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BIOMASS FORMATION BY DIFFERENT COLLECTIONS OF *PLEUROTUS* SPP. DURING SUBMERGED GROWTH

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Many species of the genus *Pleurotus* (Fr.) P. Kumm. (Oyster mushrooms) are known as valuable edible and medicinal fungi, cultivated worldwide. The studied 18 different collections of five *Pleurotus* species (*P. ostreatus*, *P. cornucopiae*, *P. eryngii*, *P. pulmonarius*, and *P. cystidiosus*) during submerged growth in malt extract medium formed round shaped fluffy pellets and disperse mycelium. After seven days of cultivation, they accumulated up to 29.46 *g*/*L* biomass, while after 14 days the amount increased up to 40.4 *g*/*L*, reaching the value of commercial *P. ostreatus* 22 strain. More than 30.0 *g*/*L* biomass was formed in nine from 12 *P. ostreatus* collections, among which Armenian (SB24 and SB25), French (Po/28) and Spanish (N001) strains, as ecologically safe resources are perspective for further study to develop mushroom-based biotech products, food, pharmaceuticals and cosmeceticals.

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***Keywords*:** biomass, biotech products, edible, medicinal, mycelium, *Pleurotus* species, submerged growth.

Introduction. The submerged cultivation of mycelia is the most controlled biotechnological process that can lead to the rapid accumulation of biomass [1].

Many species of the genus *Pleurotus* (Fr.) P. Kumm. (Oyster mushrooms) are edible and widely cultivated worldwide. They are known as a source of valuable compounds with high nutritional and medicinal significance [2–11]. The medicinal properties of xylotrophic *Pleurotus* spp., as primary decomposers of wood were reported in several species, such as *Pleurotus ostreatus* (Jacq.) P. Kumm., *P. cornucopiae* (Paulet) Quél., *P. djamor* (Rumph. ex Fr.) Boedijn, *P. eryngii* (DC.) Quél., *P. giganteus* (Berk.) Karun. & K.D. Hyde, *P. levis* (Berk. & M.A. Curtis) Singer, *P. pulmonarius* (Fr.) Quél., and *P. tuber-regium* (Fr.) Singer. They are producers of phenolic compounds, terpenoids, polysaccharides, fatty acids, proteins, lectins, diterpenoid eryngiolide A, ergothioneine and other biomolecules with antitumor, antimicrobial, antioxidant, hypoglycemic, hypocholesterolemic, hepato-protective, neurotoxic, cardioprotective and other therapeutic effects [2, 12–22].

Due to high nutritional values, *Pleurotus* spp. are not only gourmet food, but also nutraceuticals (i.e., nutrition and pharmaceuticals) considered as food or part of the food with health-enhancing dietary and therapeutic values [2, 11, 23–25].

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It was reported, that the inclusion of 10 or 20 *g* of *P. ostreatus*/*kg* in the diet of Japanese quails was effective in delaying the lipid oxidation of breasts and enhancing the color, pH, water-holding capacity, cooking loss weight and texture to define meat quality [26].

It is known that mycelial biomass exceeds fruiting bodies (mushrooms) by the content of bioactive compounds, proteins, lipids, polysaccharides and carotenoids [1, 2]. Moreover, submerged cultivation of mycelia allows obtaining environmentally safe different health improving biotech products. Among Oyster mushrooms, mycelial biomass and cultural broth of *P. ostreatus* are considered as rich sources of innovative biomedical compounds to develop nutraceuticals, nutriceuticals, pharmaceuticals and cosmeceuticals [2, 3, 6, 7, 27, 28].

The aim of the current work was to study the biomass formation in different collections of *Pleurotus* species during their submerged growth.

Materials and Methods. The collection of 18 strains of five *Pleurotus* species (*P. ostreatus*, *P. cornucopiae*, *P. eryngii*, *P. pulmonarius*, and *P. cystidiosus* O. K. Mill. 1969) from different geographic origin (Armenia, Iran, Italy, Spain, Russia, and France) were studied (Tab. 1). It included 12 wild and commercial strains of *P. ostreatus*, two strains of *P. cornucopiae*, two strains of *P. eryngii*, and one strain each of *P. pulmonarius* and *P. cystidiosus*, as well as four strains received from culture collections of Fungal Biodiversity Institute, The Netherlands (CBS-KNAW) and University of Goettingen, Germany (UG). Several strains of *P. ostreatus*, isolated from the territory of Armenia have been genetically identified [29, 30].

Table 1

The studied collections of Pleurotus spp. and biomass formation during submerged growth

Species	Strains	Catalogue number	Origin	Host plant	Biomass ± SD, *g*/*L*	
					7 days	14 days
P. cornucopiae	Pc/2*	8904	Spain	Deciduous tree	13.53±0.34	18.96±0.26
	105*	8905	UG	Deciduous tree	11.33±0.125	15.8±0.22
P. cystidiosus	65*	9200	CBS-KNAW	–	9.86±0.205	16.4±0.29
P. eryngii	Pe/6C	9001	Armenia	–	6.5±0.294	12.23±0.21
	110*	9002	UG	–	8.23±0.287	16.9±0.25
P. ostreatus	SB8•	9101	Armenia	Deciduous tree	23.23±0.403	32.4±0.36
	SB24•	9111	Armenia	Stump *Quercus* sp.	13.4±0.327	35.6±0.37
	SB25•	9112	Armenia	Stump *Ulmus* sp.	19.6±0.294	40.4±0.26
	SB26•	9113	Armenia	Stump *Morus alba*	13.23±0.403	33.8±0.26
	SB30•	9116	Armenia	Deciduous tree	10.36±0.262	20.6±0.33
	Po/28	9120	France	Deciduous tree	12.2±0.294	36.4±0.36
	Po/29	9121	Italy	Deciduous tree	18.66±0.34	33.6±0.4
	Po/31-1C*	9123	Armenia	Commercial strain	13.9±0.327	20.6±0.37
	Po/31-2S*	9124	Armenia	Commercial strain	21.2±0.356	32.8±0.22
	N001*	9125	Spain	Deciduous tree	18.3±0.216	34.4±0.4
	1056	9122	Iran	*Fagus* sp.	18.26±0.287	28.4±0.29
	22	9119	Russia	Commercial strain	29.46±0.34	40.4±0.25
P. pulmonarius	508	–	UG	–	11.25±0.25	19.1±0.41

Note: (*) strains not published in the Catalogue [29]; (•) – genetically identified strains.

Mycelia of studied collections were cultivated in 4° Balling liquid malt extract medium (50 *mL* in 100 *mL* Erlenmeyer flasks). The flasks were inoculated by five 5 mm^3 inocula and incubated on the shaker (Heidolph Unimax 1010, 200 *rt*/*min*) in dark, at 25°C, during 7 and 14 days.

The cultural liquid (CL) samples were separated from mycelial biomass by a filtration method using FILTRAK FN 18 paper filter. Obtained biomass samples were washed by distilled water, air dried at room temperature and weighted. The final weight of biomass was recalculated per *g*/*L*.

Results and Discussion. During submerged cultivation, collections of *Pleurotus* spp. formed round shaped fluffy pellets and dispersed mycelium (Fig. 1). After 7 days of growth, the cultures accumulated from 6.5 to 29.46 *g*/*L* biomass, which increased up to 40.4 *g*/*L* after 14 days (Figs. 2 and 3, Tab. 1). A relatively small amount of biomass was formed by *P. cornucopiae* 105 and Pc/2, *P. pulmonarius* 508, *P. cystidiosus* 65, as well as *P. eryngii* Pe/6C and 110 strains. The lowest amount (65 *g*/*L* and 12.23 *g*/*L*) of biomass were detected in *P. eryngii* Pe/6C strain on 7th and 14th days of growth, respectively, while the highest amount (up to 40.4 *g*/*L*) in *P. ostreatus* strains (Tab. 1).

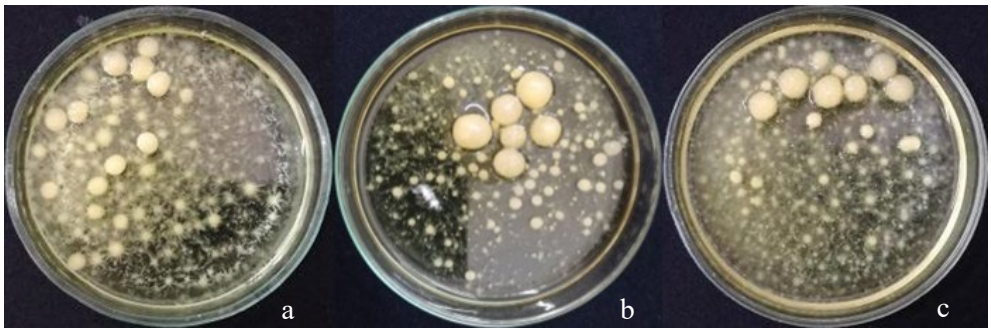

Fig. 1. Morphology of pellets and disperse mycelium in *P. cornucopiae* Pc/2 (a), *P. eryngii* Pe/6C (b), and *P. ostreatus* SB30 (c) species/strains after 14 days of submerged growth.

Fig. 2. Biomass accumulation in *P. cornucopiae* 105 (a), *P. cystidiosus* 65 (b), *P. eryngii* 110 (c), *P. ostreatus* N001 (d), and *P. pulmonarius* 508 (e) species/strains during 14 days of submerged growth.

According to biomass formation during two weeks of cultivation, the studied collections of *Pleurotus* spp. were divided into three groups: I, <10 *g*/*L*; II, 10–20 *g*/*L* and III, >20 *g*/*L* (Tab. 2). The first group included only two species and three strains of *P. eryngii* Pe/6C, 110 and *P. cystidiosus* 65 after seven days of cultivation, while the second group – three species and 12 strains (*P. cornucopiae* 105; Pc/2;

P. pulmonarius 508; *P. ostreatus* Po/28, SB26, SB24, Po/31-1C, Po/29, SB25, SB30, N001, 1056) after 7 and 14 days of growth, respectively. The third group includes only *P. ostreatus* strains: three (Po/31-2S, SB8 and 22) and 12 (SB8, SB24, SB25, SB26, SB30, Po/31-2S, Po/31-1C; Po/28, Po29, N001, 22, 1056) after 7 and 14 days of growth, respectively (Tab. 2).

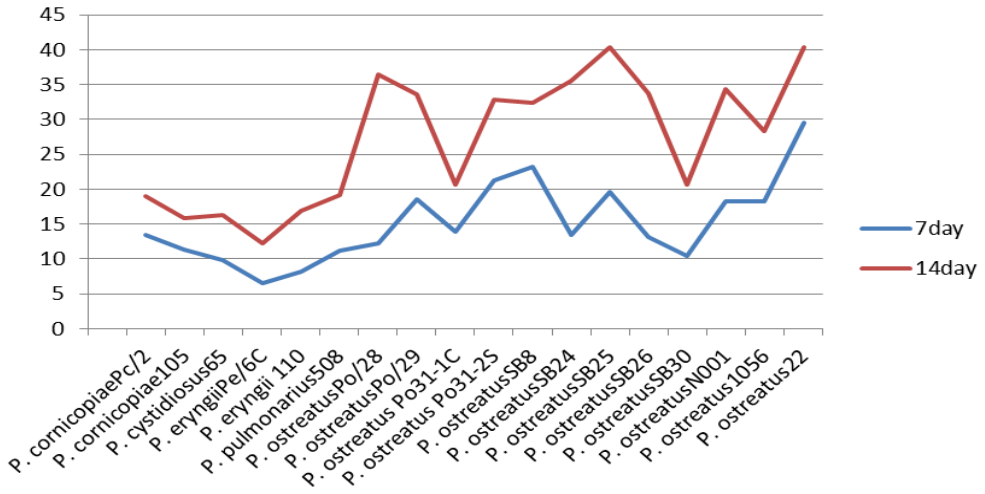

Fig. 3. Biomass (*g*/*L*) formation in studied collections of *Pleurotus* spp. during 7 and 14 days of submerged growth.

All studies species/strains mainly belonged to the second group, except Armenian (SB24, SB25), French (Po/28) and Spanish (N001) strains of *P. ostreatus* which formed up to 40.4 *g*/*L* biomass, as commercial strain 22 and included in the third group (Tab. 2). Among these strains the highest amount of biomass (33.8–40.4 *g*/*L*) after 14 days of cultivation was accumulated by Armenian strains SB24, SB25, and SB26 isolated from *Ulmus* sp., *Quercus* sp. and *Morus alba* trees, respectively. Iranian strain 1056, isolated from *Fagus* tree formed relatively low (28.4 *g*/*L*) amount of biomass (Tab. 2).

Table 2

The groups of studied Pleurotus spp. collections according to biomass formation

Group, *g*/*L*	Cultivation time	
	7 days	14 days
I , <10	*P. eryngii*: Pe/6C and 110	–
	P. cystidiosus: 65	–
II, 10–20	–	*P. eryngii*: Pe/6C and 110
	–	*P. cystidiosus* 65
	P. cornucopiae: 105 and Pc/2	*P. cornucopiae*: Pc/2 and 105
	P. pulmonarius: 508	*P. pulmonarius* 508
	P. ostreatus: Po/28, SB26, SB24, Po/31-1C, Po/29, SB25, SB30, N001, and1056	–
III, >20	*P. ostreatus*: Po/31-2S, SB8, and 22	*P. ostreatus*: SB8, SB24, SB25, SB26, SB30, Po/31-2S, Po/31-1C; Po/28, Po29, N001, 22, and 1056

Conclusion. The studied *Pleurotus* spp. collections formed round shaped fluffy pellets and disperse mycelia during their submerged growth. After 7 days of cultivation, they accumulated from 6.5 to 29.46 *g*/*L* biomass which increased from 12.23 to 40.4 *g*/*L* during 14 days of growth. More than 30 *g*/*L* biomass was detected on the 14th day in nine from 12 strains of *P. ostreatus*. Among these collections Armenian (SB24, SB25), French (Po/28) and Spanish (N001) strains, as ecologically safe resources are considered perspective for production of different biotech products, such as healthy food, pharmaceuticals and cosmeceuticals.

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REFERENCES

1. Buchalo A.S., Babitskaya V.G., et al. Biological Features of Medicinal Macromycetes in Culture (eds. S.P. Wasser). Kyiv, Alterpres. *Corresp. Member NAS Ukraine* **1** (2011), 212.
2. Badalyan S.M. Medicinal Aspects of Edible Ectomycorrhizal Mushrooms. In: *Zambonelli A., Bonito G. (eds) Edible Ectomycorrhizal Mushrooms. Current Knowledge and Future Prospects*. Heidelberg, Springer–Verlag. *Soil Biology* **34** (2012), 317–334. https://doi.org/10.1007/978-3-642-33823-6_18
3. Corrêa R.C., Brugnari T., et al. Biotechnological, Nutritional and Therapeutic Uses of *Pleurotus* spp. (Oyster Mushroom) Related with its Chemical Composition: a Review on the Past Decade Findings. *Trends Food Sci. Technol*. **50** (2016), 103–117. https://doi.10.1016/j.tifs.2016.01.012
4. Fu Z., Liu Y., Zhang Q. A Potent Pharmacological Mushroom: *Pleurotus eryngii*. *Fungal Genom. Biol.* **6** (2016), 139. https://doi.org/10.4172/2165-8056.1000139
5. Masri H.J., Maftoun P.M., et al. The Edible Mushroom *Pleurotus* spp.: II. Medicinal Values. *Int. J. Biotech. Well. Indus.* **6** (2017), 1–11. https://doi.org/10.6000/1927-3037.2017.06.01.1
6. Badalyan S.M., Barkhudaryan A., Rapior S. Recent Progress in Research on the Pharmacological Potential of Mushrooms and Prospects for their Clinical Application. In: *Agrawal D.C.., Dhanasekaran M. (eds) Medicinal Mushrooms: Recent Progress in Research and Development*. Singapore, Springer Nature (2019), 1–70. https://doi.org/10.1007/978-981-13-6382-5_1
7. Badalyan S.M., Morel S., et al. Mushrooms as Promising Therapeutic Resources: Review and Future Perspectives. In: *Agrawal D.C.., Dhanasekaran M. (eds) Mushrooms with Therapeutic Potentials: Recent Advances in Research and Development*. Singapore, Springer Nature (2023), 1–54. https://doi.org/10.1007/978-981-19-9550-7_1
8. Khatun S., Islam A., et al. Research on Mushroom as a Potential Source of Nutraceuticals: a Review on Indian Perspective. *Am. J. Experim. Agricult*. **2** (2012), 47–73. https://doi.org/10.9734/AJEA/2012/492
9. Gupta S., Summuna B., et al. Edible Mushrooms: Cultivation, Bioactive Molecules, and Health Benefits. In: *Bioactive Molecules in Food, Reference Series in Phytochemistry* (eds. J.M. Mérillon & K.G. Ramawat). Springer–Verlag (2018), 1–33. https://doi.org/10.1007/978-3-319-54528-8_86-1

10. Kalač P. *Edible Mushrooms. Chemical Composition and Nutritional Value.* Amsterdam, Academic Press (2016), 236.
11. Flores G.A., Girometta C.E., et al. Diversity of *Pleurotus* spp. (Agaricomycetes) and Their Metabolites of Nutraceutical and Therapeutic Importance. *Int. J. Med. Mushrooms* **25** (2023), 1–20. http://doi.org/10.1615/IntJMedMushrooms.2023048295
12. Vamanu E. *In vitro* Antimicrobial and Antioxidant Activities of Ethanolic Extract of Lyophilized Mycelium of *Pleurotus ostreatus* PQMZ91109. *Molecules* **17** (2012), 3653–3671. https://doi.org/10.3390/molecules17043653
13. Wang S., Bao L., et al. Isolation, Identification, and Bioactivity of Monoterpenoids and Sesquiterpenoids from the Mycelia of Edible Mushroom *Pleurotus cornucopiae. J. Agric. Food Chem.* **61** (2013), 5122–5129. https://doi.org/10.1021/jf401612t
14. Ma G., Yang W., et al. Purification, Characterization and Antitumor Activity of Polysaccharides from *Pleurotus eryngii* Residue. *Carbohydr. Polym*. **114** (2014), 297–305. https://doi.org/10.1016/j.carbpol.2014.07.069
15. Owaid M.N., Al-Saeedi S.S.S., Al-Assaffii I.A.A. Antimicrobial Activity of Mycelia of Oyster Mushroom Species (*Pleurotus spp.)* and Their Liquid Filtrates (*in vitro). J. Med. Bioengin* **4** (2015), 376–380. https://doi.org/10.12720/jomb.4.5.376-380
16. Jayasuriya W.B., Wanigatunge C.A., et al. Hypoglycaemic Activity of Culinary *Pleurotus ostreatus* and *P. cystidiosus* Mushrooms in Healthy Volunteers and Type 2 Diabetic Patients on Diet Control and the Possible Mechanisms of Action. *Phytother. Res.* **29** (2015), 303–309. https://doi.10.1002/ptr.5255
17. Tao Q.Q., Ma K., et al. New Sesquiterpenoids from the Edible Mushroom *Pleurotus cystidiosus* and Their Inhibitory Activity against α-Glucosidase and PTP1B. *Fitoterapia* **111** (2016), 29–35. https://doi.10.1016/j.fitote.2016.04.007
18. Zhang C., Li S., et al. Antioxidant and Hepatoprotective Activities of Intracellular Polysaccharide from *Pleurotus eryngii* SI-04. *Int. J. Biol. Macromol*. **91** (2016), 568–577. https://doi.10.1016/j.ijbiomac.2016.05.104
19. Adebayo E.A., Martínez-Carrera D., et al. Comparative Study of Antioxidant and Antibacterial Properties of the Edible Mushrooms *Pleurotus levis, P. ostreatus, P. pulmonarius* and *P. tuber-regium*. *Int. J. Food. Sci. Technol*. **53** (2017), 1316–1330. https://doi.10.1111/ijfs.13712
20. Yen M.T., Chang Y.H., et al. Extraction of Ergothioneine from *Pleurotus eryngii* and *P. citrinopileatus* (Agaricomycetes) and Preparation of its Product. *Int. J. Med. Mushrooms* **20** (2018), 381–392. https://doi.10.1615/IntJMedMushrooms.2018025953
21. Badalyan S.M., Rapior S. The Neuroprotective Potential of Macrofungi. In: *Medicinal Herbs and Fungi – Neurotoxicity vs. Neuroprotection* (eds. D. Agrawal & M. Dhanasekaran). Singapore, Springer Nature (2021), 37–77. https://doi.10.1007/978-981-33-4141-8_2
22. Badalyan S.M., Barkhudaryan A., Rapior S. The Cardioprotective Properties of Agaricomycetes Mushrooms Growing in the Territory of Armenia (Review). *Int. J. Med. Mushrooms* **23** (2021), 21–31. https://doi.10.1615/IntJMedMushrooms.2021038280
23. Badalyan S.M., Zambonelli A. The Potential of Mushrooms to Develop Healthy Food and Biotech Products. In: *Fungi and Fungal Products in Human Welfare and Biotechnology* (eds. T. Satyanarayana & Deshmukh SK). Singapore, Springer Nature (2023), 307–344. https://doi.org/10.1007/978-981-19-8853-0_11
24. Badalyan S.M., Zambonelli A. Biotechnological Exploitation of Macrofungi for the Production of Food, Pharmaceuticals and Cosmeceuticals. In: *Advances in Macrofungi: Diversity, Ecology and Biotechnology* (1st ed., eds. K.R. Sridhar & S.K. Deshmukh). Boca Raton, CRC Press (2019), 199–230. https://doi.org/10.1201/9781003191278
25. Barros L., Cruz T., Baptista P. Wild and Commercial Mushrooms as Source of Nutrients and Nutraceuticals. *Food Chem. Toxicol*. **46** (2008), 2742–2747. https://doi.org/10.1016/j.fct.2008.04.030

26. Reis F.S., Martins A., et al. Functional Foods Based on Extracts or Compounds Derived from Mushrooms. *Trends Food Sci. Technol*. **66** (2017), 48–62. https://doi.org/10.1016/j.tifs.2017.05.010
27. Vargas-Sánchez R.D., Torrescano-Urrutia G.R., et al. Effect of Dietary Supplementation with *Pleurotus ostreatus* on Growth Performance and Meat Quality of Japanese Quail. *Livest. Sci*. **207** (2018), 117–125. https://doi.org/10.1016/j.livsci.2017.11.015
28. Badalyan S.M., Barkhudaryan A., Rapior S. Medicinal Macrofungi As Cosmeceuticals: a Review. *Int. J. Med. Mushrooms* **24** (2022), 1–13. https://doi.org.1615/IntJMedMushrooms.2022043124
29. Badalyan S.M., Hughes K. Genetic Variability of *Flammulina velutipes* and *Pleurotus ostreatus* Collections from Armenia. In: *Science and Cultivation of Edible and Medicinal Fungi.* Proceed. XVI ISMS Int. Congr. USA, Florida, Miami Beach (2004), 149–154.
30. Badalyan S.M., Gharibyan N.G. *Characteristics of Mycelial Structures of Different Fungal Collections*. Yerevan, YSU Press (2017), 176.

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ԿԵՆՍԱԶԱՆԳՎԱԾԻ ԱՌԱՋԱՑՈՒՄԸ *PLEUROTUS* SPP. ՏԱՐԲԵՐ ՀԱՎԱՔԱԾՈՒՆԵՐԻ ԽՈՐՔԱՅԻՆ ԱՃԵՑՄԱՆ ԸՆԹԱՑՔՈՒՄ

Սնկային միցելիումի խորքային աճեցումը թույլ է տալիս կառավարել կենսատեխնոլոգիական գործընթացները, ստանալով հնարավորինս մեծ քանակի կենսազանգված: Ուսումնասիրվել է *Pleurotus* (Fr.) P. Kumm. ցեղի հինգ տեսակների (*P. ostreatus, P. cornucopiae*, *P. eryngii, P. pulmonarius* և *P. cystidiosus*) 18 շտամների կենսազանգվածի կուտակման դինամիկան խորքային աճեցման պայմաններում: *Pleurotus* spp. շտամները ձևավորել են կլորավուն, մազմզոտ պելետներ և դիսպերս միցելիում: Ուսումնասիրված հավաքածույում առավել մեծ կենսազանգված առաջացրել են *P. ostreatus* շտամները: Աճման յոթերորդ օրը այն կազմել է մինչև 29.46 *գ/լ*, իսկ 14-րդ օրը՝ 40.4 *գ/լ,* հասնելով արտադրական 22 շտամի ցուցանիշին: Ուսումնասիրված *P. ostreatus* 12 շտամներից 9-ը, մասնավորապես հայկական (SB24, SB25), Ֆրանսիական (Po/28) և Իսպանական (N001), որոնց առաջացրած կենսազանգվածը մեծ է եղել 30.0 *գ/լ*-ից, որպես էկոլոգիապես մաքուր հումք, հանդիսանում են հեռանկարային սնկային ծագման կենսատեխարտադրանքների՝ առողջարար սննդի, բուժիչ և կոսմետիկ միջոցների ստացման համար:

С. М. БАДАЛЯН, Н. Г. ГАРИБЯН

ОБРАЗОВАНИЕ БИОМАССЫ НЕКОТОРЫМИ КОЛЛЕКЦИЯМИ *PLEUROTUS* SPP. В ГЛУБИННОЙ КУЛЬТУРЕ

Глубинное культивирование мицелия грибов является наиболее экономичным и контролируемым биотехнологическим процессом, с помощью которого можно добиться быстрого роста мицелиальной биомассы. Исследована

динамика накопления биомассы 18 штаммами 5 видов (*P. ostreatus, P. cornucopiae*, *P. eryngii, P. pulmonarius*, *P. cystidiosus)* рода *Pleurotus* (Fr.) P. Kumm. в условиях глубинной культуры. Штаммы видов *Pleurotus* сформировали округлые ворсистые пеллеты и дисперсный мицелий. Среди исследованных культур наибольшую биомассу образовали штаммы *P. ostreatus:* на 7 сутки роста она составила 29.46 *г/л*, а на 14 сутки – 40.4 *г/л*, наравне с коммерческим штаммом *P. ostreatus* 22. Более 30.0 *г/л* биомассы формировали 9 из 12 исследованных штаммов *P. ostreatus*. Среди них штаммы *P. ostreatus*, в частности армянские (SB24 и SB25), французский (Po/28) и испанский (N001), в качестве экологически чистого сырья, являются перспективным для получения различных биотехпродуктов грибного происхождения пищевого, лекарственного и косметического значения.