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IM. WINCENTEGO POLA W LUBLINIE

DEVELOPMENT OF NATURAL SCIENCES IN COUNTRIES OF THE EUROPEAN UNION TAKING INTO ACCOUNT THE CHALLENGES OF XXI CENTURY

Collective monograph

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Lublin, Poland
2018

THE RARE AND BIOTECHNOLOGICALLY IMPORTANT MUSHROOM SPECIES IN THE IBK COLLECTION

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INTRODUCTION

Over 10 thousand species of *Basidiomycota* and *Ascomycota* are macrofungi, or mushrooms. Mushrooms play an important role in natural environment decomposing a huge lignocellulose biomass and plant litter, forming mycorrhiza, etc. They also have considerable economic significance as objects of mushroom growing industry and as 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 Mushroom Culture Collection (acronym IBK) was established at the M.G. Kholodny Institute of Botany, NASU in 1966 on the basis of the Department of Mycology[1]. A founder and first curator of the IBK Collection was Dr. Professor A.S. Bukhalo (1932–2014) who defined the strategy for development of collection funds and proposed basic principles of selection and preservation of mushroom diversity.

The Collection includes a total number of 1 110 strains belonging to 186 species 88 genera *of Basidiomycota* and *Ascomycota*. The IBK Collection is the largest official specialized culture collection of macromycetes in Ukraine and one of the largest in number of species and strains in Europe. According to Decree of the Cabinet of Ministers of Ukraine № 1709 of 19 December 2001, the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany was registered as a scientific object of national heritage of Ukraine. The IBK collection is also included in the international database of WFCC, the curator of the collection – Dr. Sci. N.A. Bisko (http://www.wfcc.info/ccinfo/index.php/collection/by_id/1152).

[1] Bisko N.A., Lomberg M.L., Mytropolska N.Yu., Mykchaylova O.B. The IBK mushroom culture collection. K.: M.G. Kholodny Institute of Botany, National Academy of Sciences of the Ukraine; Alterpres, 2016. 120 p.

The Culture Collection activities are focused on preservation of genofund of macrofungi in pure culture and fundamental research on biology and biotechnology of edible and medicinal mushrooms. Dikaryotic strains of macromycetes from different taxonomic and ecological groups of broad geographical origin are maintained in the Collection. The collection is of great environmental importance for ex situ conservation of the fungal genetic resource. Introduction to culture and preservation of rare and endangered species of fungi in Ukraine are emphasized, particularly those species which are listed in the Red Data Book of Ukraine.

A special attention has been paid to the introduction of species and strain diversity of edible and medicinal mushrooms. Unlike other macromycetes collections, in the IBK Collection contains a large number of strains commonly cultivated worldwide by producers of edible mushrooms and dietary and pharmacological substances.

Culture collection of mushrooms 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.

1. Methods of isolation, identification and storage

Isolation of pure cultures from fruit body tissue or basidio- and ascospores are made using conventional and modified methods. For isolation and preservation of cultures, wort, malz, compost, potato-dextrose and other agar media, including added herbal extracts, are used.

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. It has been established that during cultivation period on reference agar nutrient medium, taxonomically significant at species level are: ability to form teleomorph stage; presence and type of asexual sporification; presence, shape and location on the mycelium of clamp connections, chlamydospores; 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. The micromorphology of basidiomycetes and ascomycetes in IBK collection of mushrooms was

stadied using a scaning electron microscopy[2]. The mycelium samples were fixed with osmium tetroxide vapor (1% solution) for 96 h. After drying, the samples were covered with gold in a vacuum spray gun JII – 4X with rotation. The specimens were examined using a scanning electron microscope JEOL JSM-6060 LA (Jeol, Japan) and studied at magnification from × 100 to ×18.000.

Methods of storage of pure cultures of ascomycetes and basidiomycetes are determined by peculiarities of morphology, ecological and biological properties of the fungi that develop in culture mainly as asporous vegetative mycelium. Cultures are preserved in refrigerator at 4±1°C.

2. List of rare and biotechnological important fungi

The nine species of mushrooms of *Ascomycota* and *Basidiomycota* divisions that included in the Red Data Book of Ukraine are saved in collection IBK. There are *Gyromitra slonevskii* (1 strain), *Morchella steppicola* (2 strains), *M. crassipes* (3 strains), *Anthurus archeri* (1 strain), *Agaricus bresadolanus* (2 strains), *Fomitopsis officinalis* (3 strains), *Hericium coralloides* (3 strains), *Grifola frondosa* (19 strains), *Sparassis crispa* (4 strains)[3].

Only three species of the genus *Sparassis* (*S. crispa*, *S. laminosa* and *S. nemecii*) are known in Ukraine and are supported in the collections of IBK but only one of these species (*S. crispa*) contains to the Red Data Book of Ukraine[4]. However, *S. laminosa* and *S. nemecii* are rare species which also to need the protection. In addition, *S. nemecii* was included to the Red Lists of some Europe and Asia countries with the Endangered status. Its new location was found on the territory of the National Natural Park "Hutsulshchyna" in Ukraine.

There are also four strains of *Pleurotus nebrodensis* with different geographical origin are saved in the IBK collection. These species has been

[2] Buchalo A.C., Mykchaylova O.B., Lomberg M.L., Wasser S.P. Microstructures of vegetative mycelium of Macromycetes in pure cultures. K.: M.G. Kholodny Institute of Botany, 2009. 224 p.

[3] Червона книга України. Рослинний світ / за ред. Я.П. Дідуха. К.: Глобалконсалтинг, 2009. 900 с.

[4] Mykchaylova O.B., Gryganskyi A.P., Lomberg M.L., Bisko N.A. The study OF MORPHOLOGICAL AND CULTURAL PROPERTIES OF SPARASSIS CRISPA (SPARASSIDACEAE, POLYPORALES). *UKRAINIAN JOURNAL OF ECOLOGY*. 2017. № 7(4). P. 550–558.

recently the only until a representative of the kingdom of mushrooms which introduced in the Red List of IUCN. Since the area to which this mushroom is growing is less than 100 square km, its population is very fragmented and a continuous decrease it localities with mature individuals is observed. It is classified as a Critically Endangered species It should be noted that there are three strains of *Fomitopsis officinalis* – a fungus that is considered to be missing in our country (Regionally Extinct, RE) are saved in IBK culture collection. Previously, it grows in the Carpathian and Western Ukrainian forests, however, for over 50 years this species of fungus has not been found on the territory of Ukraine[5]. In the North America, the 12 countries of Europe and the Russian Federation (Siberia, the Far East), *F. officinalis* is presented predominantly as a species threatened with extinction. *Anthurus archeri* was included in the Red Data Book of Ukraine. But it should be noted that its status of protected species is being discussed now. This is due to the fact that this species is invasive for European countries.

Also numerous strains of 123 biotechnologically important species of mushrooms are stored in the IBK collection, such as: *Agaricus bisporus* (53 strains), *Antrodia cinnamomea* (3 strains), *A. camphorate* (1 strain), *Auricularia auricula-judae* (7 strains), *Coprinus comatus* (12 strains), *Cyclocybe aegerita* (14 strains), *Fistulina hepatica* (7 strains), *Flammulina velutipes* (32 strains), *Fomes fomentarius* (14 strains), *Fomitopsis betulina* (24 strains), *F. penicola* (8 strains), *Ganoderma applanatum* (21 strains), *G. lucidum* (39 strains), *Hericium alpestre* (1 strain), *H. erinaceus* (18 strains), *Heterobasidium annosum* (1 strain), *Hypsizygus marmoreus* (14 strains), *Inonotus obliquus* (6 strains), *Irpex lacteus* (22 strains), *Kuehneromyces mutabilis* (7 strains), *Laetiporus sulphureus* (34 strains), *Lentinula edodes* (68 strains), *Lepista nuda* (9 strains), *Lycoperdon perlatum* (9 strains), *Macrolepiota procera* (15 strains), *Morchella conica* (10 strains), *M. esculenta* (13 strains), *Phallus impudicus* (10 strains), *Pleurotus eryngii* (35 strains), *P. ostreatus* (172 strains), *Polyporus squamosus* (11 strains), *Psilocybe cubensis* (2 strains), *Schizophyllum commune* (24 strains), *Trametes versicolor* (14 strains) and many others.

[5] Mykchaylova O.B., Bisko N.A., Sukhomlyn M.M., Lomberg M.L., Pasaylyuk M.V., Petrichuk Y.V., Gryganskyi A.P. Biological peculiarities of a rare medicinal mushroom Fomitopsis officinalis (Fomitopsidaceae, Polyporales) on agar media and plant substrates. *Regulatory Mechanisms in Biosystems*. 2017. № 8(4). P. 469–475.

3. The influence of media composition, biostimulants and cultivation parameters on growth rate and teleomorph production

Research of growth characteristics and the search for optimal substrates is an important factor in ensuring the vitality of the culture of mushrooms. Our group in collaboration with researchers from various research centers conducted an experimental study on optimizing media for mushrooms from IBK collection. The main parameters of medium, which studied were: sources of nitrogen and carbon, the ratio of nitrogen to carbon, pH of nutrient media, critical and optimal temperature and concentration of biostimulants[6].

Selection of optimal sources of nitrogen and carbon, their concentration and ratio, as well as pH of nutrient media are very important indicators, which allows choosing the optimal conditions for growth and preservation of mushrooms culture. 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 same strain of *Ganoderma lucidum, G. applanatum*[7], *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 strain of *Morchella conica, M. esculenta* and other preferred ammonium phosphate as a source of nitrogen[8].

An important trend of our research group is the search for stimulators of growth and biosynthetic activity of rare and biotechnologically important strains. For example, in a current research we selected a natural growth biostimulators for rare fungi that are poorly growing on standard media.

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[6] Бисько Н.А., Бабицкая В.Г., Бухало А.С., Круподерова Т.А., Ломберг М.Л., Михалова О.Б., Пучкова Т.А., Соломко Э.Ф., Щерба В.В. Биологические свойства лекарственных макромицетов в культуре: в 2 т. К.: Институт ботаники им. Н.Г. Холодного НАН Украины, 2012. Т. 2. 459 с.

[7] Круподьорова Т.А. Біологічні особливості Ganoderma applanatum (Pers.) Pat. та Ganoderma lucidum (Curtis:Fr.) P. Karst. в культурі: автореф. дис. … канд. біол. наук: 03.00.21 «Мікологія». К., 2009. 20 с.

[8] Бисько Н.А., Бабицкая В.Г., Бухало А.С., Круподерова Т.А., Ломберг М.Л., Михалова О.Б., Пучкова Т.А., Соломко Э.Ф., Щерба В.В. Биологические свойства лекарственных макромицетов в культуре: в 2 т. К.: Институт ботаники им. Н.Г. Холодного НАН Украины, 2012. Т. 2. 459 с.

Thus culture of rare and medicinal fungi *F. officinalis* have very slowly radial growth rate (0,4–1,9 mm/day). Various vegetable biostimulants have been used to enhance growing of this culture. The husk of sunflower was the most favorable substrate for growth of vegetative mycelium. This substrate overgrown completely by mycelium on the 30th day of the experiment. The larch chips overgrew 50% by mycelium *F. officinalis* on the 30–40th day of the experiment. The *F. officinalis* fruit bodies grew only on two substrates – husk of sunflower and larch chips. In the same time, we studied the growth rate and morphology of the strains of valuable and rare *S. crispa* on six standard agar nutrient media: malt extract agar, potatoes extract agar, malt agar with the addition of pine sawdust, malt agar with the addition of larch sawdust and glucose-peptone-yeast extract agar. According to the radial growth rate of *S. crispa* cultures can be placed to the very slowly growing mushrooms, growth rate of 0,5–2,8 mm/day. We found the selective media such as malt agar with the addition of pine sawdust and larch sawdust as most favorable for the vegetative growth and generative stage for all strains.

Our collaborators study new synthetic compounds of essentials metals with high biological activity. Thus we investigated the influence of the metals citrates (copper, manganese, iron and zinc), obtained using aquananotechnology, on the biology of *Ganoderma lucidum*, *Pleurotus ostreatus*, *Cordyceps militaris*, *Ophiocordyceps sinensis*, *Trametes versicolor* in culture and on their biosynthetical properties. For example, the highest biomass production of *T. versicolor* was obtained on a liquid medium with the copper citrate (Cu^{2+} 4mg/l). In this trial, the biomass of *T. versicolor* increased by 79,9%, relative to control medium. In the same time, the zinc citrate raised the productivity by 28,3% of biomass of *G. lucidum*, in a relationship to the control trial[9].

4. Verification of isolated cultures

The correct identification of each strain of isolated culture was one of the most important aspects of the work. Identification of fungal isolates was made using the complex of morphological, micromorphological, and genetic characteristics. The teleomorph stage is the most essential criterion for the identification of cultures, but very often mushrooms require special

[9] Al-Maali G.A. The influence of metal citrates obtained by aquananotechnology on growth of the strains of medical macromycetes Ganoderma lucidum 1900 and Trametes versicolor 353. *Ukrainian Botanical Journal*. 2015. № 72(4). P. 393–397.

methodological techniques, that stimulate the formation of fruit bodies. We defined composition of special substrates, which stimulated the formation of fruiting bodies of many species from IBK collection of mushrooms, such us: species from genus *Coprinus, Hericium* and *Pleurotus, Auricularia auricula-jude,Cyclocybe aegerita, Grifola frondosa*, *Sparassis crispa, Fistulina hepatica, Lentinula edodes, Flamulina velutipes* and many others[10].

Vegetative mycelia microstructures are important factor for determine species identity of the culture. The result of this study is presented in our monograph "Microscopy of vegetative mycelium of macromycetes in pure culture"[11]. In more recent investigations we paid special attention to rare species, such a *F. officinalis, Grifola frondosa* and *Sparassis crispa.* Thus in all *S. crispa* cultures we observed the hyphae with regular one-sided gapless clamp connections, numerous secretory cells on the surface of the hyphae, anastomoses, filamentous strands and films[12] (Fig. 1). Vegetative micelia *F. officinalis* predominantly consists of thin-walled, moderately branched, hyaline hyphae with regularly septas (Fig. 2). On the hyphae, we observed numerous single clamps without gap and very rare medallion-type clamps and a small amount of anastomoses. For the first time, we detected specific inlaid hyphae. The thickened ellipsoid blastoconidia were formed on a vegetative mycelium of *F. officinalis* during longed cultivation for more than 30 days[13]. Mycelium of *G. frondosa* formed apical and intercalary chlamydospores on hyphae, which usually have no clamp connections.

It is important to note that clamp connections and type of anamorphic structures of species different genera (*Agaricus, Pleurotus, Lentinus, Trametes, Coprinus, Morchella, Lycoperdon, Oudemansiella, Hericium,*

[10] Ломберг М.Л. Лікарські макроміцети у поверхневій та глибинній культурі: автореф. дис. … канд. біол. наук: 03.00.21 «Мікологія». К., 2005. 20 с.

[11] Buchalo A.C., Mykchaylova O.B., Lomberg M.L., Wasser S.P. Microstructures of vegetative mycelium of Macromycetes in pure cultures. K.: M.G. Kholodny Institute of Botany, 2009. 224 p.

[12] Mykchaylova O.B., Gryganskyi A.P., Lomberg M.L., Bisko N.A. The study of morphological and cultural properties of Sparassis crispa (Sparassidaceae, Polyporales). *Ukrainian Journal of Ecology*. 2017. № 7(4). P. 550–558.

[13] Mykchaylova O.B., Bisko N.A., Sukhomlyn M.M., Lomberg M.L., Pasaylyuk M.V., Petrichuk Y.V., Gryganskyi A.P. Biological peculiarities of a rare medicinal mushroom Fomitopsis officinalis (Fomitopsidaceae, Polyporales) on agar media and plant substrates. *Regulatory Mechanisms in Biosystems*. 2017. № 8(4). P. 469–475.

Sparassis and many others) are the same in submerged culture and on agar media.

The molecular-genetic method is the most important way that use to verification of strains from IBK collection of mushrooms.

At this stage we are working on a complete determination of the nucleotide sequences of the internal transcribed spacer (ITS1, 5.8S and ITS2 regions of rRNA) for all strains from IBK collection of mushrooms. The data on confirmed sequences of strains/species were registered in NCBI GenBank.

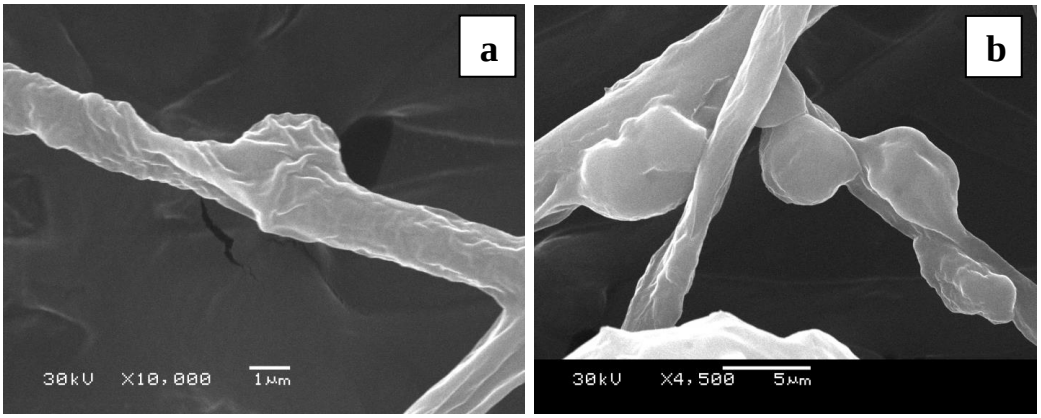

Fig. 1. *Sparassis crispa*: a – gapless clamp, b – secretory cells (stroke size: a – 2 µm, b – 5µm)

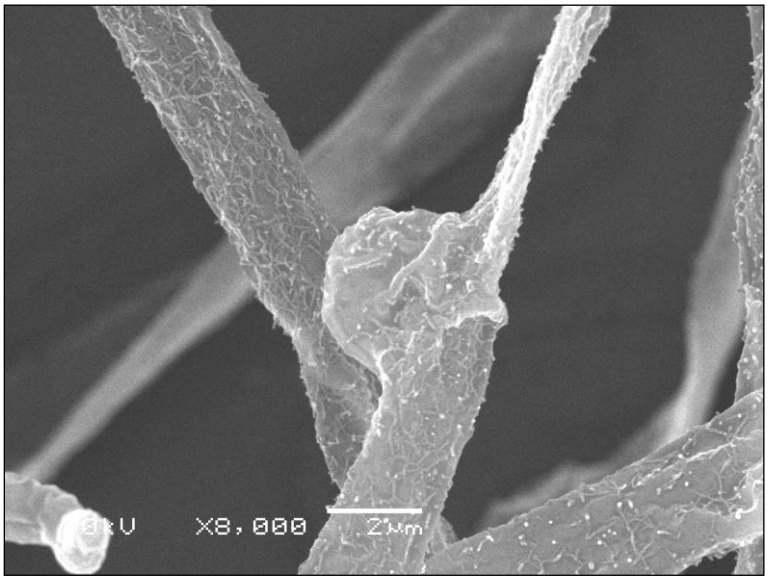

Fig. 2. *Fomitopsis officinalis:* gapless clamp and inlaid hyphae (stroke size: 2 µm)

5. Medicinal properties and biological active substances

Many mushrooms are widely known for their medicinal properties, which are associated with the presence of various biologically active substances in their biomass and cultural liquid, such as: intro- end extracellular polysaccharides, melanins, vitamins, essential lipids and amino acids, triterpens and many others[14].

Numerous researchers connected antitumor activity of medicinal mushrooms with their intro- end extracellular polysaccharides. The ability to synthesize the polysaccharides by some biotechnologically perspective mushrooms strains from IBK collections was studied. It has been shown that 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, Lentinula edodes* – producers of polysaccharides from IBK collection (Tabl. 1). Also, the physicochemical qualities of the polysaccharides of these strains have been studied[15]. In addition, 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*[16].

In addition, we studied the sorption of heavy metal preparations based on the cell wall. It was demonstrated that the chitin-glucan complex of the *Pleurotus ostreatus* possessed the best sorption properties. So, we have

[14] Бухало А.С., Бабицкая В.Г., Бисько Н.А., Вассер С.П., Дудка И.А., Митропольская Н.Ю., Михайлова О.Б., Негрейко А.М., Поединок Н.Л., Соломко Е.Ф. Биологические свойства лекарственных макромицетов в культуре: в 2 т. К.: Альтерпрес, 2011. Т. 1. 212 с.

[15] Scherba V.V., Babitskaya V.G. Polysaccharides of xylotrophic basidiomycetes. *Applied biochemistry and microbiology*. 2008. № 44(1). P. 78–83.

[16] Al-Maali G.A. The influence of different compounds of trace elements on the biomass and synthesis of exopolysaccharides of mycelium Trametes versicolor (Polyporaceae, Polyporales). *Biosystems Deversity*. 2017. № 25(4). P. 289–296.

developed methods for isolation and purification of the chitin-glucan complex with high sorption activity[17].

Many fungi contain high-quality lipids, including essential fatty acids, phospholipids and ergosterol. We found that a number of mushrooms from our collection are good producers of lipids. Thus culture of *Trametes versicolor, T. zonatus, T. pubescens, Daedaleopsis confragosa, Phanerocheton chrysosrium* have a high level of lipids in their mycelium, wherein they synthesized a large amount of biomass. But, the best producers of lipids are some strains of *Laetiporus sulphureus*, which biomass contains about 20% lipids. It should be noted that in general, the fungi of the order *Polyporales* contains more lipids than the fungi of the order *Agaricales*[18].

The nutritional value of basidial fungal lipids is determined by their composition. So a study of the fatty acid composition of showed that the fungi almost do not contain fatty acids with an odd number of atoms, as well as branched chain fatty acids. These properties of lipids of basidiomycetes favorably distinguish them from lipids of bacterial and yeast origin.

[17] Бісько Н.А., Ровбель Н.М., Гончарова І.А. Біосорбція важких металів міцелієм вищих базидіальних грибів. *Український ботанічний журнал*. 2004. № 61(6). С. 52–56.

[18] Бисько Н.А., Бабицкая В.Г., Бухало А.С., Круподерова Т.А., Ломберг М.Л., Михалова О.Б., Пучкова Т.А., Соломко Э.Ф., Щерба В.В. Биологические свойства лекарственных макромицетов в культуре: в 2 т. К.: Институт ботаники им. Н.Г. Холодного НАН Украины, 2012. Т. 2. 459 с.

Table 1

Efficacy of the process of growth and synthesis of polysaccharides by some species of mushrooms, which cultivated in a bioreactor on an optimized culture medium

Kinetic parameters	Time of cultivation, h													
	12	24	36	48	60	72	84	96	108	120	132	144	156	168
Ganoderma lucidum 16														
Y, g/l h	0,04	0,08	0,21	0,23	0,28	0,30	0,27	0,18	0,08	0,05	0,05	0,05	0,04	0,04
P (ECP), mg/g·h	25,0	23,0	55,0	55,0	48,0	45,0	42,0	38,0	40,0	38,0	32,0	32,0	31,0	32,0
П (ICP), mg/g·h	1,33	3,58	5,41	6,83	12,83	16,08	15,41	12,93	10,83	10,08	9,58	9,16	8,83	8,33
EC (ECP), %	-	30,00	45,00	38,80	35,00	33,08	28,57	25,78	25,00	24,87	23,07	21,86	20,90	20,00
EC (ICP), %	-	5,16	4,38	4,89	9,24	11,77	10,45	8,57	6,63	6,49	6,90	6,24	5,87	5,11
X (ECP), mg/l·h	-	16,0	50,0	66,6	75,0	83,3	25,0	8,3	8,3	8,3	-	-	-	-
X (ICP), mg/l·h	-	5,11	8,17	12,70	49,66	56,50	12,91	-	-	-	-	-	-	-
Z (ECP), h^{-1}	0,07	0,02	0,055	0,051	0,037	0,036	0,021	0,008	-	-	-	-	-	-
Z (ICP), h^{-1}	-	0,006	0,009	0,009	0,024	0,025	0,011	-	-	-	-	-	-	-
μ, h^{-1}	-	-	0,033	0,032	0,033	0,027	0,011	0,008	-	-	-	-	-	-
Crinipellis schevczenkovi 43														
Y, g/l h	0,125	0,183	0,150	0,208	0,336	0,250	0,150	0,116	0,108	0,070	0,070	0,070	0,060	0,030
P (ECP), mg/g·h	-	30,0	24,12	28,68	26,93	26,98	25,64	24,93	26,31	27,57	27,58	27,41	27,41	26,04
P (ICP), mg/g·h	-	3,66	4,23	6,50	7,33	7,75	8,16	7,33	6,66	6,41	6,25	6,00	5,83	5,53
EC (ECP), %	-	24,30	20,00	26,25	25,80	22,07	20,93	20,43	21,10	22,11	22,53	22,52	21,17	21,36
EC (ICP), %	-	2,97	3,50	5,95	7,02	6,34	6,66	6,00	5,34	5,14	5,10	4,92	4,71	4,58
X (ECP), mg/l h	-	33,3	16,6	83,3	91,66	16,6	16,6	16,6	33,3	33,3	16,6	16,6	25,0	-
X (ICP), mg/l h	-	5,10	6,91	23,58	32,93	8,77	14,17	-	-	-	-	-	-	-
Z (ECP), h^{-1}	-	0,025	0,013	0,036	0,024	0,027	0,013	0,016	0,055	0,055	0,027	0,023	0,027	-
Z (ICP), h^{-1}	-	0,004	0,005	0,010	0,009	0,014	0,012	-	-	-	-	-	-	-
μ, h^{-1}	-	0,061	0,045	0,048	0,031	0,004	0,009	0,006	-	-	-	-	-	-

Notes: Y – carbon source consumption rate; P – productivity of polysaccharides synthesis; EC – economic factor of using a carbon source for the synthesis of polysaccharides; X – rate of polysaccharides synthesis; Z – specific rate of polysaccharides synthesis; μ – specific rate of producer; ECP – extracellular polysaccharides; ICP – intracellular polysaccharides

In general, the quality fatty acid composition of the studied fungi from IBK collection was similar. Thus, most part of the fatty acids has a chain of 14–18 carbon atoms. In addition, unsaturated fatty acids accounted for 60–80% of total fatty acids content. The highest amount of unsaturated fatty acids was detected in biomass of some strain of *Ganoderma lucidum*, *Laetiporus sulphureus*, *Abortiporus biennis.* An important component of fungal lipids is ergosterol, which is provitamin D. Among the fungi of our collection, mushrooms of the genus *Ganoderma* contain the greatest amount of ergosterol[19]. It should be noted, that the strains of *L. sulphureus* contains a high amount of carotenoids.

Also, we investigated numerous factors that influence on the lipogenesis of selected strains of *Ganoderma lucidum* and *Laetiporus sulphureus.* The result of this study was the developed optimal medium for the cultivation of biomass these strains with increased lipid content.

Melanins are a large and most interesting group of natural antioxidants, which undergo reversible oxidation and redaction and display the properties of donors or acceptors of electrons and protons. Two strains of *Phellinus robustus*[20] and *Inonotus obliquus*[21] were selected as highly effective producers of melanins. The part of this study was used to study the physico-chemical properties of melanins of these strains, such as the chemical compositions, and spectrum of absorption in the infrared and ultraviolet range. Also, we detected new inhibitors of melanin synthesis, namely kojic acide. It was proved that the melanins of these fungi have a high sorption activity with respect to ions of heavy metals, in particular lead, copper, zinc and nickel.

Antimicrobial drugs have long been used for prophylactic and therapeutic purposes, but the drug-resistant bacterial strains have creating serious treatment problems. Mushrooms have been recognized as functional foods and as a source for the development of medicines, including antibacterial

[19] Бабицкая В.Г., Черноок Т.В., Щерба В.В., Пучкова Т.А., Филимонова Т.В., Осадчая О.В. Характеристика липидов глубинного мицелия грибов. *Труды Белорусского государственного университета*. 2009. № 4(1). С. 24–28.

[20] Bisko N.A., Shcherba, V.V., Mitropolskaya, N.Y. Study of melanin complex from medicinal mushroom Phellinus robustus (P. Karst.) Bourd. et Galz. (Aphyllophoromycetideae). *International Journal of Medicinal Mushrooms*. 2007. № 9(2). P. 177–184.

[21] Babitskaya V.G., Scherba V.V., Ikonnikova N.V., Bisko N.A., Mitropolskaya N.Y. Melanin complex from medicinal mushroom Inonotus obliquus (Pers.: Fr.) Pilat (Chaga)(Aphyllophoromycetidae). *International Journal of Medicinal Mushrooms*. 2002. № 4. P. 139–145.

end antifungal drugs. Thirteen species and sixteen strains of fungi from IBK collection were used in the study of antimicrobial activity against 12 test culture of bacteria and fungi, such as *Bacillus subtilis, B. mycoides, B. pumilis, Leuconostoc mesenteroides, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Comamonas terrigena, Pseudomonas aeruginosa, Aspergillus niger, Saccharamyces cerevisiae*. The results show that 81% of the cultures studied showed antimicrobial activity. It is important to note that 44% of strains synthesize substances that inhibit the growth of meticillin-resistant *Staphylococcus aureus. Flammulina velutipes* and *Rhodocollybia maculate* were the most active against this culture of *S. aureus.* Also, 38% of studied strains suppressed growth of *Leuconostoc mesenteroides,* which have a resistance against glycopeptides antibiotics. The strain of *Armillaria sp*. had the strongest activity against this pathogen microorganism[22].

Cytokinins are polyfunctional phytohormones that are involved in plant growth and development regulation. It is known that phytopathogenic fungi synthesize high concentrations of cytokinins in culture, but until now there are few data on the cytokinines occurrence and functioning in basidial mushrooms. We screened the mushrooms cultures from IBK collection for the presence of cytokinins in the biomass. It was found that mycelium of 13 species of mushrooms (*Morchella esculenta, Fomitopsis officinalis, Sparassis crispa, Grifola frondosa, Pleurotus nebrodensis, Lentinula edodes, Hericium coralloides, H. erinaceus, Trametes versicolor, Cyclocybe aegerita, Ganoderma lucidum, Pleurotus ostreatus, Flammulina velutipes*) contains different concentrations of cytokinins. The biomass of *Morchella esculenta* contained the greatest amount of cytokinins (26,98 μg/g of dry weight). It should be noted that the biomass of this fungus did not contain zeatin ribozide and isopentenyladenosine. Some authors indicate that these two cytokines have therapeutic and drug activity. Therefore, *Hericium coralloides* can be considered the most promising producer of cytokinins, because the highest amount of zeatin ribozide was in biomass. Thus total content of cytokinins in mycelium of *H. coralloides* was 15,62 μg/g of dry

[22] Дьяков М.Ю., Камзолкина О.В., Штаер О.В., Бисько Н.А., Поединок Н.Л., Михайлова О.Б., Тихонова О.В., Толстихина Т.Е., Васильева Б.Ф., Ефременкова О.В. Морфологические признаки природных штаммов некоторых видов базидиомицетов и биологический анализ антимикробной активности в условиях глубинного культивирования. *Микология и фитопатология*. 2010. № 44(3). С. 225–239.

weight, while the zeatin ribozide was 26% of the total number of cytokinins[23].

CONCLUSIONS

The IBK mushrooms culture collection involves a total number of 1110 strains belonging to 186 species 88 genera *of Basidiomycota* and *Ascomycota*, including rare and biotechnological important species. Morphological and physiological characteristics of cultures were investigated, and about 100 species were studied using scanning electron microscopy. Criteria for the correct identification of culture were established. Growth and morphogenesis of cultures were investigated under submerged cultivation. It was shown that clamp connections and type of anamorphic structures of different species are the same in submerged culture and on agar media.

One the basis of the IBK mushrooms culture collection strains of medicinal mushrooms that appear promising biotechnological application as producers of fruiting bodies, dietary supplements and metabolites were selected, including same strains of *Ganoderma applanatum, Hericium erinaceus, Laetiporus sulphureus, Lentinus lepideus, Phellinus robustus, Sparassis crispa* and many others species.

Original technologies for obtaining a biomass with high amount medicinal substances (melanins, extra- and intracellular polysaccharides, lipids, vitamins and etc.) from some fungi was created, such as *Crinipellis schevczenkovi, Ganoderma lucidum, Lentinula edodes, Lentinus tegrinus, Trametes versicolor.*

SUMMARY

The IBK mushrooms culture collection includes a total number of 1110 strains belonging to 186 species 88 genera *of Basidiomycota* and *Ascomycota*. Morphological, physiological, biochemical characteristics of cultures were investigated for about 100 species, including rare and

[23] Vedenicheva N.P., Al-Maali G.A., Mytropolska N.Y., Mykhaylova O.B., Bisko N.A., Kosakivska I.V. Endogenous cytokinins in medicinal Basidiomycetes mycelial biomass. *Biotechnologia Acta*. 2016. № 9(1). P. 55–63.

Vedenicheva N.P., Al-Maali G.A., Mykhaylova O.B., Lomberg M.L., Bisko N.A., Shcherbatiuk M.M., Kosakivska I.V. Endogenous Cytokinins Dynamics in Mycelial Biomass of Basidiomycetes at Different Stages of Cultivation. *Int J Biochem Physiol*. 2018. № 3(2). P. 000122.

biotechnological important species. The biotechnological perspective strains were selected, and the conductions of their submerged cultivation and fructification were developed.

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