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SCREENING OF *PLEUROTUS OSTREATUS* STRAINS AS POTENTIAL PECTIN LYASE PRODUCERS

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Subject. *Pectin is a major component of the cell walls of fruits and vegetables, contributing to the high viscosity of raw materials and complicating technological processes in the food industry. An effective approach to pectin degradation is the application of pectinases, among which pectin lyase is the most widely used. Basidiomycetes of the genus Pleurotus are promising producers of pectinases.* ***Purpose.*** *The aim of this study was to investigate pectin lyase synthesis by P. ostreatus strains cultivated in submerged culture on various synthetic media.* ***Methods.*** *The study used three strains of P. ostreatus (Fr.) P. Kumm.: P. ostreatus 226, P. ostreatus 229, and P. ostreatus 331, obtained from the Cap Fungi Collection of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (IBC). The strains were cultivated on Norcrans and Czapek media, and on a peptone-yeast medium containing 0.5% pectin as the carbon source. Cultivation was carried out under static conditions at 28 ± 1°C for 14 days. At the end of cultivation, pH was measured potentiometrically; biomass yield and dry matter content were determined gravimetrically; protein concentration and pectin lyase activity were assessed spectrophotometrically. Statistical analysis was performed using Duncan's test.* ***Results.*** *All strains demonstrated the highest biomass yields on the peptone-yeast medium with pectin (C_biomass = 1.3–1.6 g/dm³). The highest pectin lyase activity was recorded for P. ostreatus 226 cultivated in SNP medium, reaching 115.1 ± 17.6 U/g protein. P. ostreatus 331 exhibited the lowest values across all media.* ***Scope of the results.*** *The findings may be used to optimise cultivation conditions for Pleurotus ostreatus strains in the production of pectin lyase for application in the food industry.*

Key words: *pectin, pectin lyase, Pleurotus ostreatus, cultivation, enzymatic activity, food industry*

СКРИНІНГ ШТАМІВ *PLEUROTUS OSTREATUS* ЯК ПОТЕНЦІЙНИХ ПРОДУЦЕНТІВ ПЕКТИНЛІАЗИ

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Предмет. *Пектин є важливим компонентом клітинних стінок плодів та овочів, що обумовлює високу в'язкість сировини та ускладнення технологічних процесів у харчовій промисловості. Для розкладання пектину ефективним методом є використання пектиназ, серед яких найбільше застосування має пектинліаза. Базидієві макроміцети роду Pleurotus є перспективними об'єктами для синтезу пектинліаз.* ***Мета.*** *Метою дослідження було вивчення синтезу пектинліази штамами P. ostreatus у глибинній культурі на різних синтетичних середовищах.* ***Методи.*** *Об'єктами досліджень були 3 штами виду P. ostreatus (Fr.) P. Kumm.: P. ostreatus 226, P. ostreatus 229, P. ostreatus 331 отримані з Колекції шапинкових грибів Інституту ботаніки ім. М.Г. Холодного НАН України (ІБК). Штами культивували на середовищах Норкранс і Чапека, пептоно-дріжджовому середовищі, у яких джерелом вуглецю використовували 0,5% пектин. Культивування здійснювали в статичного режиму за температури 28 ± 1ºС упродовж 14 діб. В кінці культивування визначали рівень pH потенціометрично; вихід біомаси і кількість сухих речовин гравіметрично; вміст білків та активність пектинліази спектрофотометрично. Статистичну обробку даних виконували за допомогою критерію Дункана.* ***Результати.*** *Усі штами відзначилися найвищим виходом біомаси після культивування на пептоно-дріжджовому середовищі з пектином ($C_{біомаси}$ = 1,3–1,6 г/дм³). Найвища активність пектинліази спостерігалась для штаму P. ostreatus 226, який культивували у*

середовищі СНП – 115,1 ± 17,6 од/г$_{білків}$. Штам P. ostreatus 331 демонстрував найнижчі показники на всіх середовищах. ***Сфера застосування результатів.*** *Результати дослідження можуть бути використані для оптимізації умов культивування штамів Pleurotus ostreatus з метою виробництва пектинліази для харчової промисловості.*

Ключові слова: *пектин, пектинліаза, Pleurotus ostreatus, культивування, ферментативна активність, харчова промисловість*

Statement of the Problem. Pectin is a high-molecular-weight polysaccharide found in the cell walls of fruits and vegetables, with galacturonic acid as its principal monomer [1]. The presence of pectin compounds increases the pulp content and viscosity of raw materials and intermediate products, thereby complicating technological processes in the food industry [2]. Moreover, growing consumer attention to food quality in recent years has posed new challenges for the food industry and necessitated the search for innovative solutions [3].

Due to the complex chemical nature of pectin, enzymatic treatment with pectinases remains the most effective method for its degradation. Compared to physical and mechanical methods, enzymatic approaches offer significant advantages, including high substrate specificity, efficient hydrolysis, and mild reaction conditions [4, 5].

Among pectinases, particular attention has been given to lyases—specifically, pectin lyase (EC 4.2.2.2) and pectate lyase (EC 4.2.2.10). Pectin lyase is the most widely used in industrial applications and catalyzes the cleavage of α-1,4-glycosidic bonds in highly methylated pectin via β-elimination, producing 4,5-unsaturated oligogalacturonides [4]. These enzymes are typically categorized as acidic or alkaline, based on their optimal pH range. Alkaline pectin lyases are primarily employed in the textile, oil extraction, and pulp and paper industries, whereas acidic pectin lyases are more relevant to food processing [6].

A wide range of microorganisms, including fungi and bacteria, are known producers of pectinases [7]. While bacterial strains predominantly synthesize alkaline pectin lyases [1], fungi are capable of producing enzymes with optimal activity in the acidic pH range [8]. Therefore, fungal strains are more suitable for applications in the food industry.

Traditionally, pectin lyase production is carried out by surface cultivation of micromycetes. For example, Amande et al. cultivated *Aspergillus tamarii*, *A. terreus*, *A. piperis*, *A. parasiticus*, and *Mucor piriformis* on bean, mango, and plantain peels. The highest pectin lyase activity was recorded for *M. piriformis* on plantain peels (10,852.5 U/g substrate) and for *A. parasiticus* on a combined substrate (1:1:1) at 9600.5 U/g substrate [9]. However, submerged (deep) cultivation is also a viable strategy. Poturcu et al. grew *A. niger* in a medium containing wheat bran and pectin (initial pH 4.0, 40 °C, 100 rpm), achieving a maximum enzyme activity of 5 U/g on day 5 [10]. *A. brasiliensis* cultivated in a synthetic medium with 42 g/dm³ pectin (initial pH 5.5, 30 °C, 180 rpm) exhibited pectin lyase activity of 31 U/cm³, which increased to 46 U/cm³ when orange peel (160 g/dm³) was used as the substrate [11]. Similarly, Yadav et al. reported a specific activity of 0.08 U/mg when *A. flavus* was cultivated in a synthetic liquid medium with 0.5% pectin [12].

Pectin lyase is classified within the PL1, PL3, and PL9 families, according to the Carbohydrate-Active enZYmes (CAZy) database [13]. Micromycetes often possess multiple genes from these families, correlating with high enzymatic activity [14]. Recently, increasing attention has been given to basidiomycetes (macromycetes), which also show potential for pectinolytic enzyme production. Although their enzymatic profiles remain poorly characterized, the presence of PL1 and PL3 genes in representatives of *Trametes*, *Pleurotus*, and other basidiomycetes suggests their possible involvement in the degradation of wood-derived pectin components [15]. Research on pectinases from macromycetes remains limited. Rashad et al. investigated pectin lyase production by *P. ostreatus* grown on lemon peels [16], while Xie et al. used hibiscus stems, comfrey, and cotton seed husks as substrates for cultivating *P. eryngii* [17]. These findings highlight the relevance of further studies on pectin lyase synthesis by *P. ostreatus*.

Therefore, the aim of this study was to investigate the synthesis of pectin lyase by *Pleurotus ostreatus* strains under submerged cultivation on different synthetic media.

Materials and Methods. Three strains of *Pleurotus ostreatus* (Fr.) P. Kumm – *P. ostreatus* 226, *P. ostreatus* 229, and *P. ostreatus* 331 – obtained from the Collection of Cap Fungi of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (IBK) [18], were used as objects of study.

Fungal growth and enzymatic activity were evaluated in three types of synthetic media, all supplemented with pectin:

- Modified Norcrans medium (MNP, g/dm^3): pectin – 5.0, NH_4NO_3 – 1.0, KH_2PO_4 – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.5, $FeSO_4 \cdot 7H_2O$ – 0.005, $ZnSO_4 \cdot 7H_2O$ – 0.0044, $CaCl_2$ – 0.0055 [19];
- Peptone-yeast medium (PYM, g/dm^3): pectin – 5.0, peptone – 3.0, yeast extract – 3.0, $MgSO \cdot 7H_2O$ – 0.25 [20];
- Synthetic pectin medium (SPM, g/dm^3): pectin – 5.0, KH_2PO_4 – 1.0, K_2HPO_4 – 1.0, NH_4NO_3 – 3.0, $MgSO4 \cdot 7H_2O$ – 0.6 [19].

The initial pH of all media was adjusted to 6.8 ± 0.2. Inoculation was performed by transferring three agar discs containing a seven-day-old fungal culture previously grown on barley malt extract. Cultivation was conducted under static conditions in 250 cm^3 Erlenmeyer flasks containing 50 cm^3 of culture medium, incubated at 28 ± 1ºC for 14 days [21].

Upon completion of cultivation, the fungal biomass was separated from the culture liquid by filtration and dried to constant weight at 105 ± 5°C to determine the absolute dry biomass content (ADB, g/dm^3). The pH of the culture liquid was measured potentiometrically [19]. Protein concentration was determined using the Lowry method [22]. Dry matter content was evaluated gravimetrically by drying to constant weight at 105 ± 5°C [19].

Pectin lyase activity was measured in the culture liquid using a 1% pectin solution as the substrate. One unit of pectin lyase activity (PLU, units) was defined as the amount of enzyme required to catalyse the formation of 1 μmol of unsaturated galacturonide per minute per gram of protein [23].

The economic coefficient on dry matter (EC_{dm},%) was calculated as described by [19]. Pectin lyase productivity (υ, units/(g_{ADB})) was calculated using the following formula [24]:

$$\upsilon = \frac{PLU}{ADB} \quad (1)$$

All experiments were performed in triplicate. Statistical analysis was carried out using Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$. Index "1" indicates significant differences between strains grown on the same medium; index "2" indicates differences between media for the same strain. Data analysis and graphical visualization were performed using Microsoft Excel (USA).

Results and discussion. The study evaluated the effect of three variants of the culture medium on the growth (ADB), acidity of the culture liquid (pH) and dry matter assimilation (EC_{dm}) of three *P. ostreatus* strains (226, 229 and 331). The main parameters of cultivation of the studied P. ostreatus strains are presented in Table 1.

In all experimental variants, a decrease in pH of the culture medium was observed relative to the initial values (6.6–7.0). There were no statistically significant differences in the final pH between strains grown on the same medium, indicating a similar acidification pattern during mycelial growth. However, the final pH differed significantly between media. The most pronounced acidification occurred in the PYM medium (pH 4.3–4.9), whereas the highest pH values were recorded in the MNP medium (pH 5.0–5.8).

Dry biomass accumulation varied depending on the nutrient medium. The highest biomass concentrations were observed in PYM, with all strains reaching 1.3–1.6 g/dm^3, and no statistically significant inter-strain differences. In contrast, in MNP, *P. ostreatus* 226 exhibited reduced growth (0.9–1.1 g/dm^3), while *P. ostreatus* 229 and *P. ostreatus* 331 maintained biomass levels comparable to those on PYM. In the SPM medium, biomass production was

reduced only in *P. ostreatus* 229 (1.0–1.2 g/dm^3), while the other strains performed similarly to the PPD variant.

Table 1

Cultivation indicators of P. ostreatus

Medium	P. ostreatus 226	P. ostreatus 229	P. ostreatus 331
pH			
PYM	4,5 ± 0,1$^{a1, c2}$	4,4 ± 0,1$^{a1, c2}$	4,7 ± 0,2$^{a1, b2}$
MNP	5,7 ± 0,6$^{a1, a2}$	5,8 ± 0,1$^{a1, a2}$	5,1 ± 0,1$^{a1, a2}$
SPM	5,1 ± 0,1$^{a1, a2}$	4,9 ± 0,1$^{a1, b2}$	5,2 ± 0,1$^{a1, a2}$
ADB, g/dm^3			
PYM	1,5 ± 0,1$^{a1, a2}$	1,4 ± 0,1$^{a1, a2}$	1,5 ± 0,1$^{a1, a2}$
MNP	1,0 ± 0,1$^{b1, b2}$	1,4 ± 0,1$^{a1, a2}$	1,3 ± 0,1$^{a1, b2}$
SPM	1,4 ± 0,1$^{a1, a2}$	1,1 ± 0,1$^{b1, b2}$	1,3 ± 0,1$^{ab1, b2}$
EC_{dm},%			
PYM	21,3 ± 0,9$^{b1, b2}$	26,2 ± 1,8$^{a1, b2}$	18,8 ± 0,7$^{c1, b2}$
MNP	23,8 ± 1,2$^{b1, ab2}$	31,0 ± 1,8$^{a1, a2}$	26,9 ± 2,2$^{b1, a2}$
SPM	29,7 ± 4,6$^{a1, a2}$	29,8 ± 5,6$^{a1, a2}$	25,8 ± 5,3$^{a1, a2}$
Notes: PYM - Peptone-yeast medium, MNP - Modified Norcrans medium, SPM - Synthetic pectin medium			

Among the cultivation parameters evaluated, the economic coefficient on dry matter (ECdm) exhibited the highest variability, indicating the medium's significant influence on the metabolic efficiency of the fungus. EC_{dm} reflects the ability of the organism to convert substrate dry matter into fungal biomass; therefore, it serves as an important indicator of cultivation efficiency. The highest EC_{dm} values were recorded in MNP and SPM media, with maxima in *P. ostreatus* 229 (29.2–32.8% in MNP and 24.2–35.4% in SPM) that correspond to more efficient substrate utilization and biomass production in MNP and SPM. In contrast, the lowest EC_{dm} was observed in *P. ostreatus* 331 grown on PYM medium (18.8%), significantly lower than under other conditions.

Analysis of pectin lyase activity in the culture liquids (Fig. 1) revealed a substantial influence of the culture medium on enzyme synthesis levels. The data presented in Fig. 1 demonstrate significant differences in PLU values among *P. ostreatus* strains depending on the cultivation medium. The highest activity was recorded in *P. ostreatus* 226 grown on SNP (115.1 ± 17.6 units/g protein), exceeding the corresponding values for *P. ostreatus* 229 and *P. ostreatus* 331 by 11.7% and 45.1%, respectively. On all media, *P. ostreatus* 331 exhibited the lowest υ values: 33.2 ± 4.1 units/g on PPD, 62.2 ± 16.8 units/g on MNP, and only 13.3 ± 11.6 units/g on SPM. These results suggest a low basal expression level of pectin lyase in this strain.

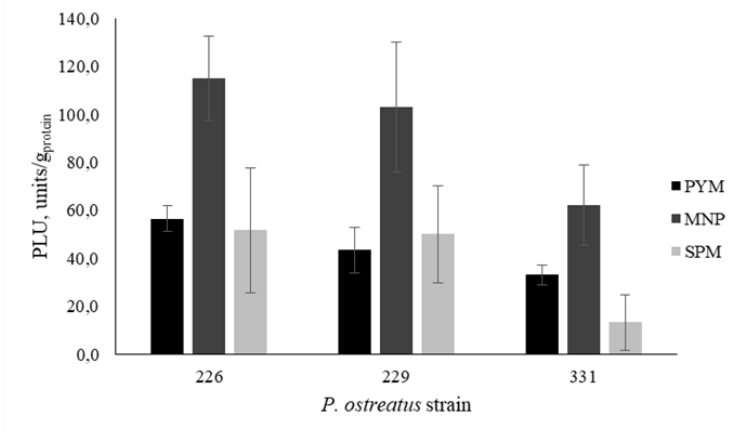

Fig. 1. Specific pectin lyase activity of *P. ostreatus* strains

Overall, the MNP medium supported the highest productivity across all strains, whereas SMP yielded the lowest average activity. This trend may be attributed to the specific composition of the media, particularly the presence of Ca^{2+} ions in SNP, which are known to stimulate pectin lyase synthesis [25].

As shown in Fig. 2, the highest productivity of pectin lyase synthesis (expressed as units per gram of dry biomass) was observed in *P. ostreatus* 226 cultivated on SNP (34,366.7 ± 6,105.6 units/g). On the same medium, *P. ostreatus* 229 reached 26,906.1 ± 5,836.7 units/g, while *P. ostreatus* 331 yielded only 19,479.2 ± 5,520.7 units/g. Thus, *P. ostreatus* 226 demonstrated the highest efficiency of enzyme accumulation in Norcrans medium. The lowest productivity was consistently recorded in *P. ostreatus* 331, with a minimum in SPM, further confirming the weak induction of pectinolytic activity in this strain.

These findings align with previous studies highlighting the role of medium components in modulating pectinase production. For instance, the presence of pectin as a carbon source has been shown to significantly enhance pectinase activity in various fungi, including *A. niger*. Moreover, factor such as nitrogen source, has been reported to affect enzyme yields [26, 27].

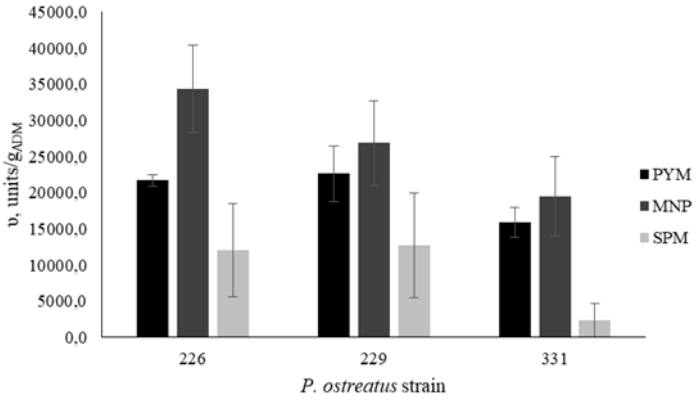

Fig. 2. Productivity of pectin lyase synthesis by P. ostreatus strains

Conclusions. *Pleurotus ostreatus* 229 demonstrated the highest efficiency of substrate assimilation in MNP and SPM media (up to 35.4%), indicating a strong capacity for utilizing available carbon sources. However, *P. ostreatus* 226 was characterized by the highest pectin lyase synthesis rates on Norcrans medium containing pectin (115.1 ± 17.6 units/g protein), surpassing the activity of the other strains. In contrast, *P. ostreatus* 331 showed minimal enzymatic activity regardless of the medium, reflecting a limited potential for pectin lyase synthesis under the tested conditions.

These results suggest that cultivation of *P. ostreatus* 226 on pectin-enriched Norcrans medium is a promising strategy for further biotechnological optimization and for the development of enzyme preparations intended for application in the food industry.

References

1. Bai, Y., Wang, J., Yan, Y., Zhan, Y., Zhou, Z., & Lin, M. (2025). A Low-Temperature-Active Pectate Lyase from a Marine Bacterium for Orange Juice Clarification. Microorganisms, 13 (3), 634. https://doi.org/10.3390/microorganisms13030634.

2. Kumar, A., Yadav, M., & Sehrawat, N. (2019). Microbial enzymes and additives for the food industry. 307 p.

3. Espejo, F. (2021). Role of commercial enzymes in wine production: A critical review of recent research. Journal of Food Science and Technology, 58(1), 9-21. https://doi.org/10.1007/s13197-020-04489-0.

4. Zheng, L., Xu, Y., Li, Q., & Zhu, B. (2021). Pectinolytic lyases: a comprehensive review of sources, category, property, structure, and catalytic mechanism of pectate lyases and pectin lyases. Bioresources and Bioprocessing, 8, 1–13. https://doi.org/10.1186/s40643-021-00432-z.

5. Mahesh, M., Arivizhivendhan, K. V., Maharaja, P., Boopathy, R., Hamsavathani, V., Sekaran, G. (2016). Production, purification and immobilization of pectinase from Aspergillus ibericus onto functionalized nanoporous activated carbon (FNAC) and its application on treatment of pectin containing wastewater. Journal of Molecular Catalysis B: Enzymatic, 133, 43–54. https://doi.org/10.1016/j.molcatb.2016.07.012.

6. Li, P. W., Ma, J., Wei, X. F., Zhang, Z. Y., Wang, R. M., Xiao, J., & Wang, J. Q. (2022). Modification and application of highly active alkaline pectin lyase. Amb Express, 12 (1), 130. https://doi.org/10.1186/s13568-022-01472-0.

7. Tasgin, E., Nadaroglu, H., Babagil, A., & Demir, N. (2020). Immobilization of purified pectin lyase from Pseudomonas putida onto magnetic lily flowers (Lilium candidum L.) nanoparticles and applicability in industrial processes. Molecules, 25 (11), 2671. https://doi.org/10.3390/molecules25112671.

8. Liu, C., Qin, X., Liu, B., Xu, X., Deng, A., Zhang, Y., Zhang, Z., Zhang, W. (2022). High-yield production of acidic pectin lyase PNLZJ5B for juice processing. Letters in Applied Microbiology, 75 (4), 1055-1062. https://doi.org/10.1111/lam.13781.

9. Amande, T. J., Ado, B. V., & Adebayo-Tayo, B. C. (2022). Production of fungal pectin lyase and polygalacturonase from fruit wastes by solid state fermentation. Biotech J Int, 36(4), 48–56. https://doi.org/10.9734/bji/2022/v26i4656.

10. Poturcu, K., Ozmen, I., Biyik, H. H. (2017). Characterization of an alkaline thermostable pectin lyase from newly isolated Aspergillus niger _WHAK1 and its application on fruit juice clarification. Arabian Journal for Science and Engineering, 42, 19–29. https://doi.org/10.1007/s13369-016-2041-6.

11. Pili, J., Danielli, A., Nyari, N. L., Zeni, J., Cansian, R. L., Backes, G. T., & Valduga, E. (2018). Biotechnological potential of agro-industrial waste in the synthesis of pectin lyase from Aspergillus brasiliensis. Food Science and Technology International, 24 (2), 97–109. https://doi.org/10.1177/1082013217733574.

12. Yadav, S., Dubey, A. K., Anand, G., Yadav, D. (2013). Purification and characterization of pectin lyase secreted by Aspergillus flavus MTCC 10938. Applied Biochemistry and Microbiology, 49, 400–405. https://doi.org/10.1134/s0003683813040145.

13. Yadav, K., Dwivedi, S., Gupta, S., Tanveer, A., Yadav, S., Yadav, P.K., Anand, G., Yadav, D. (2023). Recent insights into microbial pectin lyases: a review. Process Biochemistry, 134, 199–217. https://doi.org/10.1016/j.procbio.2023.10.008.

14. Zeuner, B., Thomsen, T. B., Stringer, M. A., Krogh, K. B., Meyer, A. S., Holck, J. (2020). Comparative characterization of Aspergillus pectin lyases by discriminative substrate degradation profiling. Frontiers in Bioengineering and Biotechnology, 8, 873. https://doi.org/10.3389/fbioe.2020.00873.

15. Ruiz-Dueñas, F.J., Barrasa, J.M., Sánchez-García, M., Camarero, S., Miyauchi, S., Serrano, A., Linde, D., Babiker, R., Drula, E., Ayuso-Fernández, I. Pacheco, R. (2021). Genomic analysis enlightens Agaricales lifestyle evolution and increasing peroxidase diversity. Molecular biology and evolution, 38(4), 1428-1446. https://doi.org/10.1093/molbev/msaa301.

16. Rashad, M. M., Abdou, H. M., Shousha, W. G., Ali, M. M., & El-Sayed, N. N. (2011). Purification and characterization of the pectin lyase produced by Pleurotus ostreatus grown on lemon pulp waste. 1377–1384.

17. Xie, C., Yan, L., Gong, W., Zhu, Z., Tan, S., Chen, D., Hu, Z., Peng, Y. (2016). Effects of different substrates on lignocellulosic enzyme expression, enzyme activity, substrate utilization and biological efficiency of Pleurotus eryngii. Cellular Physiology and Biochemistry, 39(4), 1479–1494. https://doi.org/10.1159/000447851.

18. Bisko, N., Lomberg, M., Mykchaylova, O., & Mytropolska, N. (2020). IBK Mushroom Culture Collection. Version 1.2. The IBK Mu-shroom Culture Collection of the M.G. Kholodny Institute of Botany. M. G. Kholodny Institute of Botany. https://doi.org/10.15468/dzdsqu.

19. Bukhalo AS. Higher edible basidiomycetes in pure culture. Kyiv: Naukova Dumka; 1988. 144.

20. Mykchaylova, O., Poyedinok, N. (2021). Antimicrobial Activity of Fomitopsis Officinalis (Vill.) Bondartsev & Singer in Pure Culture. Innovative Biosystems and Bioengineering, 5 (4), 220–227. https://doi.org/10.20535/ibb.2021.5.4.246668.

21. Lomberg, M., Krupodorova, T., Krasinko, V., Mykchaylova, O. (2023). The antibacterial activity of culture filtrates and mycelia of selected strains of macromycetes from the genus Hericium. Botanica Serbica, 47(2), 241–249. https://doi.org/10.2298/botserb23022411.

22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. 193(1). 265–275.

23. Çelik, S. Y., Demir, N., Demir, Y., Adiguzel, A., Gulluce, M. (2014). Production of pectin lyase from Geobacillus pallidus p26, purification, characterization and fruit juice application. Acta Chimica Slovaca, 7 (1), 57–63. https://doi.org/10.2478/acs-2014-0011.

24. Dagbagli, S., Goksungur, Y. (2008). Optimization of ß-galactosidase production using Kluyveromyces lactis NRRL Y-8279 by response surface methodology. Electronic Journal of Biotechnology, 11(4), https://doi.org/10.2225/vol11-issue4-fulltext-12.

25. Yadav, S., Yadav, P. K., Yadav, D., Yadav, K. D. S. (2009). Pectin lyase: a review. Process Biochemistry, 44(1), 1-10. https://doi.org/10.1016/j.procbio.2008.09.012.

26. Jalil, M. T. M., Zakaria, N. A., Salikin, N. H., & Ibrahim, D. (2023). Assessment of cultivation parameters influencing pectinase production by Aspergillus niger LFP-1 in submerged fermentation. Journal of Genetic Engineering and Biotechnology, 21 (1), 45. https://doi.org/10.1186/s43141-023-00510-z.

27. Linde, G. A., Magagnin, G., Costa, J. A. V., Bertolin, T. E., Colauto, N. B. (2007). Column bioreactor use for optimization of pectinase production in solid substrate cultivation. Brazilian Journal of Microbiology, 38, 557-562. https://doi.org/10.1590/S1517-83822007000300033.