

ABSTRACT&REFERENCES

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LEVEL OF CYCLIN D1 PROTEIN IN PERIPHERAL BLOOD LYMPHOCYTES OF CHORNOBYL CLEAN-UP WORKERS IN REMOTE PERIOD AFTER RADIATION EXPOSURE

p. 4-9

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Aim: to assess changes of products of Cyclin D1 protein in lymphocytes of peripheral blood of Chernobyl clean-up workers as remote results of the action of ionizing irradiation.

Methods: there were examined 120 Chernobyl clean-up workers in the remote period after radiation exposure and 45 persons of the control group. For assessing mitogen-induced levels of Cyclin D1, the micro-method of cultivating erythrocytes of whole blood was used. The quantitative assessment of the spontaneous and mitogen-induced levels of Cyclin D1 in lymphocytes of peripheral blood (PB) was realized using the reagents FITC Mouse Anti-Human Cyclin D1 Antibody Set (BD, USA) by the method of flow cytometry.

Research results: there was determined the dose-dependent increase of the spontaneous level of Cyclin D1 in PB lymphocytes of Chernobyl clean-up workers. High

values of the parameter were established in the subgroup of Chernobyl clean-up workers, irradiated in the diapason of doses as 500–1000 mSv. Maximal values of Cyclin D1 level in PB lymphocytes were observed in Chernobyl clean-up workers at exacerbation of the bronchial-pulmonary pathology, bronchial asthma in anamnesis and in reconvalescents of acute radiation sickness with the radiation doses $D \geq 500$ mSv. After mitogen stimulation of lymphocytes, there was noted the decrease of Cyclin D1 level in the group of Chernobyl clean-up workers and the increase in persons of the control group.

Conclusions: the research revealed the differences in products of Cyclin D1 in PB lymphocytes of Chernobyl clean-up workers and persons of the control group. The revealed changes of the spontaneous and mitogen-induced levels of Cyclin D1 in PB lymphocytes of Chernobyl clean-up workers with the somatic pathology reflect disorders in regulation processes of proliferation and cellular cycle. The obtained data add ideas about mechanisms of the radiation-induced disorder of the cellular cycle that may be a manifestation of genome instability and become a trigger factor of carcinogenesis in the remote period after radiation exposure

Keywords: Cyclin D1, cellular cycle, genome instability, lymphocytes, ionizing irradiation, CAES accident

References

1. Guerra, L., Cortes-Bratti, X., Guidi, R., Frisan, T. (2011). The Biology of the Cytolethal Distending Toxins. *Toxins*, 3 (3), 172–190. doi: <http://doi.org/10.3390/toxins3030172>
2. Saini, D., Shelke, S., Mani Vannan, A., Toprani, S., Jain, V., Das, B., Seshadri, M. (2012). Transcription profile of DNA damage response genes at G0 lymphocytes exposed to gamma radiation. *Molecular and Cellular Biochemistry*, 364 (1-2), 271–281. doi: <http://doi.org/10.1007/s11010-012-1227-9>
3. Tewari, S., Khan, K., Husain, N., Rastogi, M., Mishra, S. P., Srivastav, A. K. (2016). Peripheral Blood Lymphocytes as In Vitro Model to Evaluate Genomic Instability Caused by Low Dose Radiation. *Asian Pacific Journal of Cancer Prevention*, 17 (4), 1773–1777. doi: <http://doi.org/10.7314/apjcp.2016.17.4.1773>
4. Ilyenko, I. M., Bazyka, D. A., Chumak, S. A., Lohanovskiy, K. M. (2012). Osoblyvosti ekspresii heniv-rehuliatoriv apoptozu ta klitynnoho tsyklu limfotsytiv peryferychnoi krovi pry porushenniakh kohnityvnykh funktsii u uchashnykh likvidatsii naslidkiv avarii na Chornobylskii AES. *Problemy radiatsiinoi medytsyny ta radiobiologii*, 17, 163–176.

5. Wang, H.-Y., Chen, Y.-B., Gong, S.-L., Qu, L. (2012). The expression of β -catenin, cyclin D1 and c-myc mRNA on thymus tissue exposed irradiation. 2012 International Conference on Biomedical Engineering and Biotechnology. Macao, 1822–1825. doi: <http://doi.org/10.1109/icbeb.2012.425>
6. Mahdey, H. M., Ramanathan, A., Ismail, S. M., Abraham, M. T., Jamaluddin, M., Zain, R. B. (2011). Cyclin D1 Amplification in Tongue and Cheek Squamous Cell Carcinomas. *Asian Pacific Journal of Cancer Prevention*, 12 (9), 2199–2204.
7. Casimiro, M. C., Velasco-Velázquez, M., Aguirre-Alvarado, C., Pestell, R. G. (2014). Overview of cyclins D1 function in cancer and the CDK inhibitor landscape: past and present. *Expert Opinion on Investigational Drugs*, 23 (3), 295–304. doi: <http://doi.org/10.1517/13543784.2014.867017>
8. Jirawatnotai, S., Hu, Y., Livingston, D. M., Sicinski, P. (2012). Proteomic identification of a direct role for cyclin d1 in DNA damage repair. *Cancer Research*, 72 (17), 4289–4293. doi: <http://doi.org/10.1158/0008-5472.can-11-3549>
9. Hitomi, M., Yang, K., Stacey, A. W., Stacey, D. W. (2008). Phosphorylation of cyclin D1 regulated by ATM or ATR controls cell cycle progression. *Molecular and Cellular Biology*, 28 (17), 5478–5493. doi: <http://doi.org/10.1128/mcb.02047-07>
10. Shimura, T., Kobayashi, J., Komatsu, K., Kunugita, N. (2014). DNA damage signaling guards against perturbation of cyclin D1 expression triggered by low-dose long-term fractionated radiation. *Oncogenesis*, 3 (12), e132–e132. doi: <http://doi.org/10.1038/oncsis.2014.48>
11. Chaves-Ferreira, M., Krenn, G., Vasseur, F., Barinov, A., Gonçalves, P., Azogui, O. et. al. (2016). The cyclin D1 carboxyl regulatory domain controls the division and differentiation of hematopoietic cells. *Biology Direct*, 11 (1). doi: <http://doi.org/10.1186/s13062-016-0122-9>
12. Shimura, T., Ochiai, Y., Noma, N., Oikawa, T., Sano, Y., Fukumoto, M. (2013). Cyclin D1 overexpression perturbs DNA replication and induces replication-associated DNA double-strand breaks in acquired radioresistant cells. *Cell Cycle*, 12 (5), 773–782. doi: <http://doi.org/10.4161/cc.23719>
13. Bazyka D. A., Kubashko A. V., Ilyenko I. M., Belyaev O. A., Pleskach O. J. (2015). Ekspressiia bilka Cyclin D1 ta heniv CCND1 i PNKP u mononuklearakh peryferychnoi krovi uchasnykiv likvidatsii naslidkiv avarii na ChAES z riznym stanom imunitetu. *Problemy radiatsiinoi medyt-syny ta radiobiologii*, 20, 269–282.
14. Pestell, R. G. (2013). New roles of cyclin D1. *The American Journal of Pathology*, 183 (1), 3–9. doi: <http://doi.org/10.1016/j.ajpath.2013.03.001>
15. Casimiro, M. C. Pestell, R. G. (2012). Cyclin D1 induces chromosomal instability. *Oncotarget*, 3 (3), 224–225. doi: <http://doi.org/10.18632/oncotarget.476>
16. Kovalenko, A. N. (2012). Chernobylskye ocherky klynytsysta. Nikolaev: ChHU ym. Petra Mohyly, 347.
17. Lou, X., Zhang, J., Liu, S., Xu, N., Liao, D. J. (2014). The other side of the coin: the tumor-suppressive aspect of oncogenes and the oncogenic aspect of tumor-suppressive genes, such as those along the CCND-CDK4/6-RB axis. *Cell Cycle*, 13 (11), 1677–1693. doi: <http://doi.org/10.4161/cc.29082>
18. Shimura, T., Kakuda, S., Ochiai, Y., Nakagawa, H., Kuwahara, Y., Takai, Y. et. al. (2010). Acquired radioresistance of human tumor cells by DNA-PK/AKT/GSK3 β -mediated cyclin D1 overexpression. *Oncogene*, 29 (34), 4826–4837. doi: <http://doi.org/10.1038/onc.2010.238>
19. Shimura, T., Fukumoto, M., Kunugita, N. (2013). The role of cyclin D1 in response to long-term exposure to ionizing radiation. *Cell Cycle*, 12 (17), 2738–2743. doi: <http://doi.org/10.4161/cc.25746>
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- DOI: 10.15587/2519-8025.2019.165608**
- SEASONAL DYNAMICS OF FLOWERING OF *CALLUNA VULGARIS* (L.) HULL AND ITS CULTIVARS IN CONDITIONS OF KYIV REGARDING TO PECULIARITIES OF THE FLOWER STRUCTURE**
- p. 10-13**
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- The aim of our study was to research the connection between the flower structure and the duration of flowering of *Calluna vulgaris* (L.) Hull and its cultivars. Our tasks were to investigate the flower structure of bud bloomers and double-flowered *Calluna vulgaris* (L.) Hull cultivars, to compare it with the structure of *C. vulgaris* flower; to analyze the data of phenological observations of *C. vulgaris* and its 10 cultivars flowering phase during 2015–2018.*
- The methods were phenological observations and microscopic examinations.*
- Results. Common features for *C. vulgaris* and cultivars concerning to their flowering are racemose inflorescences, an arrangement of flowers, acropetal succession. The flower structure of ‘Dirry’, ‘Mullion’, ‘Golden Carpet’,*

'Winter Red' is similar to that of *C. Vulgaris*. Among this group, 'Winter Red' finishes blooming the first and 'Dirry' is the last due to its compound inflorescence. An increased number of petals, full sterility and long flowering period characterize flowers of double-flowered cultivars. Bud bloomers 'Alicia', 'Bonita' belong to *f. diplocalyx* Jansen. They are characterized by different flowering periods. 'Alicia' blooms the longest (85 ± 7 days) among investigated *C. vulgaris* cultivars and 'Bonita' blooms about 53 days.

Conclusions. *C. vulgaris* cultivars that have the same flower structure as species begin to bloom, as well as common heather, in the third decade of July. The duration of *C. vulgaris* cultivars flowering period depends also on the peculiarities of the inflorescence structure. Therefore, in order to predict the duration of cultivars flowering, it is necessary to study the features of their inflorescences structure. Double-flowered cultivars are worth to be used more often in gardening due to their high decorativeness and long flowering period. The issue of preserving the color of bud bloomers flowers needs further research. When using the cultivars of *C. vulgaris* with different types of flowers and duration of flowering, we can create landscapes of high and long decorative features

Keywords: *Calluna vulgaris*, cultivar, flower, corolla, calyx, bloom, decorative features

References

- Kurlovich, T. V. (2016). Morfobiologicheskie osobennosti i dekorativnye kachestva belorusskikh sortov vereska obyknovennogo *Calluna vulgaris* (L.) Hull [Morphobiological features and decorative qualities of Belarusian varieties of common heather (*Calluna vulgaris* (L.) Hull)]. Floriculture: history, theory, practice. Minsk: Konfido, 262–265.
- Wilson, D. (2016). Bud bloomers: a brief overview. *Heather News Quarterly*, 39 (1), 4–6. Available at: <http://www.heathsandheathers.com/CHS/HNQWinter20162.pdf>
- Calluna* cultivars list of Heather Society. Available at: <https://www.heathersociety.org/category/heathers/calluna-heathers>
- Schröder, J. (2005). The explosion of bud-flowerers. *Heathers: yearbook of the Heather Society*, 2, 17–18. Available at: <http://www.heathsandheathers.com/CHS/HNQWinter20162.pdf>
- Borchert, T., Eckardt, K., Fuchs, J., Kruger, K., Hohe, A. (2009). «Who's who» in two different flower types of *Calluna vulgaris* (Ericaceae): morphological and molecular analyses of flower organ identity. *BMC Plant Biology*, 9 (1), 148. doi: <http://doi.org/10.1186/1471-2229-9-148>
- Behrend, A., Borchert, T., Müller, A., Tänzer, J., Hohe, A. (2012). Malformation of gynoecia impedes fertilisation in bud-flowering *Calluna vulgaris*. *Plant Biology*, 15 (1), 226–232. doi: <http://doi.org/10.1111/j.1438-8677.2012.00619.x>
- Behrend, A., Borchert, T., Hohe, A. (2015). "The usual suspects"- analysis of transcriptome sequences reveals deviating B gene activity in *C. vulgaris* bud bloomers. *BMC Plant Biology*, 15 (1), 8. doi: <http://doi.org/10.1186/s12870-014-0407-z>
- Jansen, J. (1935). Over eenige in ons land aangetroffen vormen van *Calluna vulgaris*. *Nederlandsch kruidkundig archief. Serie 3*, 45, 126–128. Available at: <http://natuurtijdschriften.nl/download?type=document&doid=548950>
- Volod'ko, I., Lunina, N., Svitkovskaya, O., Gayshun, V., Ryzhenkova, Yu. (2009). Opyt pervichnoi introdukcii dekorativnyh rastenii aborigennoi flory Belarusi [The experience of the primary introduction of ornamental plants of the native flora of Belarus]. *Taras Shevchenko National University of Kyiv Bulletin. Introduction and Conservation of Plant Diversity*, 19-21, 59–61.
- Sannikov, S. N., Petrova, I. V., Cherepanova, O. E. (2016). Al'ternativnye gipotezy proishozhdeniya vereska *Calluna vulgaris* (L.) Hull [Alternative hypotheses of the origin of heather *Calluna vulgaris* (L.) Hull]. *Eco potential*, 2 (14), 28–40.
- Kokhno, M. A. (Ed.) (2002). *Dendroflora Ukrainy. Dykorosli kultyrovani dereva i kushchi. Pokrytonasinni [Dendroflora of Ukraine. Wild cultivated trees and shrubs. Angiosperms]*. Kyiv: Fitosotsiotsentr, 317.
- Yezhel, I. M. (2014). Vydy rodyny Ericaceae Juss. Pravoberezhzhja Polissia Ukrainy: ekologo-morfologichni osoblyvosti, perspektyvy vykorystannia [Species of Ericaceae Juss. of Right Bank of the Polissya of Ukraine: ecological and morphological features, prospects of use]. Kyiv, 200.
- Yezhel, I. M. (2012). Zalezhnist' biometrychnykh pokaznykiv *Calluna vulgaris* (L.) Hull (Ericaceae Juss.) vid kyslotnosti g'runtiv na Pravoberezhnomu Polissi Ukrainy [Dependence of *Calluna vulgaris* (L.) Hull (Ericaceae Juss.) biometric indicators of soil acidity on the Right Bank Polissya of Ukraine]. *Taras Shevchenko National University of Kyiv Bulletin. Introduction and Conservation of Plant Diversity*, 30, 19–22.
- Lapin, P. I. (Ed.) (1975). *Metodika fenologicheskikh nablyudeniy v botanicheskikh sadakh SSSR [Methods of phenological observations in USSR botanical gardens]*. Moscow, 27.
- Zyman, S. M., Mosiakin, S. L., Bulakh, O. V., Tsarenko, O. M., Felbaba-Klushyna, L. M. (2004). *Iliustrovani dovidnyk z morfolohii kvitkovykh Roslyn [Illustrated reference book on the morphology of flowering plants]*. Uzhhorod: Medium, 156.
- Veremchuk, O. A., Moiseev, D. V. (2014). Makro- i mikroskopicheskie priznaki pobegov vereska obyknovenn-

nogo i ih proiavljaemost' pri razlichnoi stepeni izmel'chenosti [Macro and microscopic features of shoots of common heather and its manifestation with varying degrees of grinding]. Pharmacy Bulletin, 3 (65), 49–54. Available at: <https://elibrary.ru/item.asp?id=22586321>

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POLYMORPHISM OF GENE HEMAGGLUTININ AND ITS INFLUENCE ON THE PROPERTIES OF INFLUENZA VIRUS A H1N1 AND H7N9 STRAINS

p. 14-17

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Influenza virus A is a causative agent of zoonotic and anthroponosis diseases. Virulence and contagious properties of the influenza virus are dependent on the presence of hemagglutinin. The membrane glycoprotein hemagglutinin has an important part in the adhesion and invasion of the influenza virus to the cell, as well as in the formation of immunity of the host organism. Hemagglutinin forms pandemic properties of influenza strains. Systematics and characterization of the influenza virus strains are based in particular on the type of hemagglutinin. The most contagious strains of the influenza A virus are strains H1N1 and H7N9. Polymorphism of hemagglutinin and its effect on the properties of influenza strains makes it relevant to study its variability.

The aim of the study. The purpose of the study was to determine the polymorphism of the hemagglutinin encoding gene of strains of the influenza A H1N1 and H7N9 strains, to determine its effect on the polymorphism of the amino acid sequences of hemagglutinin and the properties of the strains by bioinformatic research methods.

Materials and methods. The nucleotide sequences of hemagglutinin of influenza A H1N1 and H7N9 strains and products of its translation were analyzed by cluster analysis. The properties of hemagglutinin were defined by determining its domains.

Results and discussion. As a result of the study, the polymorphism and genetic distances between the alleles of the gene encoding hemagglutinin of the influenza virus have been calculated and its translation has been carried out. The polymorphism and amino acid distances between the products of its translation were detected; a comparative analysis between the nucleotide and amino acid sequences was performed. The influence and effect of synonymous codons in the nucleotide sequences of the alleles of the hemagglutinin encoding gene on the hemagglutinin polymorphism was shown. The domains of the test samples of hemagglutinin were identified.

Conclusions. As a result of studies, the absence of the influence of the polymorphism of the hemagglutinin encoding gene on the hemagglutinin polymorphism was shown. The absence of the effect of polymorphism on the amino acid sequences of hemagglutinin of influenza A H1N1 and H7N9 strains, as well as on the domain composition and, thus, on the properties of strains was determined

Keywords: polymorphism, hemagglutinin, Influenza A virus, H1N1, H7N9, cluster analysis

References

- Gamblin, S. J., Skehel, J. J. (2010). Influenza Hemagglutinin and Neuraminidase Membrane Glycoproteins. *Journal of Biological Chemistry*, 285 (37), 28403–28409. doi: <http://doi.org/10.1074/jbc.r110.129809>
- Efron, B. (1979). Bootstrap Methods: Another Look at the Jackknife. *The Annals of Statistics*, 7 (1), 1–26. doi: <http://doi.org/10.1214/aos/1176344552>
- Sauer, A.-K., Liang, C.-H., Stech, J., Peeters, B., Quéré, P., Schwegmann-Wessels, C. et. al. (2014). Characterization of the Sialic Acid Binding Activity of Influenza A Viruses Using Soluble Variants of the H7 and H9 Hemagglutinins. *PLoS ONE*, 9 (2), e89529. doi: <http://doi.org/10.1371/journal.pone.0089529>
- Smith, T. F., Waterman, M. S. (1981). Identification of common molecular subsequences. *Journal of Molecular Biology*, 147 (1), 195–197. doi: [http://doi.org/10.1016/0022-2836\(81\)90087-5](http://doi.org/10.1016/0022-2836(81)90087-5)
- Brockwell-Staats, C., Webster, R. G., Webby, R. J. (2009). Diversity of influenza viruses in swine and the emergence of a novel human pandemic influenza A (H1N1). *Influenza and Other Respiratory Viruses*, 3 (5), 207–213. doi: <http://doi.org/10.1111/j.1750-2659.2009.00096.x>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, 28 (10), 2731–2739. doi: <http://doi.org/10.1093/molbev/msr121>
- Taubenberger, J. K., Kash, J. C. (2010). Influenza Virus Evolution, Host Adaptation, and Pandemic For-

mation. *Cell Host & Microbe*, 7 (6), 440–451. doi: <http://doi.org/10.1016/j.chom.2010.05.009>

8. Webster, R. G., Bean, W. J., Gorman, O. T., Chambers, T. M., Kawaoka, Y. (1992). Evolution and ecology of influenza A viruses. *Microbiological Reviews*, 56 (1), 152–179.

9. Wilks, S., de Graaf, M., Smith, D. J., Burke, D. F. (2012). A review of influenza haemagglutinin receptor binding as it relates to pandemic properties. *Vaccine*, 30 (29), 4369–4376. doi: <http://doi.org/10.1016/j.vaccine.2012.02.076>

10. Kumar, S., Tamura, K., Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Briefings in Bioinformatics*, 5 (2), 150–163. doi: <http://doi.org/10.1093/bib/5.2.150>

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SPATIAL DISTRIBUTION, DIMENSIONAL-MASS AND AGE STRUCTURE OF DAB OF THE GLOSS *PLATICHTHYS LUSCUS* POPULATION OF SHABOLATSK LIMAN

p. 18-23

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The Shabolatsk liman is situated in the Southern-Western Black Sea side and relates to the type of semi-open, saline-water liman-lagoons.

Since the middle XX century essential changes have occurred in the liman hydroecosystem that resulted in the essential transformation of the basin biota.

*The aim of the study was in revealing changes in the spatial distribution, dimensional-mass and age structure of the population of dab gloss *Platichthys luscus* in the Shabolatsk liman.*

The composition and population structure of ichthyocenosis of Shabolatsk liman, bio-ecological characteristics of most mass types of fish on the example of dab gloss were studied.

*The studies of the spatial distribution of the population of dab gloss *Platichthys luscus* in the Shabolatsk liman demonstrated that gloss fattens along the whole liman water area, but in the autumn-winter period concentrates in the deep-water saline Southern-Western part of the liman.*

*The conducted studies of the dimensional-mass and age structure of the *Platichthys luscus* population in the Shabolatsk liman allowed to establish that the population was presented by five age groups like in the previous*

period, but the share of fishes of the older age decreased. Dimensional-mass indices and fatness of dab decreased. It has been established, that the loss of the essential share of the spawning herd in 1992, desalination and worsening of the fodder base resulted in the stable depression of the dab population in liman in the following years.

*Taking into account food inclinations of *Platichthys luscus* (the base of gloss nutrition is mollusks, cancroids and fish (gobies and silversides)), at forming the polyculture in the Shabolatsk liman, dab gloss may be recommended as one of main consumers of zoobenthos that doesn't come in food competition with other fish types*

Keywords: *Shabolatsk liman, ichthyocenosis formation, dab gloss, population, fatness, mass*

References

1. Burhaz, M. I. (2018). Osoblyvosti formuvannia ikhtiotsenozu Shabolatskoho lymanu v umovakh antropohennoi transformatsii vodoimy. Odessa, 20.

2. Erdogan, N., Duzgunes, E. Ogut, H.; Briand, F. (Ed.) (2010). Black Sea fisheries and climate change. Climate forcing and its impacts on the Black Sea Marine Biota. No 39 in CIESM Workshop Monographs. Monaco: CIESM, 113–120.

3. Shekk, P. V., Kriukova, M. I. (2010). Ocenka kormovoi bazy i perspektivy ispolzovaniia Shabolatskogo limana dlia pastbishchnoi marikultury. *Visnik zaporizkogo nacionalnogo universitetu*, 1, 1126–1135.

4. Çiloğlu, E. (2005). Determination of the Recruitment to Stock and Reproduction Period for Flounder (*Platichthys flesus luscus* Pallas, 1811) along the Trabzon-Rize Coast, Eastern Black Sea. *Turkish Journal of Veterinary and Animal Sciences*, 29 (1), 43–48.

5. Priakhin, Iu. V., Shnickii, V. A. (2006). *Metody rybokhoziaistvennykh issledovaniy*. Krasnodar: Kubanskii gosuniversitet, 214.

6. Kriukova, M. I. (2012). *Statystychni metody v biolohichnykh doslidzhenniakh*. Odessa: ODEKU, 118.

7. Kiriliuk, M. M., Shekk, P. V. (1976). Izuchenie pitaniia ryb Chernogo moria «Biologo-fiziologicheskie osnovy pitaniia morskikh ryb na raznykh stadiiakh razvitiia (kambalovye, kefalevye, bychkovy). Otchet o vypolnenii NIR v 1976 g. No. Gosregistr. 0173.7142571. Odesskoe otделение IUGNIRO. Odessa, 59.

8. Tarnavskii, M. P. (1960). Kambala-glossa, bychki i drugie ryby v promysle Molochnogo limana. *Trudy instituta gidrobiologii An USSR*, 35, 213–225.

9. Demchenko, V. O. (2004). Ikhtiofauna ta pokaznyky yakosti vody Molochnogo lymanu v zviazku z rybohospodarskym vykorystanniam vodoimy. Kyiv, 24.

10. Nazarov, V. M. (1966). Osobennosti rosta, sozrevaniia i plodovitosti chernomorskoii kambaly glossy. *Voprosy morskoi biologii*. Kyiv: Naukova dumka, 89–91.

11. Krotov, A. V. (1941). Rybokhoziaistvennoe ispolzovanie chernomorskikh limanov. Rybnoe khoziaistvo, 7, 87–89.

12. Serdiuk, A. V. (1975). Povyshenie effektivnosti rybokhoziaistvennogo ispolzovaniia solenykh limanov severo-zapadnoi chasti Chernogo moria putem akklimatizatsii i razvedeniia morskikh i presnovodnykh ryb «Biologicheskie osnovy povysheniia ryboproduktivnosti Tiligul'skogo limana. Otchet o vypolnenii NIR v 1975 g. No. Gosregistr. 74060258. Odesskoe otdelenie IUgNIRO. Odessa, 35.

13. Kriukova, M. I. (2013). Perspektivy razvitiia marikulturi v Shabolatskom limane v sovremennykh usloviakh. Ternopil, 169–172.

14. Orlova, L. N. (1977). Povyshenie promyslovoi produktivnosti pribrezhnoi zony severo-zapadnoi chasti Chernogo moria i Prichernomorskikh limanov «Biotekhnika vyrashchivaniia kompleksa prudovykh ryb». Otchet o vypolnenii NIR v 1977 g. No. Gosregistr. 0112.7112331. Odesskoe otdelenie IUgNIRO. Odessa, 61.

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IDENTIFICATION AND ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERIA ISOLATED FROM THE GASTROINTESTINAL TRACT OF A RABBIT

p. 24-30

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The probiotic preparations, used in rabbit breeding, contain bacteria, isolated from various niches. Therefore, these drugs are universal and recommended for different types of animals. One of the ways to increase the effectiveness of probiotics, used in breeding rabbits is the selection of strains of biologically active representatives of the obligate microflora of the gastrointestinal tract of these animals.

Purpose. Identify lactic acid bacteria, isolated from the gastrointestinal tract of a rabbit, promising for the creation of probiotic preparations. Investigate antibiotic resistance of the most biologically active isolates.

Methods. The identification of lactic acid bacteria to the genus *Lactobacillus* was carried out according to generally accepted methods. Molecular genetic identification was performed using a polymerase chain reaction. For the most promising strains, the sensitivity to antibiotics was studied using the disco-diffusion method and the minimum inhibitory concentration of antibiotics, which completely retarded the growth of bacteria.

Results. 250 bacterial isolates, isolated from the gastrointestinal tract of rabbits were identified to the *Lactobacillus* genus, 10 most active of which were selected for the further work. It has been established, that 40 % of selected isolates of lactic acid bacteria are capable of fermenting arabinose, dulcitol; 50 % – fructose; 70 % – sorbitol; 80 % – mannose, fructose; 90 % – lactose; 100 % – galactose, maltose, sucrose, glucose; 20 % – raffinose. Molecular genetic studies have established that the isolate *Lactobacillus* sp. 13/2 does not have genes typical for *L. acidophilus* and *L. helveticus* species. It has been found, that the lowest value of the minimum inhibitory concentration of antibiotics of the studied isolates was revealed to prolonged-action penicillins, which act bactericidally, disrupting the synthesis of the bacterial cell wall. The highest is for ampicillin, which is active against gram-positive bacteria that are affected by benzylpenicillin. The results indicate the absence of acquired antibiotic resistance. However, isolate L-13/2 showed resistance to oxacillin, kanamycin, streptomycin, nalidixic acid.

Findings. According to a complex of morphological, cultural, and physiological and biochemical properties, the ten most active isolates of lactic acid bacteria, isolated from the gastrointestinal tract of rabbits, of 250 ones are previously assigned to phylogenetic groups: *L. acidophilus*, *L. lactis*, *L. casei*, *L. plantarum*. For use the selected isolate *Lactobacillus* sp. 13/2 as a basis for the probiotic preparation, it is necessary to use additional identification methods to determine the species.

It has been established, that the studied isolates as a whole did not exhibit antibiotic resistance. Perspective isolate *Lactobacillus* sp. 13/2 was resistant to some antibiotics. Therefore, there is a need to study in greater detail the antibiotic resistance of the isolate under study in order to exclude the possibility of horizontal transfer of resistance genes

Keywords: lactic acid bacteria, probiotics, rabbits, antibiotic resistance, identification, polymerase chain reaction

References

1. Kovalchuk, I., Yashchuk, I. V. (2016). Suchasnyi stan ta perspektyvy rozvytku haluzi krolivnytstva v Ukraini [The modern state and prospects of development of indus-

try of the rabbit breeding are in Ukraine]. Technology of production and processing of livestock products, 5, 24–29.

2. Aksenov, Ye. O. (2016). Rozvitok krolivnictva v Ukrayini ta sviti [Development of rabbit meat in Ukraine and the world]. Scientific and technical bulletin, 116, 15–21.

3. Stephen, W. B., Stephen, M. G., Dean, H. P. (2016). Pathology of laboratory rodents and rabbits. Chichester: John Wiley & Sons, 372.

4. Antimicrobial resistance (2017). WHO. Available at: http://apps.who.int/gb/ebwha/pdf_files/wha70/a70_12-en.pdf

5. WHO Global Strategy for the Containment of Antimicrobial Resistance (2011). WHO. Available at: https://www.who.int/drugresistance/WHO_Global_Strategy_English.pdf

6. Khachatourians, G. G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal, 159, 1129–1136.

7. Kalachniuk, G. I. (1996). Probiotiki u tvarinnictvi [Probiotics in livestock breeding]. Animal husbandry of Ukraine, 5, 16–18.

8. Veterinary informational and analytical resource of Ukraine. Available at: <https://vet.in.ua/>

9. Bernbom, N., Norrung, B., Saadbye, P., Molbak, L., Vogensen, F. K., Licht, T. R. (2006). Comparison of methods and animal models commonly used for investigation of fecal microbiota: Effects of time, host and gender. Journal of Microbiological Methods, 66 (1), 87–95. doi: <http://doi.org/10.1016/j.mimet.2005.10.014>

10. Tarakanov, B. V. (2006). Metodyi issledovaniya mikrofloryi pischevaritelnogo trakta selskohozyaystvennyih zhivotnyih i ptitsyi [Methods for studying the microflora of the digestive tract of farm animals and poultry]. Moscow: Nauchnyy mir, 188.

11. Pokhilko, Yu. M., Kravchenko, N. O., Bozhok, L. V., Ageev, V. O., Dmitruk, O. M. (2015). Osoblivosti kishkovo-go mikrobocenozu molodnyaku kroliv za riznih tipiv godivli [Features of intestinal micrococoenosis of young rabbits for different types of feeding]. Agricultural Microbiology, 22, 48–52.

12. Pohilko, Yu. M., Kravchenko, N. O. (2016). Vidilennya iz travnoyi sistemi kroliv molochnokislih bakterii, perspektivnih dlya stvorennya probiotichnih preparativ [Isolation from the digestive system of rabbits lactic acid bacteria, promising for the creation of probiotic preparations]. Bioresources and nature management, 8 (5-6), 63–66.

13. Pohilko, Y. M., Kravchenko, N. O. (2017). Resistance of bacteria of the genus lactobacillus to the metabolites of the digestive system. Microbiology&Biotechnology, 2 (38), 101–111. doi: [http://doi.org/10.18524/2307-4663.2017.2\(38\).105019](http://doi.org/10.18524/2307-4663.2017.2(38).105019)

14. Pohilko, Y. M., Kravchenko, N. O. (2018). Recovery and correction of the balance of microbiota of the

gastrointestinal tract of rabbits, disabled as resulting from the use of antibiotics. Biological Resources and Nature Management, 10 (3-4), 19–31. doi: <http://doi.org/10.31548/bio2018.03.003>

15. Pohilko, Y. M., Kravchenko, N. O. (2018). Probiotic properties of bacteria of Lactobacillus genus isolated from the gastrointestinal tract of rabbits. Studia Biologica, 12 (1), 35–46. doi: <http://doi.org/10.30970/sbi.1201.535>

16. De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A. et. al. (2009). Bergey's manual of systematic bacteriology. Vol. 3. New York: Springer, 1422.

17. Egorov, N. S. (1986). Basics of Antibiotic Teaching. Moscow: Vysshaia shkola, 448.

18. Dec, M., Wernicki, A., Puchalski, A., Urban-Chmiel, R. (2015). Antibiotic susceptibility of Lactobacillus strains isolated from domestic geese. British Poultry Science, 56 (4), 416–424. doi: <http://doi.org/10.1080/00071668.2015.1058919>

19. Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E. et. al. (2006). Comparative genomics of the lactic acid bacteria. Proceedings of the National Academy of Sciences, 103 (42), 15611–15616. doi: <http://doi.org/10.1073/pnas.0607117103>

20. Oren, A., Garrity, G. M. (2018). Notification that new names of prokaryotes, new combinations, and new taxonomic opinions have appeared in volume 68, part 1, of the IJSEM. International Journal of Systematic and Evolutionary Microbiology, 68 (4), 979–981. doi: <http://doi.org/10.1099/ijsem.0.002597>

21. Marhamatizadeh, M. H., Sayyadi, S. (2019). Mining of lactic acid bacteria from traditional yogurt (Mast) of Iran for possible industrial probiotic use. Italian Journal of Animal Science, 18(1), 663–667. doi: <http://doi.org/10.1080/1828051x.2018.1552541>

22. Wang, Y., Ryu, B. H., Yoo, W., Lee, C. W., Kim, K. K., Lee, J. H., Kim, T. D. (2018). Identification, characterization, immobilization, and mutational analysis of a novel acetyltransferase with industrial potential (LaAcE) from Lactobacillus acidophilus. Biochimica et Biophysica Acta (BBA) – General Subjects, 1862 (1), 197–210. doi: <http://doi.org/10.1016/j.bbagen.2017.10.008>

23. Tamminen, M., Joutsjoki, T., Sjoblom, M., Joutsen, M., Palva, A., Ryhanen, E.-L., Joutsjoki, V. (2004). Screening of lactic acid bacteria from fermented vegetables by carbohydrate profiling and PCR-ELISA. Letters in Applied Microbiology, 39 (5), 439–444. doi: <http://doi.org/10.1111/j.1472-765x.2004.01607.x>

24. Tilsala-Timisjärvi, A., Alatossava, T. (1997). Development of oligonucleotide primers from the 16S-23S rRNA intergenic sequences for identifying different dairy and probiotic lactic acid bacteria by PCR. International Journal of Food Microbiology, 35 (1), 49–56. doi: [http://doi.org/10.1016/s0168-1605\(97\)88066-x](http://doi.org/10.1016/s0168-1605(97)88066-x)

25. Fortina, M. G., Ricci, G., Mora, D., Parini, C., Manachini, P. L. (2001). Specific identification of *Lactobacillus helveticus* by PCR with *pepC*, *pepN* and *htrA* targeted primers. *FEMS Microbiology Letters*, 198 (1), 85–89. doi: <http://doi.org/10.1111/j.1574-6968.2001.tb10623.x>
26. Ashraf, R., Shah, N. (2011). Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. *International Food Research Journal*, 18 (3), 837–853.
27. Hughes, P., Heritage, J. (2004). Antibiotic growth-promoters in food animals. *FAO Animal Production and Health Paper*, 129–152.
28. Shenderov, B. A. (2001). *Probiotiki i funktsionalnoye pitaniye* [Probiotics and functional foods]. Moscow: Grants, 288.
29. Charteris, W. P., Kelly, P. M., Morelli, L., Collins, J. K. (2001). Gradient Diffusion Antibiotic Susceptibility Testing of Potentially Probiotic Lactobacilli. *Journal of Food Protection*, 64 (12), 2007–2014. doi: <http://doi.org/10.4315/0362-028x-64.12.2007>
30. Shah, N. P. (2000). Probiotic Bacteria: Selective Enumeration and Survival in Dairy Foods. *Journal of Dairy Science*, 83 (4), 894–907. doi: [http://doi.org/10.3168/jds.s0022-0302\(00\)74953-8](http://doi.org/10.3168/jds.s0022-0302(00)74953-8)
31. Nekrasova, L. S., Svyta, V. M., Hlushkevych, T. H., Tomchuk, V. V., Zhrebko, N. M., Pokas, O. V. (Eds.) (2007). *Vyznachennia chutlyvosti mikroorhanizmiv do antybakterialnykh preparativ* [Determination of the sensitivity of microorganisms to antibacterial drugs]. Kyiv: MOZ Ukrainy, 74.