VERIFICATION AND SCREENING OF BIOTECHNOLOGICALLY VALUABLE MACROMYCETES SPECIES IN VITRO

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Abstract. The article is devoted to the *IBK* Mushroom Culture Collection as an essential basis of preservation of macrofungi genofund in pure culture and fundamental research on biology and biotechnology of edible mushrooms with medicinal properties in Ukraine. The objects of investigation are 568 strains of 28 species of edible mushrooms with medicinal properties from the *IBK* Mushroom Culture Collection. The aim of the work was the elaboration of verification criterions for the strains of edible macromycetes of *IBK* Collection for confirmation of taxonomic status of biotechnologically valuable producers. The mycological, microbiological, microscopic, molecular genetic methods were done. Methods of macromycetes isolation in pure culture, the criteria for maintaining viability and identification of different species at the vegetative growth stage are provided. 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. A complex of morphological criterions for the correct identification of the taxonomic position of cultures of 28 species edible macromycetes are proposed. It includes a presence, dislocation, and morphology of clamp connections; a type of anamorph; special hyphal structures and other characteristics; the presence of teleomorph stage; the growth rate, color, and morphology of mycelial colony. Using light and scanning elec-

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tron microscopy, new data were obtained on microstructures of vegetative mycelium of valuable species edible mushrooms with medicinal properties from genus *Pleurotus* (Fr.) P. Kumm., *Agaricus* L.: Fr., *Auricularia* Bull. ex Juss., *Coprinus* Pers., and *Lentinula edodes* (Berk.) Singer, *Hericium erinaceus* (Bull.) Pers., *Grifola frondosa* (Dicks.: Fr.) Gray, *Hypsizygus marmoreus* (Peck) H.E. Bigelow, *Lepista nuda* (Bull.: Fr.) Cooke, etc. A list of some important for biotechnology edible mushroom with medicinal properties are described. The content of the main components of fruit bodies of 11 species edible mushroom with medicinal properties maintained in the *IBK* Collection is provided. The nutritional value of these mushrooms is comparable to foodstuffs such as corn, soybeans, potato. The medicinal properties and bioactive compounds of 8th genera of macromycetes are described.

1. Introduction

Mushroom Culture Collections are the custodians of biodiversity and play a key role in the storage and supply of authentic reference material for research and development, provide starting material for life science research, development and production. Especially in biotechnology, well characterised and pure strains are essential for reproducible and safe bioprocesses [1, p. 488]. In addition, they also maintain the know-how needed for more complex identification methods and help to develop new techniques [23, p. 782]. There are more than 652 culture collections in 70 countries of the world for today. They support 1.9 million strains in total, and 215224 from them are mushroom strains. (http://www.wfcc.info/ccinfo/, accessed 12/3/2013).

In Ukraine, since the end of the 60s of the last century, the cultures of *Basidiomycota* and *Ascomycota* have been supported by the *IBK* Mushroom Culture Collection at the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine. It is a scientific object of national heritage of Ukraine and is included in the international database of the World Federation for Culture Collections – WFCC (http://www.wfcc.info/ccinfo/index.php/collection/by_id/1152) [2, p. 339].

An important direction for the work of mycologists who care for the Collection is the introduction into the culture and preservation in pure culture of edible and medicinal macromycetes which are widely used in the biotechnology of the production of fruit bodies, mycelium, enzymes, biologically active compounds, pharmacological preparations, etc. [3, p. 349; 6, p. 105].

Due to the fact that the taxonomic position of the culture producer largely determines the correct biotechnological application, the establishment of criteria for the identity of the macromycete species is **actual**.

The purpose of the work was to develop the real criteria for verifying strains of edible macromycetes from the IBK Collection to confirm the taxonomic status of biotechnologically valuable producers.

During the course of the word, common mycological, microbiological, electron microscopic, molecular genetic methods were used [3, p. 14-23; 14, p. 308; 21, p. 470-471].

Morphological and physiological cultures characteristics are investigated according to the program of the stage-by-stage screening developed by the employees Species identification of cultures by cultural-morphological and molecular genetic methods were done. Molecular genetic studies were conducted on the basis of M.G. Kholodny Institute of Botany (Kiev, Ukraine) and company LF Lambert Spawn Co. (Coatesville, USA) [21, p. 470-471]. The radial growth speed was calculated according to the described method [3, p. 347-348]. Morphological and cultural studies were carried out in Petri dishes on standard and modified agar nutrient media of different composition. The micro- and macromorphological features of vegetative mycelium were determined according to the standard methods proposed by P. Stalpers [26, p. 12-23]. Microstructures of vegetative mycelium were investigated using light microscope "MBI–15" (Russia). Preparations for scanning electron microscopy (SEM) were prepared as in Quattelbaum and Carner (1980). Micrographs were prepared using a JSM-35C (Japan) scanning electron microscope [7, p. 12; 8, p. 50].

2. Edible mushrooms as a promising source of foodstuff and supplements with medicinal properties

Mushrooms have a long history of consumption as food, and also have remarkable therapeutic properties which are mainly recognized in oriental countries [27, p. 1323]. Nutritionally they are a valuable source of health food, which is low in calories, and rich in carbohydrates, essential amino acids, fibre, important vitamins and minerals (table 1) [13, p. 289]. According to FAO and WHO (World Health Organization), they are considered rich in glutamic acid, aspartic acid and arginine [18, p. 2343]. The nutritional value of mushroom is comparable to foodstuffs such as corn, soybeans.

Table 1

The general chemical composition of some cultivated edible fungi, % to dry weight

Scientific names	Protein, g/100 g	Lipid, g/100 g	Carbo-hydrate, g/100 g	Minerals, g/100 g	Energy value, kcal/100 g
Agaricus bisporus	21,6-39,0	1,7-8,0	56,3-72,9	7,0-12,0	175-368
Auricularia judae	2,1-10,6	0,2-8,3	66,4-82,8	4,7	279-356
Flammulina velutipes	17,6	1,9	73,1	7,4	378
Grifola frondosa	27,0	3,9	33,9	9,4	360
Hericium erinaceus	31,7	4,0	49,3	9,8	374
Hypsizygus marmoreus	28,7	2,6	34,0	8,8	358
Lentinus edodes	10,0-17,5	0,6-8,0	67,5-78,0	3,7-10,0	296-392
Pholiota nameko	20,8	4,2	66,7	8,3	372
Pleurotus ostreatus	10,5-30,4	1,0-7,2	57,6-81,8	5,0-9,8	317-367
Pleurotus eryngii	29,4	2,2	50,0	9,2	356
Volvariella volvacea	25,9	2,4	63,3	8,8	276
Corn	13,6	5,6	77,9	2,9	358
Potato	7,4	0,4	79	13,2	373
Soybeans	39,8	21,8	32,7	5,7	446

Source: [10, p. 138-143]

Mushrooms are quite important natural sources used in alternative medicine. Today, in parallel with the increase in the number of diseases, alternative medicine tendency is gradually increasing due to insufficiency of synthetic medicines in these disadvantages [18, p. 2345]. Many of the macromycetes produce polysaccharides, β-glucans, lectins, lactones, terpenoids, alkaloids, sterols and phenolics with very important advantageous medicinal properties (table 2). In the last two decades there has been an upsurge on the use of mushrooms as nutraceuticals and many edible species have been thoroughly investigated and authenticated for medicinal use [13, p. 298].

Immune stimulation by medicinal and edible mushrooms occurs via innate immunity and is typically done by phagocytic cells. Recent studies showed the effect of mushroom extracts on immune function by inhibiting tumour growth. These cells ingest invading pathogens and interact with pathogen components or bioactive element for further stimulation of innate immunity and adaptive immunity by secretion of cytokines and chemokines. Figure 1 represents the mechanism of immune activation by β-glucan from mushroom [9, p. 345].

Table 2

Medicinal properties of different bioactive compounds from important cultivated edible mushroom species

Mushroom	Medicinal value	Bioactive compounds
Agaricus spp.	Anticancer, antidiabetic, antihypercholesterolemic, immunomodulatory, hepatoprotective, antiviral, antimutagenic, antiproliferative	Heteropolysaccharides, glycoproteins, peptides, phenolics derivatives, hydrolytic, lipids and oxidative enzyme, lectins, sterols
Auricularia spp.	Anti-ageing, antioxidant, antidiabetic, anticoagulant	Heteropolysaccharides, peptides, phenolics compaunds, lipids and oxidative enzyme, lectins, sterols
Grifola spp.	Anticancer, antidiabetic, antihypercholesterolemic, anti-arthritic, antiviral, anti-osteoporotic, anti-obesity, immunomodulatory	β-glucans, polysaccharide, lectine
Hericium spp.	Antihypercholesterolemic antioxidant, immunomodulatory,	Heteropolysaccharide, β-glucans, lectine
Hypsizygus spp.	Antimicrobial, antioxidant, anti-inflammation	β-glucans, phenolic and flavonoid compounds
Fllammulina spp.	Antitumor activity, antiasthmatic, antioxidant, immunomodulatory, neuroprotective activity	Dietary fiber, polysaccharides, mycosterol, sesquiterpenoids, lectins
Lentinus spp.	Antitumor, antifungal, hypolipidaemic and hypoglycaemic activities, lipoprotein cholesterol and liver glycogen, immuno-modulatory activity	Heteropolysaccharides, homogeneous, phenolic compounds, lectins
Pleurotus spp.	Anticancer, antioxidant, antitumor, antiviral, antibacterial, antidiabetic, antihyper cholesterolemic, anti-arthritic, immunomodulatory, hepatoprotective, anti-obesity	Polysaccharides, lectins, β-carotene, tocopherol, phenolic compounds, ergosterol, flavonoids, ascorbic acid

Source: [9, p. 338; 20, p. 203-205; 22, p. 257; 24, p. 449-454; 25, p. 499-501]

Many mushrooms such as *Pleurotus* species, *Agaricus blazei* Murrill, *Agaricus bisporus* (J.E. Lange) Imbach, *Flammulina velutipes* (Curtis) Singer, and *Lentinula edodes* (Berk.) Pegler are the good source of bioactive compounds, which initiate the complement immune response. Bioactive elements such as polysaccharopeptides, functional proteins ubiqui-

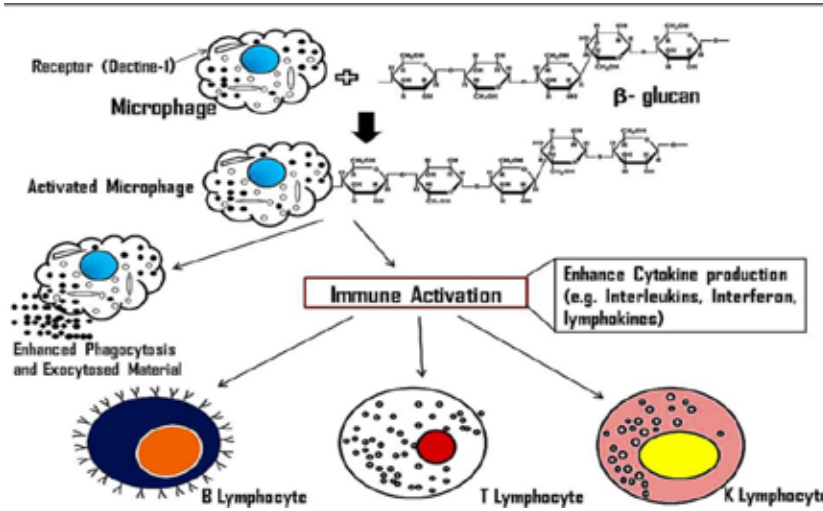

Figure 1. Mechanism of immune activation by β-glucan from mushroomn [9, p. 345]

tin-like protein, β-glucans and glycoprotein are responsible for enhancing the immune modulatory pathway as depicted in Figure 1.

Commercial production of fresh edible mushrooms is a fast growing industrial activity that can be carried out in a large scale. Its efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushrooms [19, p. 477], can convert the huge lignocellulosic waste materials into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and conserving the environment. Reducing the use of non-renewable resources is a key strategy of Circular Economy. Mycelium-based foams and sandwich composites are an emerging category of biocomposites relying on the valorization of lignocellulosic wastes and the natural growth of the living mushroom organism. Increasing attention has been paid to mycelium-based biocomposites as alternative materials to synthetic packaging and insulation panels, as well as developing a new-concept bio-inspired design. Since this is still a pioneering field, standardization

in the productive process is still in progress and only concerns the major companies [11, p. 281].

The rise of the suite of cultivated mushrooms seen on supermarket shelves today, including button mushrooms (*Agaricus bisporus*), began relatively recently in the 1960s. The majority of cultivated mushrooms (85%) come from just five genera: *Agaricus* L., *Auricularia* Bull., *Lentinula* Fr., *Pleurotus* (Fr.) P. Kumm., and *Flammulina* P. Karst.

The biotechnological use of promising edible and medicinal macromycetes species for the purpose of obtaining fruit bodies is possible only due to the presence of highly productive strains and a basic fungi collection of pure cultures.

3. The IBK Collection – a center for the preservation of valuable species of edible and medicinal macromycetes

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. For the moment the Collection holds 1250 strains belonging to 236 species of 104 genera of *Basidiomycota* and *Ascomycota* fungi, including rare and biotechnological important species with medicinal properties [4, p. 8]. This unique collection maintains dicaryotic strains of mushrooms from various taxonomic and environmental groups of a wide geographical origin [2, p. 339]. Based on the Collection strains, the scientific principles of modern technologies of artificial cultivation of edible and medicinal mushrooms, the production of highly productive strains of domestic breeding and production of mushroom spawn were developed.

A large number of strains of edible fungi are sustained in the *IBK* Collection, such as *Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes*, *Flammulina velutipes*, etc. [15, p. 23]. These species are widely cultivated throughout the world. Most of them, except for *L. edodes*, grow in the wild in Ukraine and apparently do not require additional protection measures. There are strains of 34th edible fungi species, commonly cultivated on an industrial scale worldwide, are supported at the *IBK* Collection. Some most important of them are presented in table 3. There are also indicated the countries of strains origin and suitable agar media, pH, temperature of storage for maintained cultures there.

Table 3

Some important edible mushrooms with medicinal properties preserved at the *IBK* Mushroom Culture Collection (Ukraine)

Species / common name	Number of strains	Countries of strains origin	Conditions of storage (agar media, pH, temperature)
Agaricus bisporus (J.E. Lange) Imbach / Button mushroom	53	Belgium, Czech Republic, Germany, Israel, Netherlands, Poland, Russia, USA, Ukraine	CA pH 7,0-7,5 4-8°C
A.blazei Murrill / Almond Mushroom	2	USA	CA, pH 7,0-7,5 4-8°C,
Auricularia auricula-judae (Bull.) Quel. /Juda's Ear	7	Germany, Israel, Ukraine	WA, pH 6,0-7,0 4-8°C
Auricularia nigricans (Sw.) Birkebak, Looney & Sánchez-Garcia / Black ear	2	China, Vietnam	WA, pH 6,0-7,0 4-8°C
Coprinus comatus (O.F. Müll.) Pers. / Shaggy Mane mushroom	13	China, Germany, Israel, Russia, USA, Ukraine	CA, WA, pH 6,5-7,0 4-8°C
Cyclocybe aegerita (V. Brig.) Vizzini / Black Poplar, Pioppino	15	Bulgaria, Czech Republic, Germany, Israel, Slovakia, USA, Ukraine	WA, pH 6,0-6,5 4-8°C
Flammulina velutipes (Curtis) Singer / Velvet Foot Collybia, Enoki	42	Czech Republic, Russia, Ukraine	WA, pH 6,0-6,5 4-8°C
Grifola frondosa (Dicks.) / Maitake	31	Belarus, Czech Republic, Germany, Israel, Japan, USA, Ukraine	WA, pH 6,0-6,5 4-8°C
Hericium erinaceus (Bull.) Pers. / Lion's mane	20	Belarus, China, Germany, Japan, Netherlands, Israel, USA, Ukraine	WA, pH 6,0-6,5 4-8°C
Hypsizygus marmoreus (Peck) H.E. Bigelow / Bunashimeji	16	China, Germany, Israel, Japan, Ukraine, USA	WA, pH 6,0-6,5 4-8°C

Continuation of Table 3

Species / common name	Number of strains	Countries of strains origin	Conditions of storage (agar media, pH, temperature)
Lentinula edodes (Berk.) Pegler / Shiitake	68	China, Japan, South Korea	WA, pH5,0-5,5 4-8°C
Lentinus tuber-regium (Fr.) Fr. / King tuber mushroom	2	USA	WA, pH 6,0-6,5 4-8°C
Lepista nuda (Bull.) Cooke / Blewitt	9	Belarus, Czech Republic, Israel, Ukraine	CA, WA, pH 6,0-6,5 4-8°C
Pholiota mutabilis (Schaeff.) P. Kumm. / Changeable Agaric	7	Germany, Russia, Ukraine	WA, pH 6,0-6,5 4-8°C
Pholiota nameko (T. Itô) S. Ito & S. Imai / Nameko	4	Germany, Russia, Ukraine	WA, pH 6,0-6,5 4-8°C
Pleurotus calyptratus (Lindblad ex Fr.) Sacc.	5	Czech Republic, Israel, Ukraine	WA, pH 6,0-6,5 4-8°C
Pleurotus citrinopileatus Singer / Golden Oyster, Yellow Oyster	2	South Korea	WA, pH 6,0-6,5 15-20°C
Pleurotus columbinus Quél.	2	Estonia, England	WA, pH 6,0-6,5 4-8°C
Pleurotus cornucopiae (Paulet) Rolland	7	Belarus, Czech Republic, Hungary, Russia	WA, pH 6,0-6,5 4-8°C
Pleurotus cystidiosus O.K. Mill. / Oyster mushrooms, Ohritake	6	Taiwan, USA	WA, pH 6,0-6,5 4-8°C
Pleurotus djamor (Rumph. ex Fr.) Boedijn / Pink Oyster	3	Japan	WA, pH 6,0-6,5 15-20°C
Pleurotus dryinus (Pers.) P. Kumm.	3	Czech Republic, Ukraine	WA, pH 6,0-6,5 4-8°C

End of the Table 3

Species / common name	Number of strains	Countries of strains origin	Conditions of storage (agar media, pH, temperature)
Pleurotus eryngii (DC.) Quél. / King Oyster	35	Armenia, China, Czech Republic, Israel, Germany, Japan, Korea, Thailand, Ukraine	WA, pH 6,0-6,5 4-8°C
Pleurotus nebrodensis (Inzenga) Quél / White Elf	4	Israel, Ukraine	WA, pH 6,0-6,5 4-8°C
Pleurotus ostreatus (Jacq.) P. Kumm. / Oyster, Pearl Oyster	173	Belarus, Czech Republic, Hungary, Poland, Turkmenistan, Russia, Ukraine, USA, Uzbekistan	WA pH 6,0-6,5 4-8°C
Pleurotus pulmonarius (Fr.) Quél / Lung Oyster	17	Czech Republic, Russia, Ukraine	WA, pH 6,0-6,5 4-8°C
Sparassis crispa (Wulfen) Fr. / Cauliflower Mushroom	11	Czech Republic, Germany, Russia, Slovenia, Ukraine	WA, pH 6,0-6,5 4-8°C
Stropharia rugosoannulata Farl. ex Murrill / Wine-red Stropharia, Garden Giant	7	Czech Republic, Germany, Ukraine	CA, WA, pH 6,5-7,0 4-8°C
Volvariella volvacea (Bull.) Singer / Paddy straw mushroom	1	China	WA, pH 6,0-6,5 15-20°C

Comments: CA – compost agar, WA – wort agar

The *IBK* Collection – an important resource for the development of domestic mushroom growing industry as well as biotechnologies of dietary treatment and prevention supply, food supplements, pharmaceutical and biologically active substances. [2, p. 339; 15, p. 23]. A special attention has been paid to the introduction of species and strains diversity of edible mushrooms, which are cultivated wordwide.

The main tasks that need to be solved urgently is the creation of new, more effective biotechnologies for the cultivation of edible and medicinal fungi species in order to increase their productivity. They include an improving the biotechnology of physiologically active spawn cultivation,

the selection of new, optimal fermented lignocellulosic substrates from agriculture wastes, and processing industry.

4. The main stages of verification of cultures of edible, medicinal macromycetes in the collection *IBK*

Modern technologies of edible medicinal fungi cultivation are based on fundamental knowledge of their biological properties, which allows to control the most important functions of the fungal organism and ensuring the production of fruit bodies, mycelium biomass and products of metabolism the desired quality as well as in the required amount [5, p. 23].

One of the fundamental steps towards the creation of modern biotechnology is the receipt, identification and preservation in the laboratory condition of new and already known strain producers. At present, there are different methods of isolating basidiomycetes from natural conditions using a variety of nutrient media. Their composition, as well as temperature and humidity, are important from the point of view of storing strains, provided that the mushrooms retain their biosynthetic properties and maintain the cultures purity. The choice of an effective method of selection and storage conditions is based primarily on morphological and physiological features of different basidiomycetes species. Methods of obtaining and storage of pure cultures of ascomycetes and basidiomycetes are determined by the peculiarities of morphology, ecological and biological properties of the fungi developing in culture mainly as asporous vegetative mycelium. Growth of many species on agar nutrient media is very slow, and under culture isolating, often can occur their contamination by foreign microorganisms. This makes necessity the continuous improvement and modification of the methods of macromycetes cultures isolating taking into account the specifics of individual species. All this in total ensures the success of their introduction and conservation in culture. Isolation of pure cultures from fruit body tissue or basidio- and ascospores are made using conventional and modified methods [3, p. 14; 15, p. 25]. At the *IBK* Collection pure cultures of macromycetes are preserved in big test tubes (20-22 mm in diameter) at temperatures of 4-8°C in refrigerators or indoors at temperatures 15-20°C. Replanting into fresh culture medium is carried out once a year, for thermophilic species – twice a year. As a nutrient medium, malt extract agar is mostly used (MA) (2% sugar) with pH 5,0–7,0. For certain species with increased requirements for nutrients, depending on their ecological

and trophic peculiarities, MA with added 2% straw, MA with added 1% cherry sawdust, or compost agar (CA) are used. in the absence of a foreign microflora and viability.

The microscopic visual control of cultures in absence of extraneous microflora are monitored regularly which allow to maintain the viability of cultures in the Collection. According to our observations, in this mode of replanting cultures survive and not lose their biological properties, including enzymatic and physiological activity, over decades.

One of the important activities of the IBK Collection is to confirm the taxonomic identity of strains based on cultural and morphological characteristics, as well as to maintain their authenticity in the process of preservation. Schematically, the process of obtaining and working with cultures of macromycetes in the *IBK* collection can be reduced to the following steps (Figure 2).

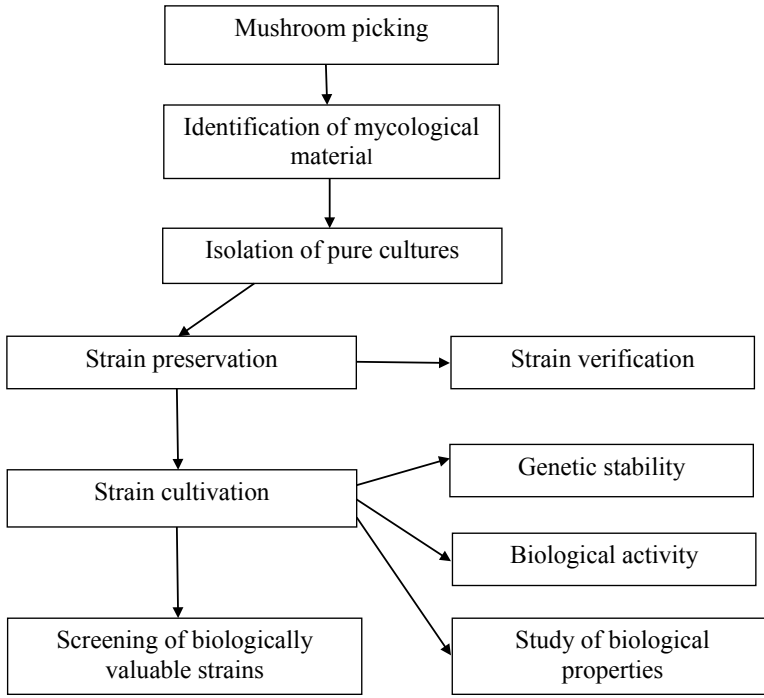

Figure 2. Schematic representation of work with strains of macromycetes in the *IBK* Mushroom Culture Collection

It has been established that during cultivation period on reference agar nutrient medium, the verification of strains at species level includes the use of a whole range of characteristics:

1. Ability to form teleomorph stage and its morphology.
2. Type of filamentous colony and its radial growth rate.
3. Presence and type of asexual sporification; presence, shape and location on the mycelium of clamp connections, chlamydospores, and others-structures of vegetative myceliu.
4. Nature of the colored test reactions in presence of certain enzymes of mushroom colony.
5. Temperature interval for mycelial growth, especially the upper critical border.

The teleomorph stage is the most important criterion for the identification of cultures, but often fungi do not form fruit bodies in a pure culture. We obtained new data on microstructures of cultures of valuable edible, medicinal fungi species from genera *Pleurotus* (Fr.) P. Kumm., *Agaricus* L.: Fr., *Auricularia* Bull. ex Juss., *Coprinus* Pers., та видів *Lentinula edodes* (Berk.) Singer, *Hericium erinaceus* (Bull.) Pers., *Grifola frondosa* (Dicks.: Fr.) Gray, *Hypsizygus marmoreus* (Peck) H.E. Bigelow, *Lepista nuda* (Bull.: Fr.) Cooke, etc. [8, p. 53].

As example of importance of mushrooms micromorphological characters in verification process we can show the new data on microstructures of *Coprinus comatus*, an edible gourmet mushroom known for its medicinal properties (figure 3, 4). The microstructures, such as typical allocysts, hyphal loops and conidial sporulation have been reported. In addition, clamp connections, anastomoses, dendroid hyphae, crystals of different shapes, including hair-like ones, were often observed on the mycelium [16, p. 221-259].

A detailed study of stamps, anamorphs and other structures of vegetative mycelium has allowed to identify more precisely and morphologically characterize a taxon presented in pure culture, which is especially important in its biotechnological application.

Comparative data on the growth of cultures on agar nutrient media of different composition were obtained. Based on the data obtained in the study of growth characteristics, all types of cultivated edible mushrooms can be divided into three main groups:

• slow-growing cultures – the radial growth rate of which did not exceed 0,05 mm/h (VR < 1,2 mm/day). To the slow-growing cultures can be

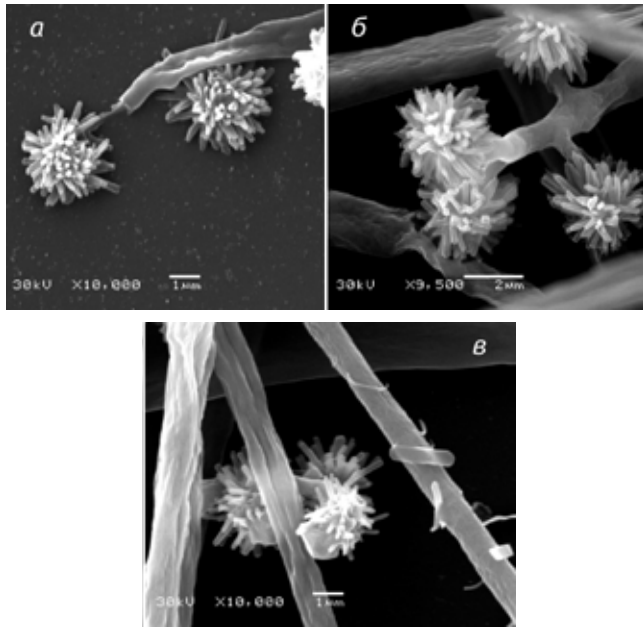

Figure 3. Morphology of *Coprinus comatus* (SEM):
а, б – denroid hyphae of strains *IBK* 2237 and *IBK* 2238;
в – denroid hyphae and cristals of strains *IBK* 2278

attributed strains from genera *Coprinus* Pers., *Sparassis* Fr., *Lepista* (Fr.) W.G. Sm., and also such valuable species of cultivated mushrooms as *Grifola frondosa*, separate strains of *Hypsizygus marmoreus*, *Stropharia rugosoannulata*, and *Lentinula edodes*.

• fast growing cultures – the radial growth rate of which is equal or exceeds 0,5 mm/h (or VR > 12 mm/day): *Pleurotus djamor IBK* 1526 и *Volvariella volvaceae IBK* 1605. Very close to this group are individual strains of the *P. ostreatus* (*IBK* 133, 530, 1017), their growth rate exceeds 11 mm/day.

• culture of fungi with an average growth rate – the average values of mycelium radial growth rate ranged from 4 to 8 mm / day. This group includes the huge majority of cultures collection: *F. velutipes*, *H. erinaceus*, *H. marmoreus*, *L. edodes*, *P. eryngii*, *Ph. mutabilis*, etc. [3, p. 349, 358].

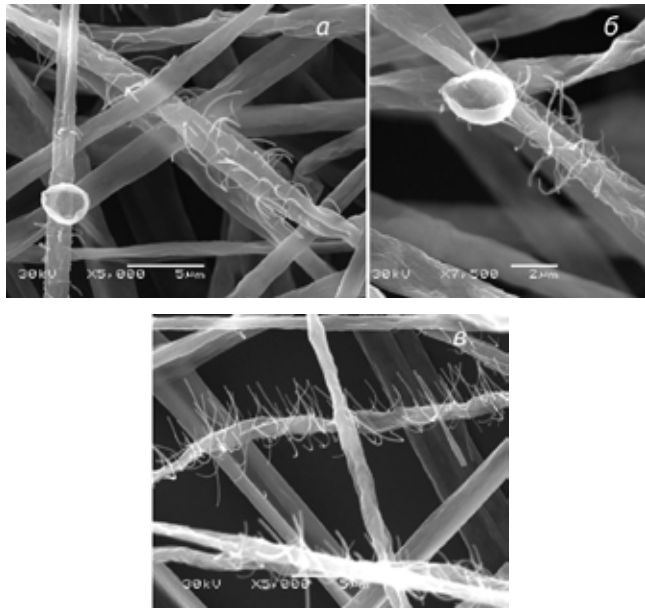

Figure 4. Morphology of *Coprinus comatus IBK* 2238 (SEM): а, б – allocysts on hyphae of strains *IBK* 2237 and *IBK* 2238 accordinally; а-в – hair-like cristals

A large group of fast-growing strains from the objects of modern world-wide mushroom production studied by us are cultures of the species *P. ostreatus*, which individual collection strains are already widely used in the mushroom industry of Ukraine. If we consider the prospects for the use in domestic mushroom production the representatives of other *Pleurotus* species, then it is worth paying attention to *P. citrinopileatus IBK* 1674 and *P. cornucopiae IBK* 106, which in a number of culture media exhibit a sufficiently high growth rate. Noteworthy is the strain of P. djamor *IBK* 1526, which is used in the amateur mushroom industry in South America and the USA. The objects of the world mushroom industry and biotechnology for obtaining drugs from fruit bodies and mycelium have been become such relatively slowly growing species of edible mushrooms as *L. edodes, H. erinaceus, H. marmoreus, G. frondosa, F. velutipes, P. eryngii,* etc [3, p. 359]. There are conditions for the teleomorph stage obtaining for these and many

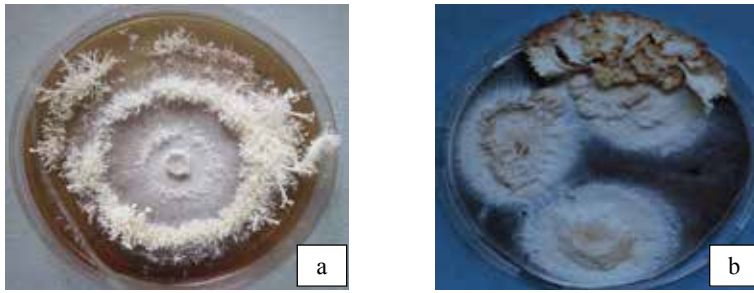

Figure 5. ***Hericium coralloides IBK*** **2332 (a): teleomorph** ***in vitro*** **on MA (15 day of cultivation).** ***Sparassis crispa IBK*** **314 (b): teleomorph on MA with pine sawdust** ***in vitro*** **(60 day of cultivation)**

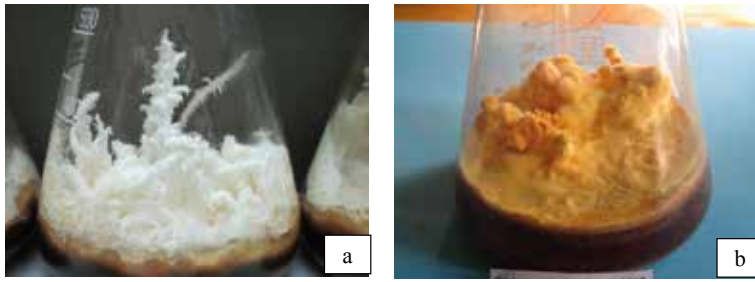

Figure 6. ***Hericium coralloides IBK*** **2332 (a): teleomorph on glucose-peptone-yeast medium** ***in vitro*** **(30 day of cultivation).** ***Cordyceps militaris IBK*** **1862 (b): teleomorph glucose-peptone-yeast medium** ***in vitro*** **(60 day of cultivation)**

others species were lined up in the *IBK* collection. The teleomorph stage of some mushrooms are given on figures 5, 6.

The strategy of screening programs that are conducted on the basis of a culture collection depends on their final goal: cultures identification, selection of producers of mycelial biomass, fruit bodies, enzymes, polysaccharides, antibiotics, pigments or other biologically active and pharmacological substances. In the *IBK* Collection based on the developed screening programs selected promising strains-producers of fruit bodies *Agaricus bisporus*, *Pleurotus ostreatus, Lentinula edodes, Flammulina velutipes*, and others. Mycelial with a faster rate of radial growth and better colony

characteristics are the morphological markers of a good mushroom breed [12, p. 5]. The former criteria are essential to ensure faster substrate colonization, resulting in more rapid completion of the production cycle, thus expedite the time to fructify [28, p. 333]. On the other hand, the thicker mycelium mat provides better ability to colonize vast agricultural lignocellulosic wastes. Utilization of these by-products for the production of mushrooms is deemed more feasible and economical [17, p. 601].

5. Cultural and morphological characteristics of some cultivated valuable edible and medicinal mushrooms from the *IBK* collection

As a result of an integrated approach, the main morphological and cultural characteristics of edible cultivated species of fungi have been established in the *IBK* Collection. These characteristics are listed below (table 4).

Table 4

Cultural and morphological characteristics of edible fungi species with medicinal properties, preserved in the *IBK*

Order/Family/Species	Morphological features
Agaricales /Agaricaceae / ***Agaricus bisporus***	Mycelial colony white, dingy with age, struck with brownish-hues, divergently rhizomorphic, with an overlayer of aerial mycelium developing in age increase in cottony, forms lacking feather-like outer edges and an overall decline in speed of growth. Crystals and chlamydospores are present on hyphae.
Auriculariales / Auriculariaceae / ***Auricularia auricula-judae***	Mycelial colony white, dense, cottony. White, becoming mottled with brown discolorations in age. Clamp connections, chlamydospores, and anastomoses are present on hyphae.
Agaricales /Agaricaceae / ***Coprinus comatus***	Mycelial colony white, fleecy, cottony, sparse, margin smooth. Asymmetrically shaped mycelia mat often forms along the outer edge of colony. Clamps are round-shaped, one-sided, mainly without slit; simple hyphal loops, branched structures with round-shaped.
Agaricales / Strophariaceae / ***Cyclocybe aegerita***	Mycelium in colony is longitudinally liner, becoming cottony, usually not aerial, white at first, soon becoming spotted brown, and eventually tan-brown. Primordia usually form on agar media. Clamps are round-shaped, one-sided, mainly without slit; anastomoses, and crystals are present on hyphae.

Continuation of Table 4

Order/Family/Species	Morphological features
Agaricales / Physalacriaceae / ***Flammulina velutipes***	Mycelial colony white, aerial mycelium longitudinally linear, becoming finely appressed and tinger light brown to spotted with golden yellow-brown zones with age. The surface roughens at the earliest stage of primordial formation. Clamps are one-sided, small, round-shaped, often; arthrospores, chlamydospores, rod-like crystals and hyphal loops are present.
Polyporales / Meripilaceae / ***Grifola frondosa***	Mycelial colony white, later light tawny-brown tones longitudinally linear, eventually thickly, cottony. The mycelium develops light tawny brown tones along the outside peripheral edge in aging. At maturity, the dense mycelia mat can be peeled directly of the agar media. Marginal hyphae 1.5-4.5 μm wide. Aeriel hyphae 1.5-3.0 μm wide, thin- to thick-walled. 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 intercallar chlamydospores form on hyphae, which usually have no clamp connections. Chlamydospores globose to broadly ellipsoid, (5-)9-15 μm diam. or 10-21×9-15 μm, not abundant
Russulales / Hericiaceae / ***Hericium erinaceus***	Mycelial colony white, whitish, forming triangular zones of collected rhizomorphs, thickly, cottony. 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 mycelia colony branched thin (≤ 1 μm width) hyphae (dichohyphidia) are formed. Intercallar and terminal chlamydospores and anastomoses between hyphae are usual. Abundant crystals of cubic or rectangular shape are sometimes present on hyphae.
Agaricales / Lyophyllaceae / ***Hypsizygus marmoreus***	Mycelial colony white, dense, cottony with tufts, edged with mycelia strands. Vegetative mycelium mostly has thin-walled, hyaline, regularly septated hyphae. Clamp connections, anamorphs, and crystals are present on hyphae. Hyphae of vegetative mycelium are incrustated. Chlamydospores are terminal and intercalary on hyphae.

End of the Table 4

Order/Family/Species	Morphological features
Agaricales / Omphalotaceae / ***Lentinula edodes***	Mycelial colony white, at first, becoming longitudinally linear and cottony-aerial in age, in response the mycelium becomes dark brown. Rarely rhizomorphic. Clamp connections have the classical form with a slit between the clamps and the septum. Rhomboid and amorphous crystals and lipid droplets are forming on hyphae.
Agaricales / Pleurotaceae / ***Pleurotus eryngii***	Mycelial colony first whitish, then cream, cottony, appressed to the substrate, with colorless droplets of exudates, rhizomorphs often present. Sometimes concentric zones of different texture of mycelia are distinct. On agar media teleomorph (primordial and fruit bodies) are forming. Vegetative mycelium consists of thin-walled hyaline and branched hyphae (2,1-5,5 μm). Clamp connection, abundant anastomoses, crystals and conidial sporulations are formed on hypha.
Agaricales / Pleurotaceae / ***Pleurotus ostreatus***	Mycelial colony white, with age cream, grayish to ivory, yellow to orange, dense, cottony, longitudinally radial, with concentric bands of different texture. On agar media teleomorph (primordial and fruit bodies) are forming.. Vegetative mycelium consists of thin-walled hyaline and branched hyphae (1,5-7,5 μm). Dolipore septa are present between the cells. Conidial sporulations single globose conidia 3-5 μm in diameter are present on hyphae.
Agaricales / Pleurotaceae / ***Pleurotus pulmonarius***	Mycelial colony is white, azonate or with concentric bands of different texture when the growth is rhythmic. As the mycelium matures, yellowish droplets of exudates are present. Teleomorphs (primordia and carpophores) form on agar media. Vegetative mycelium consists of thin-walled and branched hyphae (1,5-7,5 μm).
Polyporales / Sparassidaceae / ***Sparassis crispa***	Mycelial colony 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.

As a result of a comprehensive study of the morphological and cultural characteristics of 13 species from 9 genera of edible medicinal mushrooms, taxonomic features characteristic of each species have been established, by which the purity of the strains can be monitored at the vegetative developmental stage.

5. Conclusion

Proceeding from the extraordinary importance of correctly determining the taxonomic status of filamentous cultures of fungi-macromycetes with practical application, on the basis of the IBK strains collection, studies of the cultural-morphological properties of micellar cultures of practically important species with the use of scanning electron microscopy are carried out. 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.

The authors developed a system of criteria for the identification and verification of individual taxonomic and environmental groups of edible macromycetes *in vitro*. The study of cultural and micromorphological features, the revealing physiological and biochemical features, should be used to determine the taxonomic culture status at species level.

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