlanılan ştammlar, bioloji aktiv maddələrin potensial istehsalçıları seçilib və onların becərməsi şəraiti, qida mühitin tərkibi və bioloji aktiv maddələrin sintezinin stimullaşdırılması üsulları müəyyən olunub. Yüksək məhsuldarlığa malik olan yeməli və tibbi əhəmiyyətli göbələk ştammlarının meyvə cisminin əmələ gəlməsi üçün elektiv bitki substratları nümayiş edilib.

**Açar sözlər:** IBK göbələk kultura kolleksiyası, mikrostruktur, becərmə, sitokininlər, melaninlər, polisaxaridlər, lipidlər

**Сохранение биотехнологически важного видового разнообразия и генетических ресурсов редких и исчезающих видов грибов Украины**

**Нина А. Бисько**

**Маргарита Л. Ломберг**

**Оксана Б. Михайлова**

**Надия Ю. Митропольская**

*Институт Ботаники имени Н.Г. Холодного НАН Украины, ул. Терещенковская 2, Киев 01004, Украина*

В статье приведены данные о Коллекции культур шляпочных грибов Института Ботаники имени Н.Г.Холодного НАН Украины (IBK) - основного источника сохранения генофонда макромицетов в чистой культуре для фундаментального изучения биологии и биотехнологии съедобных и лекарственных грибов в Украине. Описаны методы выделения чистых культур макромицетов, перечислены питательные среды для сохранения вегетативного мицелия

в жизнеспособном состоянии. Охарактеризованы методы верификации путем определения нуклеотидных последовательностей штаммов, изучения их культурально-морфологических особенностей на агаризованных питательных средах и микроструктур мицелия в оптическом и сканирующем микроскопе. Приведено исследование биологически активных веществ у многочисленных макромицетов – полисахаридов, меланинов, липидов, цитокининов, изучения антимикробной активности и способности сорбировать тяжелые металлы. Приводится перечень наиболее важных для использования в биотехнологии видов съедобных и лекарственных грибов, которые сохраняются в Коллекции культур шляпочных грибов. Особое внимание уделено сохранению в Коллекции редких для Украины видов грибов, внесенных в Красную Книгу, их идентификации и верификации. Даны микроскопические

характеристики вегетативного мицелия и морфологические особенности колоний 10 редких для Украины видов макромицетов. Охарактеризованы основные параметры питательных сред, растительных биостимуляторов и условий культивирования для отдельных видов макромицетов. Подобраны штаммы коллекции – перспективные продуценты биологически активных веществ. Установлены условия их культивирования, составы питательных сред и способы стимуляции синтеза биологически активных соединений. Селектированы высокоурожайные штаммы съедобных и лекарственных грибов для выращивания плодовых тел на элективных растительных субстратах.

**Ключевые слова:** Коллекция культур шляпочных грибов IBK , микроструктуры, культивирование, цитокинины, меланины, полисахариды, липиды