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The materials include the content of the reports of students, young scientists and PhD students, and outline the results and prospects of research in various fields of Biology, Biotechnology and Biomedicine. The authors of the abstracts are responsible for the accuracy of the materials and text.

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TABLE OF CONTENTS

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Smalchuk D.[1, 2], Ivanytsia T.[1], Lacoma A.[2], Dominguez J.[2]

EFFECTIVENESS OF MYCOBACTERIOPHAGE D29 IN TARGETING INTRACELLULAR *MYCOBACTERIUM SMEGMATIS*

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Abstract. *Global public health is increasingly threatened by the emergence of multidrug-resistant tuberculosis (MDR-TB), a form of tuberculosis (TB) that does not respond to standard first-line anti-TB drugs. This resistance complicates treatment regimens, prolongs disease, increases mortality, and poses a serious public health problem. Traditional antibiotic treatments are often insufficient to kill intracellular pathogens such as Mycobacterium tuberculosis. The intracellular environment, particularly the phagolysosome, is acidic and presents a barrier to many antibiotics, limiting their effectiveness. Alternative therapeutic strategies are urgently needed to address these problems. This study investigates the potential of mycobacteriophage D29 to target and reduce intracellular mycobacterial load in macrophage cells. As a result, it was observed that bacteriophages were able to penetrate inside macrophages and retain their activity for 48 hours. The use of mycobacteriophage D29 with Mycobacterium smegmatis-infected macrophages showed that after 24 hours of incubation the amount of bacterial load decreased markedly. The trend continued after 48 hours. It should also be noted that after 48 hours, the number of bacteria in the supernatant increased along with the concentration of bacteriophage. This may be due to cell death and bacterial escape. The results show the promising potential of mycobacteriophage D29 to reduce intracellular bacterial load.*

Keywords: *tuberculosis, bacteriophages, macrophages, mycobacteriophage D29*

Introduction. The urgent problem of the emergence of multi-drug resistant tuberculosis, requires new antibiotics and therapeutic approaches [1]. Habitually, antibiotics often fail to eradicate intracellular pathogens due to limited penetration and the acidic media inside the phagolisosome. This study investigate the potential of the mycobacteriophage D29 to target intracellular mycobacteria in macrophage cells.

Material and methods. The human monocytic cell line THP-1 was used, cells were seeded at 3.5 x 10^5 per well and differentiated into macrophages using Phorbol 12-myristate 13-acetate (PMA). Mycobacteriophage D29 (MOI 10) was added to macrophages and incubated for 4, 24 and 48 hours. The presence of bacteriophages in the supernatant and intracellularly was determined by the phage plaque method.

THP-1 cell line was used, cells were seeded at 3.5 x 10^5 per well and differentiated into macrophages with PMA. Macrophages were infected with *M. smegmatis* (MOI 10) for 3 hours and then treated with Amikacin to remove extracellular bacteria. Mycobacteriophage D29 (MOI 10) was added to the infected macrophages. Bacterial counts were determined in the supernatant and intracellularly at 4 hours, 24 and 48 hours after infection. In the case of mycobacteriophages, macrophages were lysed with cold MQ water. The supernatant and lysate were centrifuged for 10 min at 4000g and passed through a 0.22 μm filter. The presence of bacteriophages was examined by the phage plaque method at the same time points.

Results and discussion. Throughout the experiment, no significant decrease in the number of macrophages was observed. At the 4-hour mark, phage concentration remained unchanged in the supernatant. However, by 24 hours, a decrease in phage concentration was noted, with phages still present at 48 hours. Intracellular phages were detected in low numbers after 4 hours, decreasing further but remaining active throughout the experiment. In the infection experiment, similar results were obtained without a significant decrease in the number of macrophages. After 4 hours, no change in bacteriophage numbers in the supernatant suggested insufficient time for infection of *M. smegmatis*. Significant increases in phage numbers were seen at 24 and 48 hours, likely due to macrophage death and bacterial release into the supernatant. Phage titers decreased significantly inside macrophages after 4 hours but showed increased activity and reduced bacterial concentrations after 24 and 48 hours. This may indicate that it takes more time for the bacteriophage to pass inside the macrophages and carry out a complete cycle than under normal conditions.

The results of this study provide valuable insights into the potential therapeutic application of mycobacteriophage D29 in targeting intracellular *M. smegmatis* within macrophage cells.

Conclusions. The ability of D29 to effectively target and diminish intracellular *M. smegmatis* suggests its promise as a valuable adjunct to traditional antibiotic therapies for treating tuberculosis. However, for phage therapy to reach its full potential, there is a crucial need to enhance the permeability and protection of bacteriophages within host cells. Ongoing efforts in developing protective particles aim to address this need, potentially

facilitating a faster and more effective accumulation of intracellular bacteriophages.

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ЕФЕКТИВНІСТЬ МІКОБАКТЕРІОФАГА D29 У НАЦІЛЮВАННІ НА ВНУТРІШНЬОКЛІТИННІ *MYCOBACTERIUM SMEGMATIS*

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Анотація. *Глобальна система охорони здоров`я все більше занепокоєна появою та загрозами, що несуть із собою мультирезистентні форми туберкульозу (МРТБ), що представляють собою різновиди туберкульозу (ТБ), які не реагують на стандартні протитуберкульозні препарати першої лінії. Ця резистентність ускладнює схеми лікування, затягує хворобу, збільшує смертність і становить серйозну проблему для громадського здоров'я. Традиційне лікування антибіотиками часто є недостатнім для знищення внутрішньоклітинних патогенів, таких як Mycobacterium tuberculosis. Внутрішньоклітинне середовище, зокрема фаголізосоми, є кислим, що є бар'єром для роботи багатьох антибіотиків і обмежує їхню ефективність. Для вирішення таких проблем терміново потрібні альтернативні терапевтичні стратегії. У даному дослідженні вивчено потенціал мікобактеріофага D29 щодо націлювання та зменшення внутрішньоклітинного мікобактеріального навантаження в клітинах макрофагів. В результаті було виявлено, що бактеріофаги здатні проникати всередину макрофагів і зберігати свою активність протягом 48 годин. Обробка мікобактеріофагом D29 макрофагів, інфікованих Mycobacterium smegmatis показало, що через 24 години інкубації кількість бактеріального навантаження помітно зменшилася. Ця тенденція зберігалася і через 48 годин. Слід зазначити, що через 48 годин разом із концентрацією бактеріофагу зростала кількість бактерій у супернатанті, що може бути пов'язано із загибеллю клітин та виходом бактерій. Отримані результати свідчать про значний потенціал мікобактеріофагу D29 щодо зменшення внутрішньоклітинного бактеріального навантаження.*

Ключові слова: *туберкульоз, бактеріофаги, макрофаги, мікобактеріофаг D29.*

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UDC 575.8

Adavoudi R., Pilot M.

DETECTION OF GREY WOLF AND GOLDEN JACKAL HYBRIDIZATION WITH DOMESTIC DOGS IN UKRAINE USING SNP MARKERS

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Abstract. *The results show that the introgression of dog-derived genetic variants into gene pools of wolves was more extensive as compared with golden jackals. It may be explained by the fact that wolf and dog are more closely related and therefore we may expect less negative fitness consequences in wolf-dog hybrids compared with jackal-dog hybrids.*

Keywords: *hybridization, local ancestry, Ukraine, genus Canis*

Introduction. From the perspective of conservation and management, hybridization between domesticated species and their wild relatives can significantly affect the evolutionary process. Hybridization in the genus *Canis* has been well-documented before.

Materials and methods. Here, we analyzed 360K genome-wide SNP loci from dogs, wolves, and golden jackals from Ukraine to detect signatures of admixture in these canids. We used Bayesian clustering approach to estimate the proportion of dog ancestry in wild canid populations. To identify ancestry blocks originating from introgression in each individual, we carried out local ancestry analysis (LAMP-LD and ELAI).

Results and discussion. Using global ancestry estimation, 26 wolves and two jackals showed evidence of admixture, with proportions of dog ancestry between 10 and 50%. The local ancestry analysis carried out for each of the 38 autosomal chromosomes, demonstrated the presence of small blocks of dog ancestry in the genomes of wild canids.

Conclusions. Our results show that the introgression of dog-derived genetic variants into gene pools of wolves was more extensive as compared with golden jackals. It may be explained by the fact that wolf and dog are more closely related and therefore we may expect less negative fitness consequences in wolf-dog hybrids compared with jackal-dog hybrids.

Адавоуді Р., Пілот М.

ВИЯВЛЕННЯ ГІБРИДИЗАЦІЇ СІРОГО ВОВКА ТА ЗОЛОТИСТОГО ШАКАЛА ІЗ ДОМАШНІМИ СОБАКАМИ В УКРАЇНІ ІЗ ВИКОРИСТАННЯМ SNP-МАРКЕРІВ

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Анотація. *Результати дослідження показують, що інтрогресія генетичних варіантів, отриманих від собак, у генофонди вовків була більшою, ніж у золотих шакалів. Це можна пояснити тим фактом, що вовк і собака більш тісно пов'язані, і тому можна очікувати менш негативних наслідків для пристосування у гібридів вовка і собаки порівняно з гібридами шакала і собаки.*

Ключові слова: *гібридизація, локальне походження, Україна, рід Canis.*

UDC 575.2

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THE EFFECT OF ENVIRONMENTAL FACTORS ON ADAPTIVE VARIATION IN GREY WOLVES (*CANIS LUPUS*) AND FREE-RANGING DOGS (*CANIS LUPUS FAMILIARIS*)

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Abstract. *Five distinct spatially structured populations of FRDs, and eight populations of wolves were found. Populations from arid biomes were identified as the most genetically isolated. Precipitation and human footprint are suggested to cause the selective pressures operating upon FDRs. In FRDs local adaptive evolution is occurring within genes involved in DNA repair and synthesis of biological products. In wolves, adaptive evolution is occurring within genes involved in response to stimulus (e.g., olfactory and immune systems), metabolic and cellular processes. Despite occupying the same habitats FRDs are undergoing greater selective pressures. FRDs local adaption is akin of colonization of novel environments. In both species, most mutations in coding regions occurred in regulatory genes (e.g., transcription factors, receptors).*

Keywords: *canid adaptive evolution, free-ranging dogs, wolf local adaptation, landscape genomics.*

Introduction. Wolves are apex predators whose population underwent a domestication process ~15,000-30,000 years ago. Free ranging dogs (FRDs) live in both urban and rural habitats and have a wide distribution overlapping with wolves. Wide distribution of both canids makes them ideal models for investigating the impact of environmental factors on genetic diversity.

Materials and methods. We used genome-wide single-nucleotide-polymorphisms (SNPs) genotypes of 370 FRDs and 232 wolves from ten biomes across Eurasia to investigate population structure, spatial autocorrelation, signatures of selection, and environmental variables causing specific selection pressures.

Results and discussion. Five distinct spatially structured populations of FRDs, and eight populations of wolves were found. Populations from arid biomes were identified as the most genetically isolated. Precipitation and human footprint are suggested to cause the selective pressures operating upon FDRs. In

FRDs local adaptive evolution is occurring within genes involved in DNA repair and synthesis of biological products.

Conclusions. In wolves, adaptive evolution is occurring within genes involved in response to stimulus (e.g., olfactory and immune systems), metabolic and cellular processes. Despite occupying the same habitats FRDs are undergoing greater selective pressures. FRDs local adaption is akin of colonization of novel environments. In both species, most mutations in coding regions occurred in regulatory genes (e.g., transcription factors, receptors).

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ВПЛИВ ФАКТОРІВ НАВКОЛИШНЬОГО СЕРЕДОВИЩА НА АДАПТИВНУ МІНЛИВІСТЬ У СІРИХ ВОВКІВ (*CANIS LUPUS*) І СОБАК, ЩО ВИГУЛЮЮТЬСЯ НА ВОЛІ (*CANIS LUPUS FAMILIARIS*)

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Анотація. *Було виявлено п'ять чітких просторово структурованих популяцій FRD і вісім популяцій вовків. Популяції з посушливих біомів були ідентифіковані як найбільш генетично ізольовані. Вважається, що опади та людський слід спричиняють вибірковий тиск, що діє на FDR. У FRD локальна адаптивна еволюція відбувається в генах, які беруть участь у репарації ДНК і синтезі біологічних продуктів. У вовків адаптивна еволюція відбувається в межах генів, залучених у відповідь на стимули (наприклад, органів нюху та імунної систем), метаболічних і клітинних процесів. Незважаючи на те, що вони займають однакові середовища існування, FRD зазнають більшого вибіркового тиску. Локальна адаптація FRD схожа на колонізацію нових середовищ. В обох видів більшість мутацій у кодуючих областях відбулися в регуляторних генах (наприклад, факторах транскрипції, рецепторах).*

Ключові слова: *адаптивна еволюція псових, собаки на вільному вигулі (FRD), локальна адаптація вовка, ландшафтна геноміка.*

UDC 579.2

Zaitsev A., Sinika V., Zinchenko O.

ANTIBIOTIC SUSCEPTIBILITY OF ABYSSAL SPORE-FORMING BACTERIA FROM THE BLACK SEA

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Abstract. *Antibiotic susceptibility of spore-forming bacteria isolated from bottom sediments of the Black Sea was studied. High level of resistance was observed in all isolates. Resistance to β-lactam antibiotics was common for most cultures.*

Keywords: *marine spore-forming bacteria, Bacillota, antibiotic resistance, Black Sea.*

Introduction. Microbial resistance to antibiotics has been remaining a global threat for last decades [1]. Resistance genes are widely distributed not only in clinical environment but also in different bacterial populations in nature. These populations can serve as important reservoirs of resistance genes, on the other hand, the presence of resistance mechanisms can be an indirect indicator of antibiotic producers because determinants for self-resistance are often clustered together with the antibiotic biosynthesis genes [2, 3]. Currently, there is limited data about resistance profiles of microbial communities of marine ecosystems.

The aim of our study was to investigate antimicrobial susceptibility of bottom sediment spore-forming bacteria isolated from the Black Sea.

Materials and methods. Susceptibility of 22 strains to 30 antimicrobials was evaluated using disk-diffusion method, EUCAST breakpoint and quality control tables were used for interpretation of results. Strains were isolated from bottom sediments taken from 3 sampling points (888 m, 1499 m and 1537 m). Multiple antibiotic resistance indices (MAR) were calculated for studied cultures according to the standard method.

Results and discussion. As a result of our study, it was shown that isolates demonstrated multiple resistance to beta-lactam antibiotics including penicillins, cephalosporins and carbapenems. The highest level of resistance (100% strains) was detected to oxacillin. Interestingly, 90% of isolates were resistant to imipenem but no one showed resistance to meropenem. 95% of

isolates were resistant to inhibitor-protected penicillin ampicillin/sulbactam. Over 90% were resistant to levofloxacin. No resistance was observed to teicoplanin, meropenem and vancomycin. Low resistance was detected to rifampicin (9,1% resistant strains), enrofloxacin (9,5%) and amoxicillin/ clavulanate (13,6%).

We analyzed the prevalence of resistance depending on the sampling location. Resistance to azlocillin, ampicillin/sulbactam, oxacillin, cefepime, cefoperazone, cefuroxime was almost the same in each sampling point. At the same time, resistance to many other β-lactam antibiotics was the highest in the deepest point (1537 m). No resistance to amoxicillin/clavulanate, carbenicillin and enrofloxacin was observed in strains isolated from the depth of 888 m.

We calculated MARs of studied cultures. There was no clear correlation between the depth of sampling and MAR index. But at the same time we could observe widening of the range of indices with the depth increase (0,5-0,7 for 888 m, 0,4-0,9 for 1499 m, 0,6-0,9 for 1537 m).

Conclusions. When analyzing the literature, we found that MAR indices of representatives of *Bacillus* genus are low in urban rivers, as well as in some seas [1, 4]. However, the MAR indices obtained in our experiments are higher than that of other studies which raises new questions for studying this phenomenon.

Perhaps increased MAR indices are associated with the accumulation of resistance mutations in sediment populations, taking into account higher depth of the sea compared to rivers. Also, external factors such as industrial pollution, livestock production and urban wastewater near the coastline and far out to sea due to ship moorings or the activity of underwater currents may contribute to resistance formation.

Зайцев, В. Сініка, О. Зінченко

ЧУТЛИВІСТЬ ДО АНТИБІОТИКІВ ДОННИХ СПОРОУТВОРЮЮЧИХ БАКТЕРІЙ ЧОРНОГО МОРЯ

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Анотація. *Досліджено чутливість до антибіотиків спороутворюючих бактерій, виділених з донних осадів Чорного моря. У всіх культур спостерігався високий рівень резистентності. Найчастіше у більшості культур виявлялася стійкість до β-лактамних антибіотиків.*

Ключові слова: *морські спороутворюючі бактерії, Bacillota, стійкість до антибіотиків, Чорне море.*

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THE INFLUENCE OF THE MICROBIAL PREPARATION DIAZOBACTERYN ON THE YIELD OF WINTER RYE

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Abstract. *The article presents experimental data on the prospects of using Diazobacterin in the cultivation of winter rye. The microbial preparation helps to activate the production process of the crop, increase the yield by 11.3% and improve the quality of products, in particular, increase the protein content in the grain by 0.35%.*

Keywords: *winter rye, Diazobacterin, chlorophyll content, bacterization.*

Introduction. Winter rye (*Secale cereal L.*) is a valuable agricultural crop. One of its main characteristics is the protein content in the grain, which ranges from 9 to 19%. Grain, grain waste and straw are used in production. However, the peculiarities of the production process of winter rye plants, including the effect of inoculation, have not been studied sufficiently. The yield of grain crops largely depends on the productivity of photosynthesis. Therefore, it is important to establish the regularities of photosynthetic apparatus functioning with the use of a microbial preparation [1, 2, 3].

Material and methods. The study of the effectiveness of Diazobacteryn application in the technology of winter rye cultivation was conducted in a small plot field experiment on cultivated sod-podzolic dusty sandy loam soil of the experimental field of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of NAAS. The experimental design included variants without inoculation and with treatment with the microbial preparation Diazobacteryn. The winter rye variety was Synthetic-38.

Planning and conducting of the field experiment, statistical processing of experimental data were performed according to generally accepted methods.

Results and discussion. The content of photosynthetic pigments in the leaves of plants is indicative of the effect of mineral fertilizers and, especially, Diazobacteryn on the production process of winter rye. Bacterization increases

the content of chlorophylls a+b (Table 1). The intensification of chlorophyll synthesis may indirectly indicate a more active course of photosynthesis and, accordingly, carbon metabolism in plants.

Table 1

Effect of Diazobacteryn on chlorophyll content, flowering phase, mg/100 g

Experimental variants	Chlorophyll content, mg per 100 g		
	a	*b*	*a+b*
Without inoculation, control	90,98±7,29	15,65±0,94	106,63±8,06
Inoculation with Diazobacteryn	113,64±4,39	20,23±1,39	133,87±5,78

Intensification of plant growth and development, improvement of root nutrition conditions, activation of photosynthesis process influenced the formation of winter rye grain yield. When analyzing the data, we can note that inoculation contributes to an increase in crop yield by 11.3% – 2.65 and 2.95 t/ ha.

Conclusions. In general, it can be noted that the effect of inoculation on the state of agrobiocenosis contributes to an increase in the productivity of winter rye. Bacterization has a positive effect on product quality - the protein content in grain increases by 0.35%.

Солохненко Р.[1], Коротка І.[2]

ВПЛИВ МІКРОБНОГО ПРЕПАРАТУ ДІАЗОБАКТЕРИНУ НА УРОЖАЙНІСТЬ ЖИТА ОЗИМОГО

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Анотація. *У статті наведено експериментальні дані щодо перспектив використання біологічного препарату Діазобактерину в технологіях вирощування жита озимого. Мікробний препарат сприяє активізації продукційного процесу культури, підвищенню врожайності на 11,3% та покращенню якості продукції, зокрема, збільшенню вмісту білка в зерні на 0,35%.*

Ключові слова: *озиме жито, діазобактерин, вміст хлорофілу, бактеризація.*

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COMPARATIVE ANALYSIS OF PROTEIN KINASES ROCK1 AND ROCK2 POTENTIAL HEME-BINDING SITES

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Abstract. *Rho-associated coiled-coil protein kinases (ROCK1 and ROCK2) are crucial regulators of cellular functions including motility, proliferation, migration, and inflammation. Despite their high sequence homology, particularly within the kinase domain, ROCK1 and ROCK2 exhibit distinct actions in specific tissues. This study aimed to explore the potential binding sites and effects of heme, a degradation product of hemoproteins that accumulates under stress conditions, on ROCK1 and ROCK2. Utilizing a comprehensive in silico approach, sequences were obtained from Uniprot and analyzed via NCBI BLASTP, with structural data derived from PDB and visualized using ChimeraX. Docking simulations conducted with CB-DOCK2 and COACH-D identified putative heme binding sites, primarily within the catalytic domains of both isoforms. Results revealed hydrophobic and electrostatic interactions between heme and kinase domains, suggesting that while direct heme binding is unlikely to be stable due to the absence of high-specificity residues like histidine and cysteine, heme could influence ROCK signaling indirectly. No significant differences were observed between ROCK1 and ROCK2 in terms of heme binding potential. This study provides foundational insights for future investigations into heme's regulatory roles in ROCK signaling pathways.*

Keywords: *Rho-kinases, heme binding, comparative analysis.*

Introduction. Rho-associated coiled-coil protein kinases are expressed throughout the body and are involved in the regulation of multiple cellular functions such as cell motility, proliferation, migration, and inflammation [1, 2]. Two isoforms of these protein kinases ROCK1 and ROCK2 are products of different genes but share 65% overall identity in amino acid sequences with the most homology in the kinase domain (92%). Enzymes have no difference in catalytic activity *in vitro* but demonstrate distinct actions in specific tissues, with some differences in their regulation by regulatory proteins or covalent modifications [2, 3]. Both isoforms contain highly conserved cysteine-rich

domain (CBD), however only ROCK1 have Cys-Pro motif that is known to bind heme in several regulatory proteins [4]. Heme as a product of hemoproteins degradation accumulates under various stress conditions such as hemolysis, trauma, hemorrhage, hypoxia. Heme has prooxidant and proinflammatory action and is degraded in heme oxygenase reaction. Inhibitor of ROCK1/2 fasudil was shown to enhance the heme oxygenase expression, but direct heme effects on ROCK activity have not been investigated [5]. Therefore, the aim of this study was to analyze *in silico* the putative heme binding sites in both isoforms and the potential effect of heme on kinases functioning.

Materials and methods. Protein annotation and sequence were obtained from Uniprot: Q13464 (ROCK1) and O75116 (ROCK2). Sequence pairwise alignment was performed by NCBI BLASTP. Protein and heme structures were taken from PDB (http://www.rcsb.org/), contact residues were analyzed by PDBsum server. For pdb-files visualization ChimeraX was used [6]. TM-align tool was used for structural alignment (https://zhanggroup.org/TM-align/). Blind docking with heme as a ligand was performed using two docking servers: CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/) and COACH-D (http://yanglab.qd.sdu.edu.cn/COACH-D/). Docking results from COACH-D were analysed by PLIP web tool (https://plip-tool.biotec.tu-dresden.de/).

Results and discussion. Both enzymes have no full solved structures. Structural alignment of total ROCK1 and 2 AlfaFold models showed low similarity however high identity was shown for kinase domains. Docking with heme revealed putative binding sites mostly in the catalytic domains of both proteins particularly in ATP-binding pockets. Hydrophobic interactions with heme were predicted in the regions analogous for both enzymes (Ile82-Val90 in ROCK1 and Ile98-Val106 in ROCK2), ionic interaction was found respectively for Lys105 and Lys121 in ROCK1 and 2. Besides kinase domain heme tended to bind to PH-domain in both enzymes including amino acid residues within zinc fingers domains: 1228-1281 (ROCK1) and 1260-1315 (ROCK2), however Cys-Pro motif wasn't near binding sites, due to its low surface accessibility.

PH-domains are located near phosphorylation sites and autophosphorylation of ROCK1 at Ser1333 and of ROCK2 at Ser1366 is known to correlate with the activation status. ROCK2 has additional sites of phosphorylation comparing to ROCK1, including Thr967, Ser1099, Ser1133, and Ser1374, all increase its activity [2]. But no additional sites of heme binding were revealed by docking to this region.

Conclusions. In conclusion, no significant differences were found in potential heme-binding sites for ROCK1 and ROCK2. Heme binding near the ATP-pocket was based mostly on hydrophobic interactions with some portion of

weak-hydrogen (Glu, Asp, Ser, Pro), hydrogen (Leu, Asp, Met, Asn) and electrostatic (Lys, Arg). In both kinases amino acids with high specificity to heme such as histidine and cysteine were not revealed in heme contact areas. Therefore, heme binding could not be stable and direct action is not highly probable. Potentially heme could affect ROCK1/2 signalling through up-regulatory protein RhoA that could be the subject for future investigation.

Сологуб-Йосеф М., Бараннік Т.

ПОРІВНЯЛЬНИЙ АНАЛІЗ ПОТЕНЦІЙНИХ ГЕМ-ЗВ'ЯЗУВАЛЬНИХ САЙТІВ ПРОТЕЇНКІНАЗ ROCK1 ТА ROCK2

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Анотація. *Rho-асоційовані протеїнкінази (ROCK1 і ROCK2) є одними з ключових регуляторів таких клітинних процесів, як рухливість, проліферація, міграція та запалення. Незважаючи на високу гомологію між послідовностями, особливо в кіназному домені, ROCK1 і ROCK2 демонструють різні дії в специфічних тканинах. Це дослідження мало на меті вивчити, проаналізувати і порівняти in silico потенційні сайти зв'язування та вплив гему, продукту деградації гемопротеїнів, який накопичується за умов стресу, на активність ROCK1 і ROCK2. Результати показали наявність гідрофобних та електростатичних взаємодій між гемом та кіназними доменами, що свідчить про можливий непрямий вплив гему на сигнальні шляхи ROCK. Значних відмінностей у потенціалі зв'язування гему між ROCK1 і ROCK2 не було виявлено, що надає перспективу для подальших досліджень регуляторної ролі гему в сигнальних шляхах ROCK.*

Ключові слова: *Rho-кінази, гем-зв'язування , порівняльний аналіз.*

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Shurhin M.., Vasylieva N.

PHYLOGENETIC ANALYSIS OF PRMABCD GENE CLUSTERS IN REPRESENTATIVES OF THE GENUS *RHODOCOCCUS*

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Abstract. *A phylogenetic analysis of the gene cluster sequences prmABCD of Rhodococcus genus representatives has been conducted using bioinformatics methods.*

Keywords: *phylogenetic analysis, prmABCD, Rhodococcus.*

Introduction. The possibilities of using the gene cluster sequences prmABCD, which include genes associated with the oxidation of n-alkanes, certain halogenated hydrocarbons, and ethers, as marker sequences for the exploration of oil and gas fields using microbiological methods were investigated. It was necessary to prove the high identity between the sequence of this gene in microorganisms found in such ecotypes. Special attention was given to representatives of the genus *Rhodococcus*, which have the ability to degrade a wide range of organic compounds, including propane, through enzymatic systems such as propane monooxygenase A (prmA).

Materials and methods. Multiple alignment was conducted in the MEGA X program using the MUSCLE algorithm (GTR+G+I model). Phylogenetic analysis was performed in the raxMLGUI2.0 program using the Maximum Likelihood method. Additionally, the sequences of the prmA genes and the protein sequence of propane monooxygenase A were analyzed. The study used sequences from strains *Rhodococcus opacus*, *Rhodococcus wratislaviensis*, *Rhodococcus jostii*, *Rhodococcus sp*., and the closest homologs from the strains *Mycobacterium goodii*, *Mycolicibacterium hodleri*, and *Gordonia sp*.

Results and discussion. High identity between them was demonstrated, indicating the possibility of using the prmA gene as a marker for propane-oxidizing bacteria in soils, with potential for the exploration of oil and gas fields. It was also noted that the gene cluster sequence prmABCD could

serve not only as a marker for oil and gas fields but also as a marker for identifying the origin of oil derivatives.

Conclusions. From the above, it can be concluded that the quantitative assessment of the prmA gene can help determine the relative abundance of propane-oxidizing bacteria in soils and has potential for the exploration of oil and gas fields.

Шургін М., Васильєва Н.

PHYLOGENETIC ANALYSIS OF PRMABCD GENE CLUSTERS IN REPRESENTATIVES OF THE GENUS *RHODOCOCCUS*

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Анотація. *Методами біоінформатики проведено філогенетичний аналіз кластерних послідовностей генів prmABCD представників роду Rhodococcus.*

Ключові слова: *філогенетичний аналіз, prmABCD, Rhodococcus.*

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Putyatin B.

COSMETIC PRODUCTS CONTAINING PROBIOTICS: TRENDS AND ISSUES

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Abstract. *Probiotics, defined as "live microorganisms that, when present in sufficient quantities, confer a health benefit on the host," are becoming increasingly in demand in the market. However, many products labeled as probiotics do not meet these characteristics. In recent years, the cosmetics industry has seen an increase in the number of products claiming to be probiotic. Although probiotics may have several potential applications in personal care products, it is still necessary to ensure proper manufacturing practices to ensure that consumers actually receive probiotic products.*

Keywords: *probiotics, cosmetics, lysates, skin, microbiome.*

Introduction. According to the U.S. Food and Drug Administration (FDA), cosmetics are defined as "a product (excluding pure soap) intended to be applied to the human body to cleanse, enhance attractiveness, or alter appearance". Increased interest in the microbes that colonize the human body has led to numerous studies attempting to manipulate the microbiome in a specific niche to benefit health. The use of beneficial microbes for this purpose has led to a significant expansion of the probiotic field. Probiotics, defined as "live microorganisms that, when administered in adequate quantities, confer a health benefit on the host," vary widely in type, amount, and use. This includes cosmetic applications, where the probiotic market is projected to grow 12 % in the next ten years. Research on probiotics for potential use in cosmetics and the development of "probiotic cosmetics" is a fairly current scientific area [1].

Materials and methods. The increase in the number of products called probiotics on the market does not necessarily indicate the successful implementation of science in the consumer cosmetics market. Many products do not meet the characteristics to be called probiotics. For a product to be considered a probiotic, it must meet three basic characteristics: 1. The strain(s) must be characterized, including genetically and phenotypically. 2. At the time of use, the product must contain sufficient live microorganisms equivalent to when the product was indicated in clinical studies to provide benefit to the

desired target site. 3. The method of delivery, dosage and duration of use should be based on human scientific data if the intended recipient is humans.

Results and discussion. Analysis of the composition of cosmetic creams showed that the main probiotic strains for creams are *Lactobacillus ferment, Lactococcus ferment lysate, Bifida ferment lysate, Bacillus coagulans, Lactobacillus, Streptococcus thermophilus ferment*. There are numerous studies supporting the benefits of specific probiotic strains for skin health. Additionally, anti-aging mechanisms suggest that the strains may help regulate pH, reduce oxidative stress, protect against photoaging, and improve skin barrier function. Technological technique - microencapsulation is used to extend the shelf life and viability of probiotics. It is difficult for the cosmetics industry to create topical formulations that maintain the viability of probiotic bacteria from production to consumer. The presence of moisture will allow the dried organisms to hydrate and reproduce or die, so oil-based formulations are necessary. The question is how easily microorganisms can escape from the oil after it is applied to the skin and become metabolically active enough to provide the necessary probiotic effect. Many creams are not produced under sterile conditions, so they often add preservatives that have a bactericidal and/or bacteriostatic effect. They could potentially not only affect the viability of the probiotic strain, but also inadvertently change the microbiota of the recipient. For safety reasons, cosmetic products are expected to have a low microbial content (below 1000 CFU/g). It is not practical for them to contain live bacteria, which means there can be no cosmetic product that is a true probiotic. However, they may still contain components derived from probiotic strains that may be beneficial. They can be bacterial lysates, enzymes and filtrates, sometimes called postbiotics, defined as “a preparation of non-living microorganisms and/ or their components that provides a health benefit to the target host”. This definition does not include purified metabolites or cell-free components, which must instead be listed according to their chemical nomenclature. Leachates without cellular components are not considered postbiotics. However, bacterial lysates and enzymes may fall into this category depending on their composition [2].

Conclusions. To date, the identification of molecules responsible for the beneficial properties of microbial products has not been sufficiently studied and applied. More research will be needed to ensure that high-quality cosmetics that meet the definitions (probiotic, prebiotic, etc.) can reach consumers.

Путятін Б.

КОСМЕТИЧНА ПРОДУКЦІЯ, ЩО МІСТИТЬ ПРОБІОТИКИ: ТЕНДЕНЦІЇ ТА ПРОБЛЕМИ

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Анотація. *Пробіотики, визначені як «живі мікроорганізми, які, якщо присутні в достатній кількості, приносять користь для здоров'я господаря», стають все більш затребуваними на ринку. Однак багато продуктів, позначених як пробіотики, не відповідають цим характеристикам. В останні роки в косметичній промисловості зросла кількість продуктів, які претендують називатися пробіотиками. Хоча пробіотики можуть мати декілька потенційних застосувань у продуктах особистої гігієни, все одно необхідно забезпечити належну практику виробництва, щоб гарантувати, що споживачі дійсно отримають пробіотичні продукти.*

Ключові слова: *пробіотики, косметика, лізати, шкіра, мікробіом.*

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IN SILICO ANALYSIS OF EPB41 PHOSPHORYLATION BY PROTEIN KINASE C UNDER HEMOLYSIS

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Abstract. *Band 4.1 protein (EPB41) belongs to the FERM domain protein family and is one of the key components of the erythrocyte cytoskeleton. In erythrocytes, EPB41 interacts with several structural and transport membrane proteins, including band 3 protein, glycophorin, and spectrin. Protein kinase C (PKC) is one of the regulators of EPB41 and is activated by reactive oxygen species. Heme is a prooxidant and is accumulated under hemolysis. Molecular docking with heme revealed potential binding sites in the active site of PKC, but in the modeled complex with EPB41, heme was bound mainly to FERM domain of EPB41. Phosphorylation site of EPB41 protein had low probability of heme binding. Increased phosphorylation of EPB41 due to heme accumulation could lead to the cytoskeleton conformational changes and destabilization of erythrocytes.*

Keywords: *EPB41, protein kinase C (PKC), hemolysis, FERM domain, oxidative stress.*

Introduction. Erythrocyte membrane protein band 4.1 (EPB41) belongs to the FERM domain-containing family and is one of the key components of erythrocyte cytoskeleton. It has erythroid and non-erythroid forms made by alternative splicing. Erythroid form is much shorter and lacks three regions including N-end 209 amino acids and two regions (616-648 and 772-805) close to C-end. In erythrocytes EPB41 binds with several structural and transport membrane proteins such as band 3, glycophorin and spectrin. Besides it's structural role EPB41 regulates ion and water transport important for erythrocyte deformability. EPB41 could be phosphorylated by several protein kinases. Protein kinase C (PKC) is one of the regulators of EPB41 and is known to be activated by reactive oxygen species. Hemolysis is accompanied with heme accumulation and oxidative stress development, therefore protein kinase could be active in various tissues. But direct effect of hemolysis on PKC activity in erythrocytes and it's interaction with EPB41 has not been investigated. The purpose of this study was to analyze the heme-binding sites in EPB41, PKC and their complex.

Materials and methods. The UniProt knowledge base (http://www.uniprot.org/) was used to obtain sequences in *.fasta format and protein annotations. Protein and heme structures were taken from PDB (http://www.rcsb.org/). Protein docking was performed by GRAMM server (https://gramm.compbio.ku.edu/). Post-translational modifications of EPB41 were analyzed using PhosphoSitePlus (https://www.phosphosite.org). Blind docking with heme as a ligand was performed by CB-DOCK2 server (https://cadd.labshare.cn/cb-dock2/).

Full structure of human EPB41 has not been experimentally solved for this moment. Therefore partial experimental structures with FERM domain 1GG3, 3QIJ were used for docking. Alphafold model of EPB41 was manually modified to remove regions not corresponding to the erythroid form. Protein kinase C has several experimental structures, monomeric 4RA4 and oligomeric 3IW4 were taken for docking studies.

Results and discussion. Heme docking to EPB41 predicted two binding areas with high scores: a pocket with His401 in FERM domain and the region out of FERM domain, close to the phosphorylation site Ser540. It is known that two sites of EPB41 are phosphorylated by PKC: Ser521 and Ser540. They are both located in 16kDa domain between FERM-domain and C-end. Phosphorylation of EPB41 results in loss of its affinity to spectrin and actin and reorganization of erythrocyte cytoskeleton.

PKC active site Asp463 also was predicted to be potential target for heme. Docking to PKC monomer revealed several variants of heme binding near the catalytic site within 5-6Å from His428 in contact area. Histidine is known to have high specific affinity to heme therefore it could form a stable complex. Heme as prooxidant could enhance production of reactive oxygen species and additional activate PKC.

Assembly of two protein chains (EPB41 and PKC) was modeled by protein-protein docking with contact area between phosphorylation sites and kinase active centre. Heme docking to this complex revealed EPB41 FERM domain as the most probable binding site.

Conclusions. According to docking results heme could bind to both FERM domain and phosphorylation sites of EPB41 through amino acid residues with high affinity. This could be significant for modifying of the protein's function during hemolysis. Protein kinase C could bind heme mostly in unbound state when its catalytic site is not occupied. Heme could enhance EPB41 phosphorylation under hemolysis that could lead to conformational changes of cytoskeletal complex and erythrocytes stability.

Посунько О., Бараннік Т.

IN SILICO АНАЛІЗ ФОСФОРИЛЮВАННЯ EPB41 ПРОТЕЇНКІНАЗОЮ С ЗА УМОВ ГЕМОЛІЗУ

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Анотація. *Білок смуги 4.1 (EPB41) належить до родини білків з доменом FERM і є одним із ключових компонентів цитоскелету еритроцитів. В еритроцитах EPB41 взаємодіє з кількома структурними та транспортними мембранними білками, зокрема із білком смуги 3, глікофорином та спектрином. Протеїнкіназа С (PKC) є одним із регуляторів EPB41 і активується реактивними формами кисню. За умов гемолізу відбувається накопичення гему, що є прооксидантом. Молекулярний докінг з гемом виявив потенційні зв'язувальні сайти в активному центрі PKC, але при моделюванні комплексу з EPB41 гем зв'язувався переважно з FERM-доменом EPB41. У сайті фосфорилювання білку EPB41 прикріплення гему було маловірогідним. Посилення фосфорилювання EPB41 через накопичення гему може призводити до конформаційних змін цитоскелету і дестабілізації еритроцитів.*

Ключові слова: *EPB41, протеїнкіназа С (PKC), гемоліз, FERM-домен, оксидативний стрес.*

UDC 578

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VIRUCIDAL ACTIVITY OF FULLERENOLS AGAINST INFLUENZA A VIRUS

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Abstract. *Influenza viruses are a constant threat, causing millions of flu cases every year. Despite existing drugs the need for new ones is constant due to the mutational potential of the virus. Fullerenes are unique carbon molecules with great antiviral potential. Therefore, the goal of this work was to determine the virucidal activity of hydrated fullerenes against the influenza A (H1N1) virus. For that, influenza virus was incubated with fullerenes of different concentrations for 15, 30 and 60 minutes with subsequent determination of infectious titer. Along with the low toxicity, fullerenols showed a pronounced dose-dependent virucidal activity regardless of the time of contact with the virus. The decrease in the infections titer was up to 8 orders of magnitude compared to the virus control. The results indicate the potential of fullerenols in the development of new antiviral agents.*

Keywords: *fullerenes, antivirals, H1N1, nanotechnology.*

Introduction. Influenza viruses are a constant health threat. Annual regional epidemics lead to millions of flu cases and hospitalizations, despite preventive measures and vaccination [1]. To date, the most common anti-influenza agents are neuraminidase inhibitors (oseltamivir, zanamivir), but due to the mutational potential of the virus, the need for new drugs is constant.

Fullerenes (C_n) are completely carbon molecules of spherical or ellipsoid shape. These molecules have unique chemical properties and are able to interact with various atoms, molecules and complexes, which is the basis of their use in the fields of biotechnology [2]. Various fullerene derivatives show pronounced antiviral activity against RNA-viruses. Fullerenols - water-soluble hydrated fullerenes - occupy a special place among derivatives, because they are biocompatible [3].

The goal of this work was to determine the virucidal activity of hydrated fullerenes against the influenza A (H1N1) virus. The objectives of the study were: to investigate the cytotoxic effect of the drug on cell culture and to determine the effectiveness against the influenza virus.

Materials and methods. The preparation of a mixture of fullerenols with a mass ratio of 78.1% C_{60}/C_{70} and 21.9% $C_{76}/C_{78}/C_{84}$ (YMFNANO®, Ukraine) was used for the study. Experiments were performed using MDCK cell culture and influenza A (H1N1) virus strain A/FM/1/47.

Cytotoxicity was determined using the MTT-assay in the range of concentrations from 0.5 to 3 mg/ml. Virucidal activity was determined by the reduction of the virus titer ($TCID_{50}$/ml) relative to the control after contact with fullerenols for 15, 30 and 60 min in the range of concentrations from 0.01 to 2 mg/ml.

Results and discussion. The obtained results of cytotoxicity evaluation indicate the presence of dose-dependent activity: with increasing concentration, the proportion of living cells becomes smaller. Based on cell viability data, the half-maximal cytotoxic concentration was calculated, CC_{50}=2.89±0.14 mg/ml.
The results of the study of virucidal activity indicate a pronounced activity of the fullerenols regardless of the time of contact with the virus. Thus, after incubation of the virus with the drug for 60, 30 and 15 minutes, it was found that at concentrations of 0.5, 1 and 2 mg/ml, fullerenols completely inactivate the virus, leading to a complete loss of its infectivity. The decrease in titer compared to the control was 8.19 orders of magnitude. At the lower studied concentrations (0.1 and 0.01 mg/ml), a similar pattern was observed for all time points: the decrease in the infectious titer was 2.5-3 orders of magnitude, relative to the virus control.

Conclusions. Thus, taking into account the low cytotoxicity, the obtained results about the pronounced virucidal activity of fullerenols can be an important contribution to the development of new strategies to fight viral infections.

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ДОСЛІДЖЕННЯ ЕФЕКТИВНОСТІ ФУЛЕРЕНІВ ПРОТИ ВІРУСУ ГРИПУ ТИПУ А

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Анотація. *Віруси грипу становлять постійну загрозу, щорічно спричиняючи мільйони випадків захворювань. Найефективніші препарати 2024 року — занамівір, осельтамівір, римантадин, амантадин і рибавірин. Фулерени — унікальні вуглецеві молекули з противірусною активністю. Мета роботи було визначення віруліцидної активності гідратованих фулеренів проти грипу H1N1. Дослідження з використанням клітин MDCK показали дозозалежну активність фулеренолів, що при концентраціях 0,5, 1 та 2 мг/мл повністю інактивували вірус. Результати свідчать про значний потенціал фулеренолів для розробки нових противірусних засобів.*

Ключові слова: *вірус, грип А, фулерен, H1N1.*

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HEMATOLOGICAL AND BIOCHEMICAL PROFILE OF DOGS WITH DISEASE CAUSED BY PROTOZOAN PARASITES *BABESIA CANIS* AND ITS TREATMENT WITH IMIDOPYRAN AND PREDNISONE

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Abstract. *Babesiosis in dogs is a tick-borne disease caused by apicomplex hemoprotozoan parasites in red blood cells. According to recent reports, the most well-known type of babesiosis found in Ukraine is the B. canis. This study includes a retrospective investigation of clinical cases of babesia infection in dogs. For this study, a complete hematological and biochemical profile of 25 dogs with babesiosis submitted to the Peredovyi Veterinary Complex, Dnipro, was obtained and analyzed.*

Treatment of dogs infected with Babesia canis with imidopyran and prednisone resulted in improvement of both biochemical and hematological parameters. There was a decrease in the levels of liver enzymes, bilirubin and alkaline phosphatase, as well as an increase in the number of red blood cells, hemoglobin, hematocrit and platelets. This indicates a decrease in intoxication, improvement of liver function and hematopoiesis, although most indicators have not yet reached normal values. It is necessary to continue monitoring the dogs' condition and apply supportive therapy to achieve full recovery of hematological and biochemical parameters.

Keywords: *babesiosis, Babesia canis, imidopyrine, prednisone.*

Introduction. The protozoan parasite *Babesia canis* is the causative agent of canine babesiosis, a severe disease characterized by red blood cell destruction, hemolytic anemia, and significant systemic disorders. Canine babesiosis is common in regions with high activity of Ixodes ticks, the main vectors of *Babesia canis*. The disease can lead to serious consequences, including organ failure and death, if not treated in a timely manner. The hematologic and biochemical profiles of dogs infected with *Babesia canis* show characteristic changes. Analysis of these parameters is critical for diagnosing and monitoring the course of the disease. The aim of the study is to comprehensively evaluate the hematological and biochemical profiles of dogs infected with the protozoan parasite *Babesia canis*, as well as to determine the effectiveness of treatment with imidopyran and prednisone.

Materials and methods. 25 domestic dogs weighing 1-10 kg aged from 2 months to 17 years were used for the study. The detection of *Babesia canis* parasites inside erythrocytes was performed using thin blood smears stained with LEUCODIF 200 fast dyes (Erba Lachema, Czech Republic) and examined under 100x magnification using a Leica DM4 optical microscope (Germany). Hematologic analysis was performed using an automated analyzer MicroCC-20 Plus (HTI, USA). The study of biochemical parameters was performed using a semi-automatic analyzer BS-3000M (SINNOWA, China).

The main period of treatment of babesiosis in dogs is the first 24 hours with imidopyran (Arterium, Ukraine, dose 7 mg/kg) and prednisone (Darnitsa, Ukraine, dose 2.2 mg/kg) with simultaneous injection. The parameters of the study of the impact of the disease on the development of hemolytic anemia and liver function were the parameters of complete blood count and liver biochemical parameters. In addition, the effect of treatment with imidopyran and prednisone on reducing the development of hemolytic anemia and improving liver function was studied.

Results and discussion. The main indicator of the development of anemia in animals is the number of erythrocytes, hemoglobin, hematocrit and platelets. The number of red blood cells before treatment (3.59 million/mL) was reduced compared to the norm (6.35 million/mL) and increased after treatment (4.20 million/mL), which indicates an improvement in the dogs' condition. However, this figure is still significantly lower than normal, which may indicate the presence of residual anemia and that the recovery process is ongoing. The hemoglobin level also improved after treatment (pre-treatment - 83.38 g/l; post-treatment - 90.92 g/l), but remains below normal (158.00 g/l). This indicates an improvement in tissue oxygenation, but the recovery process is not yet complete. The increase in hematocrit after treatment (before treatment - 22.54%; after treatment - 26.92%) indicates an improvement in the concentration of red blood cells in the blood. However, the index still remains significantly lower than normal, which confirms the presence of residual anemia. The increase in platelet count after treatment (before treatment - 38.00 thousand/mL; after treatment - 65.15 thousand/mL) indicates an improvement in the process of thrombosis. However, the platelet count remained significantly below normal (330.00 thousand/mL), which may indicate a prolonged recovery from the acute condition and the presence of residual disorders in blood coagulation.

The ALT level in dogs before treatment was below normal (40.76 U/l). After the treatment, the index decreased (34.24 U/l) and remains below normal values (52.00 U/l). This may indicate an improvement in liver function, but the value is still below normal, which may be due to prolonged exposure to infection. AST levels were elevated before treatment (51.22 U/l) and remained above normal (36.00 U/l) after treatment (44.32 U/l), although the value

decreased. This may indicate residual damage to the liver or other tissues containing AST, but there is a gradual recovery. The level of total bilirubin was significantly elevated before treatment (20.95 μmol/l) and remained elevated after treatment (15.97 μmol/l), although it decreased. This indicates a decrease in the hemolytic process and improvement in liver function, but the values are still significantly higher than normal. The level of alkaline phosphatase was elevated before treatment (86.19 U/l) and remained elevated after treatment (82.47 U/l), with a slight decrease. This may indicate some reduction in liver stress, but the value remains above normal (66.50 U/l), possibly due to residual damage.

Conclusions. Treatment of dogs infected with *Babesia canis* with imidopyran and prednisone resulted in improvement of both biochemical and hematological parameters. There was a decrease in the levels of liver enzymes, bilirubin and alkaline phosphatase, as well as an increase in the number of red blood cells, hemoglobin, hematocrit and platelets. This indicates a decrease in intoxication, improvement in liver function and hematopoiesis, although many indicators have not yet reached normal values. This indicates the need for further monitoring and supportive therapy to achieve full recovery of the dogs' hematological and biochemical status.

Невідник-Правда А., Ушакова Г.

ГЕМАТОЛОГІЧНИЙ ТА БІОХІМІЧНИЙ ПРОФІЛЬ СОБАК ІЗ ЗАХВОРЮВАННЯМ, СПРИЧИНЕНИМ ПРОТОЗОЙНИМИ ПАРАЗИТАМИ *BABESIA CANIS,* ТА ЇЇ ЛІКУВАННЯ ІМІДОПІРАНОМ ТА ПРЕДНІЗОЛОНОМ

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Анотація. *Бабезіоз у собак – кліщове захворювання, яке викликається апікомплексними гемопротозойними паразитами в еритроцитах. За останніми даними найпоширенішим в Україні збудником бабезіозу є Babesia canis. Дана роботи включає ретроспективне дослідження клінічних випадків інфекції бабезії у собак. Для цього були отримані та проаналізовані повні гематологічні та біохімічні профілі 25 собак, хворих на бабезіоз, доставлених до Ветеринарного комплексу «Передовий», м. Дніпро.*

Лікування інфікованих собак імідопіраном і преднізолоном призвело до поліпшення як біохімічних, так і гематологічних показників. Спостерігалося зниження рівня печінкових ферментів, білірубіну та лужної фосфатази, а також підвищення кількості еритроцитів,

гемоглобіну, гематокриту та тромбоцитів. Хоча більшість показників на даному етапі лікування не досягли нормальних значень, такі результати свідчили про зниження інтоксикації, поліпшення функції печінки і кровотворення. Необхідно продовжувати спостереження за станом собак та проводити підтримуючу терапію для досягнення повного відновлення гематологічних і біохімічних показників.

Ключові слова: *бабезіоз, Babesia canis, імідопіран, преднізолон.*

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INFLUENCE OF MARIGOLDS EXTRACTS ON THE MORPHOLOGICAL AND FUNCTIONAL FEATURES OF CELLS

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Abstract. *The cell viability assay is an important method of toxicology analysis. That is why the aim of this study was to investigate influence of ethanol extracts of marigolds on the morphological and functional features of cells. It was found that the viability results were dependent not only on the type of marigold, but also on the part of the plant from which the extract was obtained. Extracts from marigold flowers showed a lower toxic effect on both the morphological and functional properties of Vero cells (CC_{50} value was in range 1:45-1:286). The obtained data are important for further studies for antitumor and antiviral properties of marigold extracts.*

Keywords: *herpes simplex virus 1, plant extracts, cytotoxic effect.*

Introduction. Natural products, such as plant extracts, have shown high antiherpetic activity *in vitro* and *in vivo* and are an excellent source for the discovery and isolation of new antiviral compounds [1, 2]. The urgent problem of studying the effect of plant extracts is, first of all, the study of their toxic effects and the impact on the course of cellular life processes. That is why the aim of this study was to investigate influence of ethanol extracts of marigolds on the morphological and functional features of cells.

Materials and methods. Extraction of biologically active substances from fresh flowers and green mass of three types of marigolds was carried out using 70% ethanol. Vero cells morphology after extracts exposure at various doses was analyzed and documented using an inverted microscope (magnification 70). The influence of extracts on mitochondrial and lysosomal activity of cells were studied using MTT and neutral red assays [3, 4].

Results and discussion. This study confirms that ethanol extracts of marigolds induces changes in the cell morphology and functional features, which were dose dependent and more pronounced with an increase of extracts concentration. Extract isolated from the flowers of *Tagetes patula* marigolds turned out to be the least toxic for the cells, because the cells functioned normally (cell viability, depending on the concentration of the extract and the research method, was 55-100%). On the other hand, with the use of *T. patula* green mass extracts in the same dilutions, a significant disruption of the cell monolayer, cells rounding, granularity and destruction were observed, at the same time their mitochondrial and lysosomal activity decreased by 85-93% and 33-61%, respectively. A similar pattern was found for extracts isolated from other types of marigolds. Thus, extracts from marigold flowers showed a lower toxic effect on both the morphological and functional properties of Vero cells (CC_{50} value was in range 1:45-1:286).

Conclusions. It was found that the cell viability results were dependent not only on the type of marigold, but also on the part of the plant from which the extract was obtained. The current *in vitro* cytotoxicity screening of various marigolds offered important preliminary information for selection of plant species and their extracts for further investigations in drug development research involving their antitumor and antiviral properties.

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ВПЛИВ ЕКСТРАКТІВ ЧОРНОБРИВЦІВ НА МОРФОЛОГІЧНІ ТА ФУНКЦІОНАЛЬНІ ХАРАКТЕРИСТИКИ КЛІТИН

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Анотація. *Дослідження життєздатності клітин є важливим методом токсикологічного аналізу. Саме тому метою цієї роботи було дослідити вплив етанольних екстрактів чорнобривців на морфологічні та функціональні особливості клітин. Було виявлено, що життєздатність залежить не тільки від виду чорнобривців, але й від частини рослини, з якої було отримано екстракт. Екстракти з квіток чорнобривців показали менший токсичний вплив як на морфологічні, так і на функціональні властивості клітин Vero (значення CC50 знаходилося в діапазоні 1:45-1:286). Отримані дані є важливими для подальших досліджень*

протипухлинних та противірусних властивостей екстрактів чорнобривців.

Ключові слова: *вірус простого герпесу 1 типу, рослинні екстракти, цитотоксична дія.*

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ACTIVITY OF NO-SYNTHASE ISOFORMS IN THE LIVER OF RATS WITH TOXIC INJURY AFTER PARTIAL HEPATECTOMY

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Abstract. *The study is dedicated to evaluating the activities of inducible and constitutive isoforms of NO-synthase in the cytosolic and mitochondrial fractions of rat liver under conditions of acetaminophen-induced injury and partial hepatectomy. Partial hepatectomy after modeling a toxic injury in the liver of rats is accompanied by a decrease in the activity of the constitutive isoform of NO synthase at the initial stages of regeneration (24 and 48 hours) and enhanced activation of the inducible isoform of NO synthase in the remote period (168 hours).*

Keywords. *Nitric oxide, NO-synthase, partial hepatectomy, acetaminophen, liver.*

Introduction. Currently, the problem of the development and progression of hepatic homeostatic imbalance under the influence of hepatotoxins, particularly paracetamol (acetaminophen, APAP), remains relevant. Compensatory proliferation of hepatocytes (hyperplasia or hypertrophy) followed by liver regeneration and restoration of hepatostat in response to segmental resection, infectious diseases, chronic and acute injuries by xenobiotics, including APAP, plays a crucial role in restoring the homeostatic capability of the organ [1]. At the initiation stage of the regenerative process, it is important to maintain an optimal concentration of nitric oxide (NO) in liver cells. NO is a universal messenger that exhibits cytoprotective effects under physiological conditions, but in excessive amounts, it possesses cytotoxic properties. The enzymatic synthesis of NO is provided by NO synthase through the oxidation of the guanidine group of L-arginine. There are constitutive (cNOS) and inducible (iNOS) isoforms of NO synthase [2, 3]. Therefore, the aim of this study was to evaluate the activities of inducible and constitutive isoforms of NO synthase in the cytosolic and mitochondrial fractions of rat liver under conditions of acetaminophen-induced injury and partial hepatectomy (PH).

Materials and methods. Experimental animals were divided into two groups: control rats that underwent partial hepatectomy of 2/3 of liver tissue (C/PH) and rats that underwent resection of the organ after toxic injury by APAP (TI/PH). The study was conducted at 24 (initiation phase), 48 (proliferative phase), 72 (termination phase), and 168 (remote period) hours after PH.

Results and discussion. The research results indicate a multidirectional trend in the changes of cNOS activity in the cytosolic fraction of liver cells in rats from the C/PH and TI/PH groups. If a significant increase in cNOS activity is observed in the C/PH group during the initiation period and active cell proliferation (24 and 48 hours), then a decrease in cNOS activity compared to the control (0 hours) is recorded in the TI/PH group during the same periods. It is likely that the activation of cNOS at the initial stages of regeneration in the C/PH group is due to hemodynamic changes in the liver that occur after PH. Considering that cNOS is characterized by basal NO production under physiological conditions, a decrease in its activity during the initiation period of the regenerative process in rats of the TI/PH group may lead to a disruption of the implementation in the compensatory reaction involving NO in response to liver damage [2, 3]. Evaluating the trend of changes in iNOS activity in the cytosolic fraction of rat liver under the studied conditions, the C/PH group recorded an increase in iNOS activity at 24 and 48 hours after PH. In return, in the TI/PH group exhibited enhanced iNOS activation not only during the initial stages of liver regeneration (24 and 48 hours) but also during the remote period (168 hours). It is known that the release of pro-inflammatory cytokines (TNFα, IL-6) during the initiation of the regenerative cascade of reactions leads to the induction of iNOS [2, 3]. Accordingly, at the initial stages of regeneration, the changes shown probably occur in response to the development of inflammatory processes after surgical resection. In the remote period, the demonstrated induction of iNOS in the TI/PH group may be mediated by the occurrence of oxidative-nitrosative stress.

Regarding the changes in the activity of NO synthase isoforms in the mitochondrial fraction of liver cells, it is worth noting that in the TI/PH group, we observed an increase in both cNOS and iNOS throughout the entire experimental period. The maximum increase in iNOS activity under the conditions of conducting PH after APAP-induced toxic injury is detected during the initiation period and the remote period. Excessive generation of NO in mitochondria at 168 hours after conducting PH, when interacting with $O_2^{\bullet-}$, may be accompanied by the production of highly toxic $ONOO^-$. The latter is capable of initiating mitochondrial dysfunction with increased apoptotic or necrotic cell death [2].

Conclusions. Thus, performing partial hepatectomy after modeling a toxic injury in the liver of rats is accompanied by a decrease in the activity of

the constitutive isoform of NO synthase at the initial stages of regeneration (24 and 48 hours) and enhanced activation of the inducible isoform of NO synthase in the remote period (168 hours).

Копильчук Г., Николайчук І., Урсатий М.

АКТИВНІСТЬ ІЗОФОРМ NO-СИНТАЗИ В ПЕЧІНЦІ ЩУРІВ ІЗ ТОКСИЧНИМ УРАЖЕННЯМ ПІСЛЯ ЧАСТКОВОЇ ГЕПАТЕКТОМІЇ

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Анотація. *Робота присвячена оцінці активностей індуцибельної та конститутивної ізоформ NO-синтази в цитозольній та мітохондріальній фракціях печінки щурів за умов ацетамінофен-індукованого ураження та часткової гепатектомії. Проведення часткової гепатектомії після моделювання токсичного ураження печінки щурів супроводжується зниженням активності конститутивної ізоформи NO-синтази на початкових етапах регенерації (24 та 48 год) та посиленою активацією індуцибельної ізоформи NO-синтази у віддалений період (168 год).*

Ключові слова: *Оксид азоту, NO-синтаза, часткова гепатектомія, ацетамінофен, печінка.*

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PARATHYROID CARCINOMA WITH WIDE INVASION: CASE REPORT

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Abstract. *Parathyroid carcinoma (PC) is an uncommon malignancy originated from parathyroid parenchymal cells [1]. The chief method of treatment is surgery, including parathyroidectomy or block resection of the parathyroid gland [2]. We represent a case of parathyroid carcinoma in a patient with primary hyperparathyroidism.*

Keywords: *endocrine neoplasm, hypercalcaemia, parathyroid carcinoma.*

Introduction. Parathyroid carcinoma (PC) is a most rare endocrine malignancy. It is a functional tumor that produces an excess of parathyroid hormone (PTH), which leads to hypercalcemia with high ionised calcium serum level. The main goal of this effort is to represent clinical manifestation, diagnosing and treatment of patient with parathyroid carcinoma due to primary hyperparathyroidism.

Materials and methods. We collected the patient's clinical data, the results of imaging diagnostics, samples of pathological slides, and the pathologist's report. We also got access to the notes about surgery course and findings.

Results and discussion. The case was a 67-year-old male who suffering from cough and joint pain for about three weeks. Ultrasound imaging of thyroid (TG) and parathyroid glands (PTG) showed: a cystic lesion size 75 mm having a septum in the cavity in the right thyroid lobe. Behind the dorsal surface of left lobe, the hypoechoic mass with a size of 19 mm is detected. Blood test results: PTH - 292,1 pg/mL, ionised calcium - 1,56 mmol/L, Vit D - 47,5 nmol/L, Antithyroid peroxidase (ATPO) - 0.2 IU/ml, thyroglobulin antibody (TgAb) - less than 0.9 IU/ml.

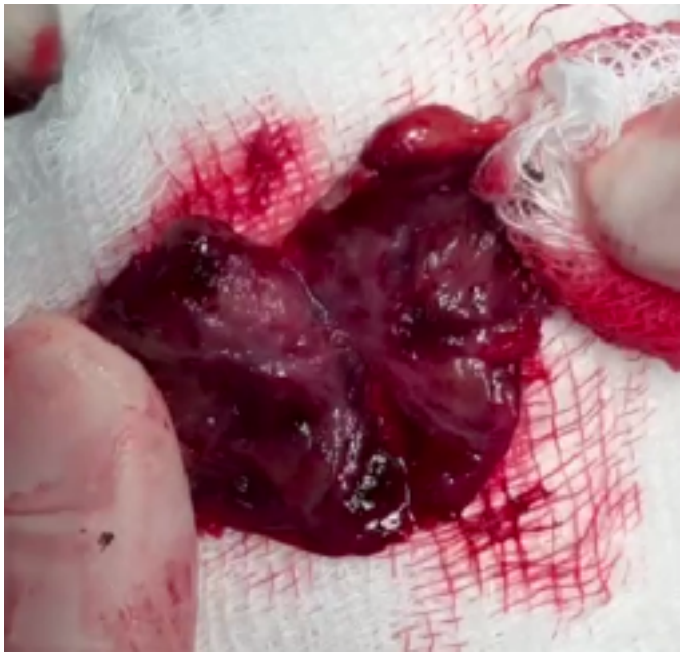

Fig. 1. Parathyroid tumor on the section.

The diagnosis was established: Primary hyperparathyroidism, visceral form. Adenoma of the left lower parathyroid gland. Nodular goiter of the 2nd grade, euthyroidism. The patient was referred for surgery. Surgery findings: Right thyroid lobe nodule 80 mm in size. A PTG tumor 25 mm in size (Fig. 1.), with visual signs of invasion into the tissue of TG, pretracheal tissue and muscles, into the muscular layer of the oesophagus, into the trachea and into the recurrent nerve. Surgery outcomes: Left-sided hemithyroidectomy with left lower parathyroidectomy, right thyroid lobe resection. Pathology report: Parathyroid carcinoma, signs of extraorganic invasion. Microfollicular thyroid adenoma. (Fig. 2.)

Conclusions. We report a case of left lower parathyroid gland carcinoma with primary hyperparathyroidism. The key clinical sighs of parathyroid carcinoma in our patient were increased PTH and ionised calcium serum level, invasion of the thyroid gland, surrounding tissue and recurrent nerve [3]. The only efficient treatment way of PC is surgical removal of a parathyroid gland tumor.

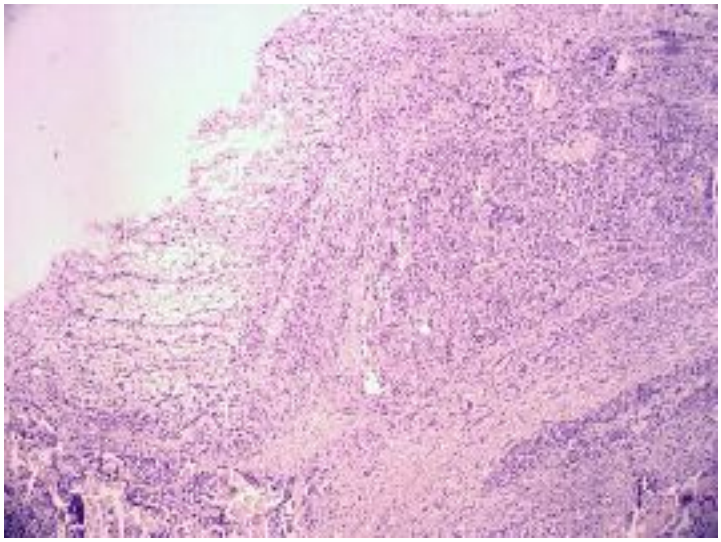

Fig. 2. Extraorganic invasion by parathyroid carcinoma (H&E, ×10).

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КАРЦИНОМА ПАРАЩИТОПОДІБНОЇ ЗАЛОЗИ З ШИРОКОЮ ІНВАЗІЄЮ: КЛІНІЧНИЙ ВИПАДОК

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Анотація. *Карцинома паращитоподібної залози (ПК) - це рідкісна злоякісна пухлина, що походить з паренхіматозних клітин паращитоподібних залоз [1]. Основним методом лікування є хірургічне втручання, включаючи паратиреоїдектомію або блокову резекцію паращитоподібної залози [2]. Ми презентуємо випадок паратиреоїдної карциноми у пацієнта з первинним гіперпаратиреозом.*

Ключові слова: *ендокринне новоутворення, гіперкальціємія, карцинома паращитоподібної залози.*

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UDC 58.085: 58.035.4

Batuieva Y., Hrimanova K.

EFFECT OF IRRADIATION WITH RED AND BLUE LIGHT ON THE MORPHOGENESIS OF CALLUS TISSUE OF THE ISOGENIC LINE *GLYCINE MAX* (L.) *MERR.* WITH A SHORT PHOTOPERIODIC REACTION

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Abstract. *The aim of the work was to investigate the effect of irradiation with red (660 nm) and blue (450 nm) light on the photomorphogenesis of the transplanted callus culture of soybean (Glycine max (L.) Merr.) in vitro. It was shown that the growth of callus tissue is inhibited during exposure to RL and BL. The prolonged effect of exposure to RL and BL is expressed in RL inhibition of callus tissue growth and absence of BL exposure, compared to the control, as well as in stimulation of various pathways of callus culture morphogenesis in vitro.*

Keywords: *photobiotechnology, photomorphogenesis in vitro, photoreceptors, selective light, soybean.*

Introduction. Photobiotechnology is a modern branch of biotechnology that studies the interaction of light with biological systems and the use of unique properties of light to regulate biological processes. One of the areas of research is photomorphogenesis - the process of vital activity and development of plants under the influence of various spectral parameters of light. Different ranges of light waves have different effects on morphogenetic processes, and this effect depends individually on the species and variety of plants. Due to growing demand for sustainable agricultural and biotechnological products, the study of the main influencing factors - light - on plant morphogenesis is relevant. The appearance of new lamps - LEDs, which provide radiation of a certain wavelength - monochromatic (selective) light and a combination of light lengths. This makes it possible to study the individual influence of light on the morphogenesis of plants and provide optimal conditions for their cultivation, became revolutionary [1, 3].

The connecting link between light and morphogenesis is the photoreceptor system of plants, the components of which are photoreceptors - specialized light-sensitive proteins. Photoreceptors participate in the initiation of cascade reactions for the gene regulation of plant life processes [4].

Nowadays five classes of photoreceptors have been identified: phytochromes, cryptochromes, phototropins, F-box proteins, and UVR8 protein. Photoreceptors also have an influence on other regulatory mechanisms of plants, which makes it difficult to study the molecular mechanism of their actions [6, 7].

In vitro culture is the best model system for studying the processes of plant growth and development. Cells of the callus tissue of higher plants in in vitro culture, along with the acquisition of new specific properties, are able to retain the properties characteristic of plants in in vivo conditions. It is known that phytochrome and cryptochrome systems of plants are involved in the perception of the photoperiodic signal, and therefore photoperiodically sensitive groups of plants can be the best system for studying the processes of photomorphogenesis. Also, the results of research on *in vitro* cultivation can be applied to countries with unfavorable climatic conditions to increase the level of agricultural production [2, 7].

Hence, the aim of our work was to investigate the processes of photomorphogenesis of the callus culture of the short-day line of soybean under in vitro conditions under the action of monochromatic blue (450 nm) and red (660 nm) light spectra.

Materials and methods. The object of the study was a callus culture of the isogenic line of Clark soybean with a genotype characterized by a short-day photoperiodic response. Soybean primary callus was obtained through the stage of aseptic seedlings cultured on Schenk-Hildebrandt hormone-free medium, after which the explants were passivated on Murashige and Skoog (MS) callus induction medium with the addition of 5 mg/l 2,4-D. The primary callus was cultivated for 2 weeks in a thermostat at a temperature of 26 °C. During this period, the callus was irradiated daily with red light (RL) and blue light (BL) for 15 minutes a day using LED matrices, simultaneously analyzing callus growth indicators - area, growth rate, and growth. After that, the callus was passivated on the MS regeneration medium containing 0.5 mg/l IAA and 0.5 mg/l BAP, and cultivated in the light for a month, analyzing the manifestation of the morphogenic potential of the control and experimental callus.

The obtained results were processed statistically using the method of dispersion analysis.

Results and discussion. According to the results, the primary soybean calluses cultivated in the darkness exhibit typical callus tissue characteristics: yellowish, amorphous, highly hydrated, fast-growing. During the photoperiod of RL and BL induction, we measured the growth response indicators of callus tissue. In our studies, it was demonstrated that the growth and growth rate of primary callus tissues under the influence of RL and BL were inhibited to the same extent, compared to the control. Morphologically, the callus tissues after 2

weeks of irradiation did not differ from the control - typical, watery, amorphous masses. The action of BL was distinguished from others, under the action of which the appearance of centers of mixotrophic areas was observed in the callus.

The study of the prolonged effect of irradiation was continued during the passivation of callus tissues on the regeneration medium. During the study of the effects of RL and BL irradiation on the morphogenetic potential of callus tissues, we noted such characteristics such as color, the appearance of necrotic spots, callusogenesis, the appearance of morphogenic structures, and rhizogenesis. The analysis of the parameters of the growth response showed that exposure to RL inhibits the growth of callus tissue, compared to the control, and under the action of BL, the growth rate of callus tissue does not differ from the control.

Mixotrophic callus was formed during the cultivation of soybean callus tissue in the light. Activation of the cryptochrome system, which is known to control the stages of chlorophyll synthesis, stimulated the formation of a bright green callus, while exposure to RL leads to the appearance of a greenish-brown color. Prolonged action of BL stimulated the manifestation of various types of morphogenesis as much as possible. Irradiation of RL also stimulated morphogenetic reactions of callus tissue, but with a predominance of callusogenesis processes.

Conclusions. Thus, under *in vitro* conditions, the influence of activation of red and blue light photoreceptors on the growth and morphogenetic characteristics of callus cultures is observed. In the period of irradiation of RL and BL, a decrease in growth and growth rate of primary callus tissues is noted, compared to the control. After transfer to the regenerative nutrient medium, the growth of callus tissue is inhibited due to the activation of phytochromes under the action of the emergency system and remains at the level of the control variant under the activation of cryptochromes under the action of the BL. The prolonged effect of exposure to RL and BL is expressed in stimulating the manifestation of various pathways of callus culture morphogenesis *in vitro*.

Батуєва Є., Гріманова К.

ВПЛИВ ОПРОМІНЕННЯ ЧЕРВОНИМ ТА СИНІМ СВІТЛОМ НА ФОТОМОРФОГЕНЕЗ КАЛЮСНОЇ ТКАНИНИ СОЇ КУЛЬТУРНОЇ З КОРОТКОДЕННОЮ ФОТОПЕРІОДИЧНОЮ РЕАКЦІЄЮ

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Анотація. *Метою роботи було дослідити вплив опромінення червоним (660 нм) та синім (450 нм) світлом на фотоморфогенез пересадкової калюсної культури сої культурної (Glycine max (L.) Merr.) в умовах in vitro. Було показано, що приріст калюсної тканини гальмується під час опромінення ЧС і СС. Пролонгований ефект впливу ЧС та СС виражається у гальмуванні ЧС приросту калюсної тканини і відсутності впливу СС, порівняно з контролем, а також у стимулюванні прояву різних шляхів морфогенезу калюсної культури в умовах in vitro.*

Ключові слова: *фотобіотехнологія, фотоморфогенез in vitro, фоторецептори, селективне світло, соя культурна.*

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UDC 606:628

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SCREENING OF MARINE SPORE-FORMING BACTERIA DEGRADING POLYETHYLENE TEREPHTHALATE

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Abstract. *The ability of 60 cultures of marine spore-forming bacteria to degrade polyethylene was studied. The activity of microorganisms was evaluated preaccording to formation of clear zone around colonies on LB agar containing bis(hydroxyethyl)terephthalate (BHET). A total of 24 active strains were found with the prevalence of species Bacillus subtilis. Strains of Bacillus reuszeri, Bacillus licheniformis and Priestia megaterium showed the highest activity.*

Keywords: *polyethylene terephthalate, biodegradation, spore-forming bacteria, Black Sea*.

Introduction. The problem of plastic pollution is actual and requires finding new solutions [Sadler et al., 2021]. It is known that microorganisms can use waste from various industries as substrates for bioconversion and decompose them into monomers. Therefore, marine spore-forming bacteria were screened to determine their ability to degrade polyethylene terephthalate.

Materials and methods. The objects of the study were sixty cultures of spore-forming bacteria isolated from the bottom sediments of the Black Sea. All studied strains are represented by nine genera. The species affiliation of another 12 strains is unknown.

Cultivation of bacteria was carried out on an agarized LB nutrient medium with the following composition (g/l): tryptone – 10; NaCl - 5; yeast extract - 5; agar - 15. 5 mM bis(hydroxyethyl)terephthalate (BHET) was added to the medium. BHET consists of terephthalic acid and ethylene glycol residues, that are PET monomers. Therefore, it is convenient to use it to check the presence of PET hydrolytic activity [Weber et al., 2021].

All cultures were plated in duplicate for each strain. Cultivation was carried out in incubators with temperature regimes of 30 ºC and 37 ºC within 1 week.

The ability of the cultures to decompose polymer additive was evaluated by the formation of a transparent zone around the colonies.

Results and discussion. The strains were assigned numbers from B1 to B60. A total of 24 strains showed a positive result. The most active were 6 strains (B1, B8, B10, B12, B16 and B19) with zones of 2 mm and more. Average activity was shown by three strains (B26, B31 and B33) that formed zones of 1.5 mm. All other strains showed low activity, forming zones 1 mm or smaller.

No clear correlation between BHET decomposition and temperature was found. Fifteen strains degraded BHET at both temperatures, four strains only at 30 °C, and five strains only at 37 °C. In 10 strains, the activity increased with increasing cultivation temperature, in 7 – on the contrary, it was higher at 30 °C, in 7 strains it did not depend on temperature.

A total of 21 strains out of 24 active ones belonged to seven species of microorganisms: *Priestia megaterium* (3 strains), *Shouchella clausii* (1 strain), *Brevibacillus reuszeri* (1 strain), *Bacillus licheniformis* (1 strains), *Bacillus subtilis* (10 strains), *Bacillus atrophaeus* (2 strains), *Sutcliffiella halmapala* (1 strain). The species affiliation of three other strains is unknown.

The highest prevalence of the ability to decompose BHET was found among representatives of the species *B. subtilis* and was equal to 83%. But they showed low activity, the average size of transparent zones did not exceed 1 mm.

Strains belonging to the species *B. reuszeri* and *B. licheniformis* showed an average prevalence of 43 and 50%, respectively. Among the *B. reuszeri* strains, one strain (B8) showed high activity, and among the *B. licheniformis* strains, two strains (B12 and B19) showed high activity.

P. megaterium strains showed a reduced frequency of prevalence (27%), but at the same time high activity (two active strains B1 and B16). This bacterium was found in a consortium capable of degrading PET [Taniguchi et al., 2019]. Results demonstrate that *P. megaterium* decompose PET degradation products such as BHET.

The species *S. clausii, B. atrophaeus* and *S. halmapala*, which were each represented by one strain, did not show significant activity.

The genus *Bacillus* is the most widespread degrader among all studied (3 species and 15 strains). The literature describes the ability of *Bacillus* to decompose polyethylene terephthalate, but with low activity, which is confirmed by experimental data.

Conclusions. The ability of marine spore-forming bacteria to decompose polyethylene terephthalate was proven, activity was shown by 24 strains out of 60. Six strains showed high activity.

No clear dependence was found between the activity of enzymes that hydrolyze BHT and the temperature regime.

Five genera of bacteria (*Priestia, Shouchella, Bacillus, Sutcliffiella* and *Brevibacillus*) have been shown to be capable of degrading

bis(hydroxyethyl)terephthalate. Representatives of the *Bacillus subtilis* species (ten strains) were most often found among the active cultures, but none of them showed high activity.

Чебанов Н., Зінченко О., Штеніков М.

СКРИНІНГ МОРСЬКИХ СПОРОУТВОРЮЮЧИХ БАКТЕРІЙ, ЗДАТНИХ ДО БІОДЕГРАДАЦІЇ ПОЛІЕТИЛЕНТЕРЕФТАЛАТУ

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Анотація. *Досліджено здатність 60 культур морських спороутворюючих бактерій до деградації поліетилентерефталату (ПЕТ). Активність мікроорганізмів оцінювали за утворенням прозорих зон навколо колоній на середовищі LB з біс(гідроксіетил)терефталатом (БГЕТ). Виявлено 24 активних штами, більшість яких належать до виду Bacillus subtilis. Найвищу активність проявили культури Bacillus reuszeri, Bacillus licheniformis та Priestia megaterium.*

Ключові слова: *поліетилентерефталат, біодеградація, спороутворюючі бактерії, Чорне море.*

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UDC 578

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CYTOTOXIC EFFECT OF NANOCOMPOSITES AT LYTIC AND LATENT EBV-INFECTION

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Abstract. *Epstein-Barr virus (EBV) is one of the most widespread viruses in the human population. It is associated with many lymphoproliferative disorders when it is in the latency state, but none of the drugs affect this state of infection.*

In this work was researched the cytotoxicity effect of nanocomposites with silver on a model of EBV-associated B-cell lymphoma in the latent and lytic states. Besides this, we also analyzed the effect of the tested nanoparticles on the mitochondrial activity and the state of the lysosomal system.

Cytotoxicity study showed the most inhibition of cell culture viability by nanocomposites in the highest tested concentration as well as determination of mitochondrial. A study of the lysosomal system showed increasing lysosomal activity by nanocomposites in low concentrations. This effect was observed only for nanocomposites with Ag from what can be concluded, that these nanocomposites potentially can affect the EBV in vitro.

Keywords: *Epstein-Barr virus, nanocomposites, cell culture, cytotoxicity.*

Introduction. Epstein–Barr virus (EBV) is a human γ-herpesvirus that infects up to 95% of the adult population. The latency state of EBV is associated with lymphoproliferative disorders such as Burkitt's lymphoma, Hodgkin's lymphoma, and other oncological diseases while the lytic cycle is needed for virus replication.

Although some antiviral agents proved to be effective inhibitors of EBV replication in vitro and were used experimentally, none of them received approval by the FDA (Food and Drug Administration) or EMA (European Medicines Agency) for the treatment of EBV infections [1]. Moreover, none of the drugs affect latent infection, which is dependent upon persistent EBV episomes [2].

Materials and methods. This work aimed to study the cytotoxicity of three nanocomposites based on La and Ce oxides alloyed by Ag (La_2O_3-CeO_2, CeO_2-Ag>5% and La_2O_3-Ag>5%) on a model of EBV-associated B-cell lymphoma. Oxides synthesis was performed in Institute for Problems of Materials Science NAS of Ukraine [3]. Cell line B95-8 was used in the experiment as a model of the virus latency state. Sodium butyrate (BioFroxx, Germany) was added to the same cell line for modeling the lytic cycle. Each nanocomposite was irradiated with a bactericidal irradiator (BactoSfera, Ukraine) with wavelength 253,7 nm for 15 and 30 minutes. Also, non-irradiated nanocomposites were used as a control. In the course of the research, we analyzed the effect of the tested nanoparticles on the proliferative activity of cells with the detection of membrane permeability (0.4% trypan blue, BioFroxx, Germany), determination of mitochondrial activity (MTT, Sigma, USA), and the state of the lysosomal system (neutral red, Thermo Scientific, Germany).

Results and discussion. Cytotoxicity study (based on the trypan blue) showed the most inhibition of cell culture viability by nanocomposites in the highest tested concentration of 1000 μg/ml (cell viability ranged from 0,0 to 31,16%). The mitochondrial activity also was inhibited by nanocomposites in concentration 1000 μg/ml and was equal from 5,84 to 27,25%. A study of the lysosomal system showed increasing lysosomal activity by nanocomposites in low concentrations (1-100 μg/ml). This effect was observed only for nanocomposites with Ag that were irradiated for 30 minutes and non-irradiated ones. It should also be noted that mitochondrial and lysosomal activities were generally higher in the case of lytic infection compared to the latent one. Based on the increasing of lysosomal activity, it can be assumed that the nanocomposites with Ag can affect the virus, because of the possibility of the EBV to reduce autophagy [4].

Conclusions. Considering the results, it can be concluded that the nanocomposites of La and Ce oxides alloyed by Ag potentially can affect the EBV in vitro based on the results of the lysosomal activity study.

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ЦИТОТОКСИЧНА ДІЯ НАНОКОМПОЗИТІВ ЗА УМОВ ЛАТЕНТНОЇ ТА ЛІТИЧНОЇ ВЕБ-ІНФЕКЦІЇ

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Анотація. *Вірус Епштейна-Барр (ВЕБ) є одним із найпоширеніших вірусів серед людей. Це пов'язано з великою кількістю лімфопроліферативних розладів, які викликаються вірусом в латентному стані, однак жоден із препаратів не впливає на цей стан інфекції.*

У роботі було досліджено цитотоксичну дію нанокомпозитів зі сріблом на моделі ВЕБ-асоційованої В-клітинної лімфоми в латентному та літичному станах. Крім цього, ми також проаналізували вплив досліджуваних наночастинок на активність мітохондрій та стан лізосомальної системи.

Дослідження цитотоксичності показало найбільше пригнічення життєздатності клітинної культури нанокомпозитами у найвищій дослідженій концентрації, так само як і пригнічення мітохондріальної активності. Дослідження лізосомальної системи показало підвищення лізосомальної активності нанокомпозитами у низьких концентраціях. Цей ефект спостерігався лише для нанокомпозитів з Ag, з чого можна зробити висновок, що вони потенційно можуть впливати на ВЕБ in vitro.

Ключові слова: *вірус Епштейна-Барр, нанокомпозити, культура клітин, цитотоксичність.*

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BIOSURFACTANTS PRODUCED BY MARINE MICROORGANISMS

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Abstract. *This report provides an overview of the recent advances in the study of biosurfactants (BSs), surface-active metabolites produced by bacteria. These metabolites have gained significant attention due to their biodegradability, low toxicity, and potential for production from renewable and cheaper substrates. The report focuses on marine BSs, highlighting their sources, characterization, and potential biotechnological applications. The research on BSs is still evolving, with promising results obtained from marine resources in terms of BS chemical diversity, effectiveness, and microbial production capacity. The report concludes by highlighting the potential of marine bioprospecting and blue biotechnology as promising areas for future research and applications.*

Keywords: *biosurfactant, bacteria, biotechnology, aquatic environment.*

Introduction. Through the process of evolution, bacteria have adapted to survive by creating and utilizing surface-active products that facilitate the adsorption, emulsification, wetting, dispersal, and solubilization of water-immiscible compounds. Because of their biodegradability, low toxicity, and capacity to be synthesized from less expensive and renewable substrates, surface active metabolites, or BSs, have drawn a lot of attention in this context. As a result, they have gained an important ecological role due to their structural and functional diversity as well as their potential for multidisciplinary applications in the environmental and industrial fields [1].

Results and discussion. Bacterial secondary metabolic products, or BSs, have hydrophobic end groups and hydrophilic water soluble ends that allow them to show their surface and emulsifying properties. A range of yeasts, bacteria, and filamentous fungi produce them either outside the cell or as a component of the cell membrane from a number of substrates, including sugars, oils, alkanes, and waste products [2]. There are three main steps to investigating the production of BS from microorganisms: isolating potential BS producers; screening for BS production; and extracting and purifying the product, which is

occasionally enhanced by chemically characterizing molecules. These investigations are becoming more and more common. Fish from the Indian Ocean, sponges, sea pens, polychaetes in the Mediterranean Sea as sources of BS producers with ideal potentialities have all been identified [3].

One common property shared by BSs is their ability to relax or decrease surface tension, which promotes solubility and allows BSs to interact with interfaces that regulate bacterial adhesion and detachment. Together with the ability to remove contaminants, all of these characteristics give BS antibacterial, antifungal, and antiviral effects [4].

The class of glycolipids has been extensively researched in the marine environment because a large range of bacteria isolated from different marine matrices, including fish gut, sea pen *Pteroeides*, and annelida, as well as polluted soils (Arctic and Antarctic sediments), produce them [5].

The diverse roles that biosurfactants play are frequently exclusive to the physiology and ecology of the microorganisms that create them. As previously said, one of the most fascinating roles from an environmental perspective is illustrated by the various tactics used by microbes to improve their bioavailability and accessibility to hydrophobic substances as a source of carbon. A suggested mechanism for the incorporation of hydrocarbons into the hydrophobic core of the BS micelles is presented. This process, known as "micelle solubilization," was investigated using alkanes as model substrates [6]. It has been observed that strains of the Mediterranean Sea connected to *Rhodococcus spp.* can lower surface tension when oily substrates are present. The isolated beta-carotene synthases (BSs) have also been shown to be the best enhancers for the biodegradation of n-hexadecane and tetradecane [7].

The best option for oil removal was found to be a glycolipidic biosurfactant with a 45% removal capacity that is produced by the Mediterranean *Bacillus spp.*, a bacteria that is connected with coral. It has been demonstrated that marine-derived lipopeptides and glycolipids are effective against a variety of bacterial infections [8]. In fact, the lipopeptide generated by *N. alba* that was isolated from the Indian Ocean shown antibacterial action against *B. subtilis, C. albicans*, and *E. faecalis*. Certain microbes have the ability to control the characteristics of their cell surface using their BS, allowing them to adhere to or separate from surfaces as needed. It's interesting to note that *P. aeruginosa* ATCC10145, which was isolated from the Gulf of Mannar, exhibits suppression of biofilm formation; this appears to be related to BSs generated by strains linked to coral mucus [3, 8].

Furthermore, it has been discovered that rhamnolipids play a new role in the induction of defense responses in plants and animals. Specifically, it has been shown that rhamnolipids directly operate as a biocide on bacteria and fungus, and they also make some Gram-positive and Gram-negative bacteria more vulnerable to certain antibiotics [2].

Conclusions. The field of BS research is still developing and needs a lot of improvement in a number of areas. The outcomes from marine resources over the past few decades are highly promising in terms of microbiological production capacity, BS effectiveness, and BS chemical diversity. Blue biotechnology and marine bioprospecting are study fields that merit attention, should be investigated, and have the potential to yield important discoveries and practical uses for people [1].

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БІОСУРФАКТАНТИ, ЩО ПРОДУКТУЮТЬСЯ МОРСЬКИМИ МІКРООРГАНІЗМАМИ

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Анотація. *У даному звіті представлено огляд останніх досягнень у вивченні біоповерхнево-активних речовин (біо-ПАР), поверхнево-активних метаболітів, що виробляються бактеріями. Ці метаболіти привернули значну увагу завдяки здатності до біологічного розкладання, низької токсичності та потенціалу виробництва з відновлюваних і дешевших субстратів. Доповідь присвячена морським біо-ПАР і висвітлює їх джерела, характеристики та потенційне коло застосування. Дослідження біо-ПАР все ще розвиваються і мають багатообіцяючі результати. Особливо це стосується біо-ПАР, отриманих з морських ресурсів, їх хімічного різноманіття, ефективності та мікробної продуктивності. Звіт завершується підкресленням значущості потенціалу морської біорозвідки та блакитної біотехнології як перспективних областей для майбутніх досліджень і застосувань.*

Ключові слова: *біосурфактант, бактерії, біотехнологія, водне середовище.*

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THE INFLUENCE OF SECONDARY EXOMETABOLITES OF *STREPTOMYCES AMBOFACIENS* ONU 561 ON PHYTOPATHOGENIC MICROMYCETES

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Abstract. *The development of microbial preparations based on antagonistically active strains of microorganisms is a very promising direction in the fight against pathogens of fusarium head and grain of wheat and barley. The aim of work was to study the effect of extracted secondary exometabolites of Streptomyces ambofaciens ONU 561 on phytopathogenic micromycetes. Extracted secondary exometabolites of strain S. ambofaciens ONU 561 in concentrations of 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml, 1000 μg/ml were applied in 5 μl on sterile discs. To study the effect of extracted exometabolites on collection strains of micromycetes and Fusarium oxysporum strains isolated from infected wheat and barley plants, Petri dishes with Sabouraud's agar medium with glucose were inoculated. After that, disks soaked extracted exometabolites, in working concentrations, were placed on the inoculations. The results were recorded every day for 10 days, measuring the sizes of the zones of no growth of micromycete strains around the disks. Both collection strains of micromycetes and phytopathogenic strains of F. oxysporum were sensitive to extracted secondary exometabolites of S. ambofaciens ONU 561. The zones of no growth of micromycete strains varied widely and depended on the micromycete strain, the exometabolite concentration and the term of registration of the results. The minimum inhibitory concentrations (MIC) of the extracted secondary exometabolites, which inhibited the growth of the micromycete collection strains, were 100 – 250 μg/ml, the phytopathogenic F. oxysporum strains were higher and were at least 250 μg/ml. The presence of compounds with antimycotic activity in the pool of extracted secondary exometabolites allows to recommend strain S. ambofaciens ONU 561 for a more detailed study of the possibility of its use to create antimycotic preparations for agriculture.*

Keywords: *Streptomyces ambofaciens ONU 561, extracted secondary exometabolites, phytopathogenic micromycetes, inhibitory influence.*

Introduction. Currently, among the diseases of cereal crops, the most dangerous is fusarium head and grain blight. Despite the fact that a large number of studies have been devoted to the study of this disease, this problem still cannot be considered solved. Unlike other grain diseases, fusarium head blight leads, in addition to a decrease in yield, to a change in grain quality. The development of pathogens leads to the accumulation of mycotoxins in the grain, which are dangerous to human and animal health [1, 2].

The disease is caused by a complex of fungi of the genus *Fusarium*, which are characterized by high polymorphism, ecological plasticity and rapidly developing resistance to fungicidal drugs [3].

Therefore, the development of strategies to combat fusarium pathogens remains very relevant. One of the promising and perspective direction is the biological method of control, which involves the development and creation of microbial preparations based on strains of microorganisms antagonistically active against *Fusarium* [4].

In this regard, the aim of this work was to study the effect of extracted secondary exometabolites of *Streptomyces ambofaciens* ONU 561 on phytopathogenic micromycetes.

Materials and methods. The object of investigation was the strain *Streptomyces ambofaciens* ONU 561, isolated from mussel shells (*Mytilus galloprovincialis*) in 2020. According to the results of determining the effect on indicator gram-positive and gram-negative bacteria and the yeast-like fungus *Candida albicans* ATCC 18804, this strain was selected as one of the most antagonistically active [5, 6]. In addition, strain *S. ambofaciens* ONU 561 showed high activity against collection strains of micromycetes [6, 7] and phytopathogenic micromycetes isolated from infected wheat and barley plants [7].

For further research, including the study of antagonistic properties, this strain of streptomycetes is supported on Gause 2 medium.

To obtain secondary exometabolites, *S. ambofaciens* ONU 561 was grown in TSB medium on a rotary shaker at 180 rpm for 3 days at 28 °C. After that, the SG medium was inoculated with the grown culture in a seed dose of 1 – 2 ml. Cultivated on a rotary shaker at 180 rpm at 28 °C for 7 days. Further manipulations for the isolation (extraction) of secondary exometabolites were carried out according to [6, 8].

The extracted secondary exometabolites were dissolved in dimethylsulfoxide (DMSO, Gaylord Chemical Corp., USA) at a concentration of 100 mg/ml to transfer from the dried state to the liquid state. Working solutions in concentrations of 25 μg/ml, 50 μg/ml, 100 μg/ml, 250 μg/ml, 500 μg/ml, 1000 μg/ml were prepared using sterile distilled water. After that, they were sterilized using membrane filters (pore diameter 0.22 μm, Millex Syringe Filter Unit, Millipore Corp.) and applied 5 μl to sterile discs (5 mm) to

determine their inhibitory effect on micromycetes collection strains and on *Fusarium oxysporum* strains (n=30) isolated from infected wheat and barley plants. Micromycete strains were prepared for the experiment as described in [7]. Disks soaked extracted exometabolites in working concentrations were placed on Petri dishes with Sabouraud's glucose agar medium, inoculated with the appropriate micromycetes strain. The study of the effect of extracted exometabolites was carried out by cultivating inoculations at 28 °C. The results were recorded every day for 10 days, measuring the sizes of the zones of no growth of micromycetes strains around the disks. It was considered that the concentration at which the growth of the studied micromycetes strain was not observed is the minimum inhibitory (suppressive) concentration (MIC). The controls were the inoculation of micromycetes strains on Sabouraud's glucose agar medium, as well as the inoculation of these strains on the specified medium, on which disks soaked DMSO at a concentration of 500 μg/ml were placed.

The study was conducted three times. To analyze the obtained results, descriptive statistics were carried out using the Microsoft Office Excel-2016 program.

Results and discussion. The *S. ambofaciens* ONU 561 strain exhibits pronounced antagonistic activity against a wide range of proto- and eukaryotic microorganisms when pre-cultivated on nutrient media of different composition [5 – 7]. However, as evidenced by the data of many publications of specialized scientists, extracts from the biomass of actinobacteria can show different antagonistic activity, which is explained by the unequal composition of exo- and endometabolites [9, 10]. Therefore, in this study determined the antagonistic effect of extracted secondary exometabolites on micromycetes collection strains and on *Fusarium oxysporum* strains isolated from infected plants.

Both collection strains and strains of *F. oxysporum* isolated from infected plants were sensitive to extracted secondary metabolites of *S. ambofaciens* ONU 561. The zones of no growth of micromycete strains varied widely and depended primarily on the micromycetes strain, the exometabolite concentration and the term of registration of the results, which does not contradict the data of similar studies [11 – 13].

So, for example, the collection strain *F. oxysporum* UKM F-54201 showed sensitivity to streptomycete exometabolites at a concentration of 250 μg/ml already on the 3rd day of observation, the zone of no growth of its growth was 6.33 ± 0.02 mm. At the same time, increasing the concentration to 1000 μg/ml did not lead to an earlier manifestation of the activity of exometabolites, which is associated with the growth rate of this micromycete strain, but affected the size of the zone of no growth, which was 7.66 ± 0.02 mm per 3 days. At the end of the observation period (10 days), under the influence of this

concentration of exometabolites, the zone of no growth increased and reached 10.0 ± 0.01 mm.

Among the collection strains, the most sensitive to exometabolites of *S. ambofaciens* ONU 561 was the strain *Alternaria alternata* UKM F-16866. Already on the second day of observation, the zone of absence of its growth was 6.66 ± 0.02 mm under the action of extracted substances at a concentration of 100 μg/ml.

The MIC of exometabolites of the studied streptomycete was 100 μg/ml against *Aspergillus flavus* UKM F-3023, *Alternaria alternata* UKM F-16866 and *Penicillium expansum* UKM F-575. As for other collection strains of micromycetes, this indicator was 250 μg/ml.

F. oxysporum strains isolated from infected wheat and barley plants also showed different sensitivity to the extracted secondary metabolites of *S. ambofaciens* ONU 561. As in the case of the collection strain *F. oxysporum* UKM F-54201, the antagonistic effect of exometabolites was noted starting from the 3rd day. Zones of no growth ranged from 6.33 ± 0.02 mm to 15.0 ± 0.01 mm, depending on the strain of isolated fusarium, the concentration of exometabolites, and the term of registration of the results.

Compared to the collection strains, the MICs that inhibited the growth of phytopathogenic *Fusarium* were higher and amounted to at least 250 μg/ml. For half of the isolated strains, the MIC was 500 μg/ml. In most cases, the best inhibitory effect, manifested in the size of the zones of no growth, was recorded on the 4th – 5th day of registration of the results without significant changes at the end of the observation term.

Conclusions. The extracted secondary exometabolites of *S. ambofaciens* ONU 561 show a pronounced inhibitory influence on micromycetes of collection strains and *F. oxysporum* strains isolated from infected wheat and barley plants, which indicates the presence of substances with antimycotic activity in the pool of extracts. Strain *S. ambofaciens* ONU 561 can be recommended for further research on the possibility of using it to create antimycotic preparations for agriculture.

Андрющенко О., Лісютін Г., Страшнова І.

ВПЛИВ ВТОРИННИХ ЕКЗОМЕТАБОЛІТІВ *STREPTOMYCES AMBOFACIENS* ONU 561 НА ФІТОПАТОГЕННІ МІКРОМІЦЕТИ

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Анотація. *Розробка мікробних препаратів на основі антагоністично активних штамів мікроорганізмів є досить перспективним напрямком у боротьбі зі збудниками фузаріозу колоса та зерна пшениці та ячменю. Метою роботи було вивчити вплив екстрагованих вторинних екзометаболітів Streptomyces ambofaciens ONU 561 на фітопатогенні мікроміцети. Екстраговані вторинні екзометаболіти штаму S. ambofaciens ONU 561 у концентраціях 25 мкг/мл, 50 мкг/мл, 100 мкг/мл, 250 мкг/мл, 500 мкг/мл, 1000 мкг/мл наносили по 5 мкл на стерильні диски. Для дослідження впливу екстрагованих екзометаболітів колекційними штамами мікроміцетів і штамами Fusarium oxysporum, виділеними із уражених рослин пшениці і ячменю, інокулювали чашки Петрі із агаризованим середовищем Сабуро з глюкозою. Після цього на посіви накладали диски, просочені екстрагованими екзометаболітами у робочих концентраціях. Облік результатів проводили щодня протягом 10 діб, вимірюючи розміри зон відсутності росту штамів мікроміцетів навколо дисків. І колекційні штами мікроміцетів, і фітопатогенні штами F. oxysporum виявилися чутливими до екстрагованих вторинних екзометаболітів S. ambofaciens ONU 561. Зони відсутності росту штамів мікроміцетів коливалися у широкому діапазоні і залежали від штаму мікроміцету, концентрації екзометаболіту і терміну обліку результатів. Мінімальні інгібуючі концентрації (МІК) екстрагованих вторинних екзометаболітів, які пригнічували ріст колекційних штамів мікроміцетів, становили 100 – 250 мкг/мл, фітопатогенних штамів F. oxysporum були вищими і становили не менше 250 мкг/мл. Наявність у пулі екстрагованих вторинних екзометаболітів речовин з антимікотичною активністю дозволяє рекомендувати штам S. ambofaciens ONU 561 для подальших досліджень щодо можливості використання його для створення антимікотичних препаратів для сільського господарства.*

Ключові слова: *Streptomyces ambofaciens ONU 561, екстраговані вторинні екзометаболіти, фітопатогенні мікроміцети, пригнічувальний вплив.*

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Petrenko K., Yarova D., Tsveklova O., Gorshkova O., Gudzenko T., Merlich A.

ANTIPROLIFERATIVE ACTIVITY OF MARINE LACTIC ACID BACTERIA WHITH BIOTECHNOLOGICAL POTENTIAL

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Abstract. *Culture liquids of marine lactobacteria containing organic acids and CFS of Enterococcus italicus ONU 547 with bacteriocins were obtained. The antiproliferative activity of the metabolic products of lactobacteria isolated from the Black Sea was shown and it was found that all of the studied strains, both from water and mussels, inhibited the growth of human laryngeal adenocarcinoma Hep-2 cells in 25-65%.*

Keywords: *marine lactobacilli, metabolic products, bacteriocins, antiproliferative activity.*

Introduction. Cancer is currently one of the leading causes of death worldwide, with 19.3 million new cases and nearly 10 million deaths in 2020 [4]. Conventional treatment includes surgery, radiation therapy, and drug therapy, but they often damage healthy cells and organs [1]. Lactic acid bacteria (LAB) are of considerable interest for research in the field of oncology for development of alternative methods of cancer treatment. The aim of this work was study of antiproliferative activity of marine lactobacteria with biotechnological potential.

Materials and methods. The strains *Enterococcus* W1.1, *Enterococcus* W2.3, *Lactobacillus* W2.4, and *Enterococcus* M7.2, which were isolated from Black Sea water and mussels, were used. Hep-2 cell culture (human laryngeal adenocarcinoma) was grown in DMEM medium. Bacterial cultural liquids containing antiproliferetive metabolites were obtained by standard methods and to test for the presence of bacteriocins in them the agar well diffusion assay was used with *Lactobacillus sakei* subsp. *sakei* JCM1157 as an indicator strain [3]. Antiproliferative activity against Hep-2 was determined by the optimized microplate calorimetric method [2].

Results and discussion. It was established that organic acids, but not bacteriocins, were present in the culture liquids of marine lactobacteria when tested by agar well diffusion assay. Using the optimized microplate method

with 1:10 ratio of supernatants and cancerous cells we studied the antiproliferative activity of supernatants of marine LAB with an initial pH value (4.24-4.95) and adjusted to pH 7. Our results showed that both types of supernatants of all the studied strains had an inhibitory effect on Hep-2 cells. Indeed, supernatants metabolites with a neutral pH of *Enterococcus* W1.1 strain reduced the number of cancerous cells in 47% compared to the control (MRS medium), while *Enterococcus* W2.3 – in 36.7%, *Enterococcus* M7.2 – in 37.3%, and *Lactobacillus* W2.4 – in 25%. According to the literature sources, lactobacteria can secrete a wide range of antitumor compounds, including bacteriocins, nucleic acids, and exopolysaccharides [1; 5; 6].

At the same time, the antiproliferative activity was more expressed by supernatants with low pH due to the presence of organic acids. Thus, the decrease of the cell number under the effect of supernatants with the initial (acidic) pH of *Enterococcus* W1.1, *Enterococcus* W2.3, *Enterococcus* M7.2, and *Lactobacillus* W2.4 strains was in 65%, 58.3%, 45.3%, and 40%, respectively. The conducted studies have shown that among the microbiota of the Black Sea there is a significant number of LAB strains with high antiproliferative activity. Therefore, the Black Sea is a promising source of LAB producing antitumor metabolites. These antiproliferative substances may have the potential to be used for development of new antitumor drugs for modern medicine.

Conclusions. All the tested strains, both from seawater and mussel liquor, inhibited the growth of human laryngeal adenocarcinoma cells Hep-2 with activity ranging from 25% to 65%. The strains *Enterococcus* W1.1 and *Enterococcus* W2.3 from the Black Sea water were the most effective.

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АНТИПРОЛІФЕРАТИВНА АКТИВНІСТЬ МОРСЬКИХ МОЛОЧНОКИСЛИХ БАКТЕРІЙ ІЗ БІОТЕХНОЛОГІЧНИМ ПОТЕНЦІАЛОМ

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Анотація. *Отримано культуральні рідини морських лактобактерій, що містять органічні кислоти та НОР Enterococcus italicus ОНУ 547, з бактеріоцинами. Показано антипроліферативну активність продуктів метаболізму лактобактерій, виділених із Чорного моря, і встановлено, що всі досліджені штами, як з води, так і з мідій,*

пригнічують ріст клітин аденокарциноми гортані людини Нер-2 на 25-65%.

Ключові слова: *морські лактобактерії, продукти метаболізму, бактеріоцини, антипроліферативна активність.*

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CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM ODESA ESTUARY OF BLACK SEA

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Abstract. *The number of lactobacteria in water and mussels of Black Sea collected in the winter period was determined. Preliminary identification of strains was carried out by studying cell morphology, Gram reaction, catalase activity, and cultural characteristics. According to the results of the PCR analysis, it was determined that 87.5% of the investigated strains of LAB of marine origin belong to the genus Enterococcus. It was found that most of the studied strains did not show inhibitory activity against each other, except for Enterococcus W1.4, Enterococcus W1.5, which inhibited the growth of the indicator strains.*

Keywords: *lactic acid bacteria, mussels, marine sources, lactobacteria, Enterococcus.*

Introduction. Lactic acid bacteria (LAB) are a diverse group of industrially important and safe microorganisms [2]. Since marine lactobacteria live in extreme and stressful conditions, they can become a rich source of new antimicrobial peptides that can be effective drugs for medicine and veterinary medicine [4].

The aim of the work was to characterize the strains of lactobacteria isolated from the Odesa estuary of Black Sea.

Materials and methods. LAB were isolated from mussels (*Mytilus galloprovincialis* Lam.) and water of Black Sea by inoculating on MRS medium with a neutral and slightly acidic pH. Standard microbiological methods were used to obtain pure cultures, to describe their cultural, morphological, tinctorial, and biochemical characteristics [1]. Isolation of DNA was performed by the heat lysis method and identification of the strains – by genus-specific PCR [3, 5].

Results and discussion. Eight new strains of lactobacteria were isolated from Black Sea: six strains from seawater and two – from mussels. LAB were present in small quantities in the examined samples taken in the winter. Thus, in 1 ml of mussel liquor, only 2x10 CFU of LAB were found,

which made up 0.4% from the total microbiota, while in 1 ml of water – $4.8x10^2$ CFU.

The growth ability of seven studied strains in the presence of 6.5% NaCl, as well as the established other cultural and morphological characteristics, suggested their belonging to the genus *Enterococcus*, which was further confirmed by genus-specific classical PCR.

We also studied the interactions of the selected strains with each other and with the indicator strain *L. sakei* subsp. *sakei* JCM1157. It was established that the isolated strains *Enterococcus* W1.1, *Enterococcus* W1.2 and *Enterococcus* W1.3 showed antagonistic activity only against *L. sakei* subsp. *sakei* JCM1157, which can indicate their ability to produce bacteriocins. Bacteria of *Enterococcus* W1.5 and *Enterococcus* W1.4 strains showed a high level of antagonistic activity against many other LAB strains, which can be a consequence of production of organic acids as factors of non-specific antagonism [1].

Conclusions. Water of Black Sea contained $4.8x10^2$ CFU/ml of bacteria of the LAB group, and the liquor of mussels – 2x10 CFU/ml. Seven out of eight strains (87.5%) were identified as members of the genus *Enterococcus*.

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ХАРАКТЕРИСТИКА МОЛОЧНОКИСЛИХ БАКТЕРІЙ ВИДІЛЕНИХ ІЗ ОДЕСЬКОЇ АКВАТОРІЇ ЧОРНОГО МОРЯ

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Анотація. *Визначено кількість лактобактерій у пробах води та мідій Чорного моря, відібраних у зимовий період. Попередня ідентифікація штамів була проведена шляхом дослідження морфології клітин, забарвленням за Грамом, визначення каталазної активності, вивчення культуральних характеристик. За результатами ПЛР-аналізу визначено, що 87,5% досліджених штамів МКБ морського походження належать до роду Enterococcus. Виявлено, що більшість досліджуваних штамів не проявляли інгібувальної активності один проти одного, окрім Enterococcus B1.4, Enterococcus B1.5, які інгібували ріст індикаторних штамів.*

Ключові слова: *молочнокислі бактерії, мідії, морські джерела, лактобактерії, Enterococcus.*

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BIOSYNTHETIC CLUSTERS OF SECONDARY METABOLITE GENES IDENTIFIED IN THE GENOME OF *BACILLUS SUBTILIS* STRAIN 200

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Abstract. *The Bacillus subtilis 200 strain, isolated from a sample of bottom sediments of the anaerobic zone of the Black Sea, demonstrated antagonistic activity against Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica, Klebsiella pneumoniae and Bacillus subtilis, especially high against Proteus vulgaris, Staphylococcus aureus, Bacillus cereus and Candida albicans. After analyzing the genome of Bacillus subtilis 200 on the antiSMASH server, 15 regions were identified in which secondary metabolites are produced. Most of the clusters belong to the NRPS and PKS type. This strain is capable of synthesizing a variety of secondary metabolites such as terpenes, glycocins, metallophores, subtylosin-A, taililanstatin A, bacillisin, and surfactin.*

Thus, the Bacillus subtilis 200 strain produces many secondary metabolites with a wide spectrum of biological activity, which can be used in the production of new drugs.

Keywords: *biosynthetic gene clusters, secondary metabolites, Bacillus subtilis.*

Introduction. It is known that bacilli produce a wide range of different secondary metabolites, including polyketides, lipopeptides, fatty acids, macrolactones, lipoamides, polypeptides, isocoumarins and carotenoids. Due to this diversity, secondary metabolites of marine bacilli can be used in many areas of human activity.

However, some of the gene clusters that produce secondary metabolites can be activated only under extreme conditions, such as high salinity and pH, or at low temperatures. That is why marine bacilli are currently one of the most interesting objects for research in the field of biotechnology.

Materials and methods. Strain *Bacillus subtilis* 200 was isolated from a sample of bottom sediments from the anaerobic zone of the Black Sea at a depth of 1537 metres and demonstrated antagonistic activity against *Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica, Klebsiella*

pneumoniae and *Bacillus subtilis,* with particularly high activity against *Proteus vulgaris, Staphylococcus aureus, Bacillus cereus* and *Candida albicans.*

Results and discussion. After analysing the *Bacillus subtilis* 200 genome, the antiSMASH server identified 15 regions in which secondary metabolites are produced.

It was found that most of the clusters belong to the NRPS (regions 2, 3, 7, 10, 12, 14 and 15) and PKS (regions 2 and 7) types. This strain is also capable of synthesising sactipeptide, ranthipeptide, epipeptide, sactipeptide, CDPS, transAT-PKS, PKS-like, T3PKS and betalactone. The products encoding regions 1 and 4 were identified as terpenes.

Region 2 contains mainly T3PKS-type peptidases, non-ribosomal peptidases and trans-acyltransferase polyketide synthases, a group of enzymes involved in the biosynthesis of polyketides, complex organic molecules with a wide range of biological activities. In Region 3, beta-lactates were identified, which in themselves have attracted considerable attention for their antimicrobial and anticancer properties. In region 5, glycocins, post-translationally glycosylated bacteriocins with antimicrobial activity, were found. Based on the results of the antiSMASH analysis, this bacteriocin was identified as sublancin 168. This bacteriocin was also identified by the BAGEL4 server (bacteriocin AOI_2) as a homologue of the bacteriocin sublancin produced by *Bacillus subtilis* 168 (P68577.1 prophage-derived bacteriocin sublancin-168) with an identity score of 100.0%.

Clusters of genes encoding metallophores were identified in region 7. Microbes often compete for a limited pool of trace elements. In response to metal deficiencies, many bacteria produce metallophores, low-molecular-weight organic compounds that bind ions with high affinity and selectivity

A gene cluster responsible for subtilosin-A was identified in region 9. It is active against a variety of Gram-positive bacteria, including *Listeria* and other pathogens. The production of mature subtilosin is based on the expression of the sbo-alb gene cluster, which includes the structural subtilosin gene sbo and genes involved in post-translational modification and processing of presubtilosin and immunity. The sequence found in the *Bacillus subtilis* 200 genome is 100.0% identical to O07623.1 (*Bacillus subtilis* 168*)*, which encodes subtilisin-A, as a result of the BAGEL4 server and designated as bacetriocin AOI_5 *Listeria*.

Region 10 encodes thailanstatin A, a promising lead compound for drug discovery and development. It demonstrates cytotoxicity against a variety of human cancer cell lines. The mechanism of action (MOA) of thailanstatin A is to inhibit splicosome assembly. Interestingly, it was previously thought that this secondary metabolite could only be obtained from *Thailandensis burkholderia* MSMB43.

Region 11 encodes bacilysin, a dipeptide antibiotic active against a wide range of bacteria and *Candida albicans*.

Regions 12, 14, and 15 encode surfactin, which is a cyclic lipopeptide commonly used as an antibiotic due to its surfactant ability.

In region 13, we found the sporulation killing factor (SKF), a ribosomally assembled and post-translationally modified sactypeptide of 26 residues. It was identified by the BAGEL4 server as bacteriocin AOI_1. Additionally, two more gene regions of the secondary metabolite gene clusters that were not identified by antiSMASH were found using the BAGEL4 server.

Bacteriocin AOI_3, predicted by BAGEL4, was identified as ComX with 100.0% identity to the sequence Q9K5K8.1 of *Bacillus mojavensis* strain.

The following sequence (AOI_4) refers to choline-like peptides (BhlA/ UviB family holin-like peptide) encoded in bacteria with similarities to bacteriocin. They demonstrate antibacterial action against gram-positive bacteria.

Conclusions. Thus, strain *Bacillus subtilis* 200 produces many secondary metabolites with a wide range of biological activities that can be used in the production of new drugs.

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БІОСІНТЕТИЧНІ КЛАСТЕРИ ГЕНІВ ВТОРИННИХ МЕТАБОЛІТІВ, ВИЗНАЧЕНІ В ГЕНОМІ ШТАМУ *BACILLUS SUBTILIS* 200

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Анотація. *Штам Bacillus subtilis 200, ізольований з проби донних відкладень анаеробної зони Чорного моря, продемонстрував антагоністичну активність до Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica, Klebsiella pneumoniae і Bacillus subtilis, особливо високу до Proteus vulgaris, Staphylococcus aureus, Bacillus cereus та Candida albicans. Після аналізу геному Bacillus subtilis 200 на сервері antiSMASH було виявлено 15 регіонів, в яких продукуються вторинні метаболіти. Більшість кластерів відноситься до типу NRPS та PKS. Цей штам здатний до синтезу різноманітних вторинних метаболітів, таких як терпени, глікоцини, металофори, субтилозин-А, таїланстатин А, бацилізин та сурфактин.*

Таким чином, штам Bacillus subtilis 200 продукує багато вторинних метаболітів з широким спектром біологічної активності, які можуть бути використані при виробництві нових лікарських засобів.

Ключові слова: *кластери біосинтетичних генів, вторинні метаболіти, Bacillus subtilis.*

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Zatynatska D., Gudzenko T., Gorshkova O., Sashchuk O.

ANTITUMOR ACTIVITY OF ACTINOBACTERIA

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Abstract. *Actinomycetes are the most powerful source for the production of secondary metabolites, antibiotics and other bioactive compounds. Analysis of the literature showed that marine actinomycetes have a significant effect on the proliferative activity of tumor cells and can be potential sources of new anticancer drugs.*

Keywords: *marine actinobacteria, exometabolites, antitumor activity.*

Introduction. The annual increase in the level of oncological morbidity in the world, and in particular in Ukraine, prompts scientists to search for new and effective solutions for the treatment of oncological diseases. Today, actinobacteria are a promising source of clinically useful anticancer drugs.

The purpose of the work was to analyze the literature devoted to the study of the antitumor activity of actinobacteria.

Results and discussion. Actinobacteria represent one of the largest groups of prokaryotic microorganisms, which includes gram-positive bacteria with a content of more than fifty-five percent guanine and cytosine, aerobic or anaerobic, filamentous, spore-forming bacteria that are widely distributed in aquatic and terrestrial environments. Actinomycetes are the most powerful source for the production of secondary metabolites, antibiotics and other bioactive compounds. Analysis of the literature showed that actinomycetes are potential sources of new anticancer drugs [1].

Recently, much attention has been paid to the isolation of rare actinomycetes from various extreme environments. It was established that each strain of actinomycetes has the ability to produce up to 20 secondary metabolites. Along with significant antimicrobial activity, secondary metabolites of marine actinobacteria also exhibit cytotoxic effects. For example, exometabolites of Black Sea strains of actinobacteria *Streptomyces* sp. Lim 9.2 and *Streptomyces* sp. Lim 10 at a concentration of 25.0 - 500.0 μg/ml showed a pronounced cytotoxic antiproliferative effect on tumor cultures of human cells - rhabdomyosarcoma (RD) and adenocarcinoma of the larynx (Hep-2). The antitumor activity of marine bacteria can be associated with campechic acid,

cyanophycin and mirabilite found in the metabolome. Campechic acid belongs to antitumor agents and is an inhibitor of tumor cell metastasis. Cyanophycin, due to its ability to inhibit the growth of tumors, has attracted attention for a long time, since one type of cyanobacterial compounds, dolastatin, has already proven itself well in clinical practice. Cytotoxic activity of exometabolites of *Streptomyces* sp. Lim 10 can be explained by the action of the apoptosis inducer staurosporine together with its structural homologues [1].

The actinomycete strain *S. bingchenggensis* ULS14 isolated from the Lagos lagoon showed cytotoxic activity. Two purified bioactive compounds were isolated from it - ULDF4 and ULDF5. The antitumor activity of ULDF5 against the HeLa cell line was higher than that of ULDF4. Cytotoxicity of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one (DMBPO) extracted from marine bacteria *Streptomyces* VITSVK5 spp. was established on Hep G2 and HEP-2 cell lines. isolated from samples collected on the Marakkanam coast of the Bay of Bengal [2].

Cytostatic compounds of marine origin are extremely diverse in chemical structure. Among them are known alkaloids, terpenes, amino carbohydrates, polyketides, non-ribosomal peptides and nucleoside compounds, etc. [3]. A number of metabolites of representatives of marine microbiota are unique in their structure. In the strain *Streptomyces* sp. KMM 9048 revealed specific variants of antitumor antibiotics of the aureolic acid group [4].

A representative of the genus *Streptomonospora* from the group of "rare actinomycetes" from the littoral of the Wadden Sea revealed new cytotoxic bacteriocins from the group of thiopeptides - litoralimycins [5]. Among microbial exometabolites of marine origin, they are unique not only in structure, but also in mechanism of action. For example, polyketide compounds of the salinisporamide group are known for representatives of the genus *Salinispora*, native to the sea, which are capable of antitumor action through specific inhibition of the proteasome. In the polyketides of the group of manumycins, produced by marine representatives of the genus *Streptomyces*, an extremely rare mechanism of action based on the type of "molecular glue" was found. They act by forming a covalent bridge between UBR7 ligase molecules and an abnormal variant of the TP53 protein characteristic of breast cancer cells, which leads to tumor cell apoptosis [6].

Conclusions. According to the literature, exometabolites of marine actinobacteria show antitumor activity in in vitro experiments and may be promising for the development of medicinal antitumor drugs.

Затинацька Д., Гудзенко Т., Горшкова О, Сащук О.

ПРОТИПУХЛИННА АКТИВНІСТЬ АКТИНОБАКТЕРІЙ

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Анотація. *Актиноміцети є найпотужнішим джерелом вторинних метаболітів, антибіотиків та інших біологічно активних речовин. Аналіз літературних даних показав, що морські актиноміцети мають значні ефекти на проліферативну активність пухлинних клітин і можуть бути потенційними продуцентами нових протипухлинних препаратів.*

Ключові слова: *морські актінобактерії, екзометаболіти, протипухлинна активність.*

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SYMBIOTIC INTERACTIONS BETWEEN PLANTS AND *BACILLUS* SPP.

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Abstract. *The interaction of various bacteria with plants is an integral part of biogeocenoses and sustainable ecosystems. As a result of such symbiotic interactions, bacteria receive organic carbon from plants in the form of sugars, and bacteria, in turn, provide a certain number of plant life mechanisms that improve plant stress resistance to both abiotic and biologically created adverse conditions. It was investigated that such PGPR as bacteria of the genus Bacillus spp. enhance the plant defense system through induced systemic resistance and thus participate in biocontrol not only by directly inhibiting phytopathogenic fungi and bacteria, but also indirectly through ISR. Bacillus spp. are able to form a stable biofilm in the rhizosphere of plants within 24 hours, and sporulation only increases the chances of storage of preparations based on these bacteria in the soil around plants with further competition with phytopathogenic microbiota. The growth-stimulating properties of these bacteria are also known due to the increase in the synthesis of a number of phytohormones in plants. Bacillus subtilis, one of the species of rhizobacteria, turned out to be very promising for use in agriculture. Its ability to increase plant resistance to stress and protect against pathogens makes it a valuable tool for increasing yields. Despite the advantages of biological preparations, their use in field conditions remains a difficult task.*

Keywords: *PGPR, Bacillus spp., ISR, biocontrol, rhizobacteria, biofilm.*

Introduction. Bacteria are the basis of the cycle of substances, especially in the cycle of carbon and nitrogen, influence the existence of living things in various ways. The interaction of plants with them promotes healthy development, and disruption of the microbiome can lead to serious problems with metabolism [4]. The role of bacteria in interaction with plants has been known for more than 100 years [7]! *Rhizobacteria* promote the growth of plants and increase their yield. PGPRs, particularly *Bacillus subtilis*, have shown promising applications in agriculture, particularly due to their ability to increase plant resistance to stress and protect against pathogens. However, the use of

biological preparations in field conditions remains problematic, which requires a deeper understanding of the interaction between bacteria and plants [2, 8].

Results and discussion. *B. subtilis* plays an important role in fixing and mobilizing nutrients. This bacterium also produces compounds that affect the homeostasis of plant growth hormones, promoting their development [5]. *B. subtilis* can help plants become more resistant to stress, particularly drought and soil salinity, by modulating plant genes and increasing their osmotic resistance. These properties make it important for crop support in conditions of changing climate and limited water resources [6]. Chemotaxis and biofilm formation on plant roots are important for rhizosphere colonization. These processes help to efficiently colonize roots and contribute to plant resistance under adverse environmental conditions [1]. Biofilms consist mainly of cells embedded in a matrix containing the exopolysaccharide EPS and the protein TasA, which are essential for successful root colonization. Biofilm formation is initiated by the expression of the SinI gene, which is activated at intermediate levels of Spo0A~P. KinC and KinD kinases in *B. subtilis* play a key role in colonization of plant roots by forming biofilm in response to signals originating from the host plant. L-malic acid and plant polysaccharides promote biofilm formation [9].

In addition, *B. subtilis* affects the expression of genes in the plant, which contributes to its colonization. Interaction with plants leads to the production of defense compounds and stimulates plant growth, making *B. subtilis* an important agent for improving the quality and yield of agricultural crops. Most studies were conducted under sterile conditions, which may not reflect real conditions in natural environments. It is important to consider the influence of natural plant microbiomes on the effectiveness of using *B. subtilis* in biocontrol and plant growth stimulation [3]. A positive effect on disease control and plant growth was observed in the interaction of nodule bacteria with *B. subtilis*, which can stimulate the synthesis of phytohormones in host plants. Studies have shown that these bacteria can promote biofilm formation and stimulate plant growth by producing phytohormones and other compounds that increase enzyme production, antioxidants, phosphorus solubilization, biocontrol activity, root nodulation, and nitrogen fixation [5].

B. subtilis, in addition to directly suppressing pathogens, can enhance plant defense by inducing ISR. This process involves ultrastructural and cytochemical changes in host cells in response to pathogen attack. This process is associated with the degradation of the cell wall, the production of glucanases and chitinases de novo, as well as with the synthesis of phytoalexins, which increase resistance to diseases. Different strains of *B. subtilis* have also shown the ability to induce the secretion of defense responses in plants, such as the synthesis of jasmonic acid (JA), ethylene, and the NPR1- regulatory gene, which promote disease resistance. Some strains produce phenylalanine-

ammonialyase (PAL), peroxidase (POD), and other proteins that help plants become more resistant to viruses. In addition, *B. subtilis* can reduce the intensity of disease caused by *Botrytis cinerea* in tomato leaves and beans, as evidenced by a reduction in the level of disease symptoms. These results emphasize the importance of research on *B. subtilis* and other *Bacillus* strains for further use in biological control of plant diseases [10].

Conclusions. A deeper understanding of the interaction between bacteria and plants in uncontrolled conditions will help to use these drugs more rationally and efficiently.

The relevance of the study of symbiotic interactions of *Bacillus* spp. is growing more and more, and the problem of successful inoculation of bacterial preparations in vivo still remains unresolved, which especially attracts attention to further research.

Ружанський Б., Галкін М.

СИМБІОТИЧНА ВЗАЄМОДІЯ МІЖ РОСЛИНАМИ ТА *BACILLUS SPP.*

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Анотація. *Взаємодія різноманітних бактерій із рослинами є невід'ємною частиною біогеоценозів та стійких екосистем. В результаті таких симбіотичних взаємодій бактерії отримують від рослин органічний карбон у вигляді цукрів, а бактерії, в свою чергу, забезпечують певний ряд механізмів життєдіяльності рослин що покращує стресостійкість рослин до як абіотичних, так і біологічно створених несприятливих умов. Досліджено, що такі PGPR як бактерії роду Bacillus spp. підвищують захисну систему рослин через індуковану системну резистентність і таким чином приймають участь у біоконтролі не тільки безпосередньо пригнічуючи фітопатогенні гриби та бактерії, але й опосередковано через ISR. Bacillus spp. здатні утворювати стійку біоплівку у ризосфері рослин протягом 24 годин, а спороутворення тільки підвищує шанси зберігання препаратів на основі цих бактерій у ґрунті навколо рослин із подальшою конкуренцією з фітопатогенною мікробіотою. Відомі також ростстимулюючі властивості цих бактерій через підвищення синтезу ряду фітогормонів у рослинах. Bacillus subtilis, один із видів ризобактерій, виявився дуже перспективним для застосування в сільському господарстві. Його здатність підвищувати стійкість рослин до стресу та захищати їх від патогенів робить його цінним інструментом для*

збільшення врожайності. Незважаючи на переваги біопрепаратів, їхнє застосування в польових умовах залишається складним завданням. Глибше розуміння взаємодії бактерій та рослин у неконтрольованих умовах допоможе більш раціоно та ефективно використовувати ці препарати. Актуальність дослідження симбіотичних взаємодій Bacillus spp. все більше зростає, а проблематика успішної інокуляції бактеріальних препаратів in vivo досі залишається не вирішеною, що особливо привертає увагу до подальших досліджень.

Ключові слова. *Ростстимулюючі ризобактерії, Bacillus sp., індукована системна резистентність, біоконтроль, ризобактерії, біоплівка.*

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MARINE BACTERIA ENZYMES

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Abstract. *The potential of marine microorganisms, in particular actinobacteria and bacteria of the Black Sea, as producers of keratinase and proteolytic enzymes, has been investigated. Analysis of literature showed promising directions of development and search of new enzymes of marine bacteria in Ukraine.*

Keywords: *marine bacteria, keratinases, proteolytic enzymes.*

Introduction. The marine environment opens up ample opportunities for the detection of unique bacteria-producers of enzymes with specific properties. Marine bacteria enzymes open up new possibilities for biotechnological use due to their uniqueness and potential of use in various fields. The aim of the work was to analyse the literature data on the study of enzymes of marine bacteria, with an emphasis on their specificity, activity and potential practical application.

Results and discussion. Data from the literature indicate that marine microorganisms are able to produce a wide range of unique enzymes with different specificity. It has been found that sea strains of actinobacteria are able to synthesize enzymes with keratinolytic activity, which opens the prospect of their use for the biodegradation of feathers by a cost-effective bioconversion of feather waste into a nutritious, balanced and easily digestible product containing free amino acids, peptides and ammonium [1]. Due to keratinolytic microorganisms, a very important environmental problem can be solved, because about 2 million tons of feathers are produced annually worldwide as a by-product of poultry farming. Due to the lack of funds and the laboriousness of processing, they have become one of the main environmental pollutants. Keratinase is a specific type of proteolytic enzymes for hydrolysis (splitting) of insoluble keratin with the release of the free amino acid oxyproline [2]. The decomposition of feathers by keratinolytic microorganisms is an effective, environmentally safe and profitable method of converting waste feathers into nutritious products. However, most of the described producers of keratinase were found among bacteria and fungi, and the number of such enzymes in

actinobacteria is much smaller. Analysis of the literature showed that of the 10 strains of actinobacteria isolated from bottom sediments in the area of the Dnieper Trench of the Black Sea shelf, hydrolytic activity toward keratin was detected in five cultures. The highest keratinase activity is found in the strain Acty 9 (12 units/ml), which allows it to be a potential producer of extracellular keratinase, due to the fact that the enzyme synthesized by it is marked by pH and thermostability [2].

The interest in the Black Sea bacteria is increasing also because they are promising producers of proteolytic enzymes. In recent years, interest in peptidase has increased, which are able to break down elastin as a specific substrate [3-6]. *Streptomyces fradiae*, *Bacillus thermopoltoteolyticus* are the most powerful producers of elastolic proteinase discovered to date, as they are 4-8 times more effective than pancreatic elastases. The disadvantages of these strains-producers include the fact that most of them are pathogenic to humans, and elastase is directly involved in the initiation of the pathogenetic process, which complicates their practical use. In 4 of the 10 studied isolates of Black Sea bacteria the activity of elastase was detected. The most active producer of elastase was the strain *Bacillus sp*. 051, isolated from the Black Sea [3]. Due to the absence of highly active elastase producers in Ukraine, a search for new microorganisms is being carried out. Different levels of enzymatic activity among ten isolated cultures have been identified. Several isolates showed significant caseinolytic, elastase, fibrinolytic and fibrinogenolytic activity, while others had less significant or did not show these properties at all. For example, *Bacillus* sp. 051 complex enzyme preparation is able to hydrolyze elastin, casein and fibrinogen, but the highest is elastase activity [3].

Conclusions. Data from the literature indicate a significant diversity of enzymatic properties of marine microorganisms, which confirms the prospects of using marine microorganisms as efficient producers of enzymes.

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ФЕРМЕНТИ МОРСЬКИХ БАКТЕРІЙ

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Анотація. *Досліджено потенціал морських мікроорганізмів, а саме актинобактерій та бактерій Чорного моря як продуцентів кератиназ та протеолітичних ферментів. Аналіз літературних даних показав перспективи розвитку подальших досліджень та пошуків нових ферментів у морських бактерій в Україні.*

Ключові слова: *морські бактерії, кератинази, протеолітичні ферменти.*

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ANTAGONISTIC EFFECT OF ACTINOBACTERIA ISOLATED FROM SPONGES *HALICLONA* SP. ON INDICATOR STRAINS OF PRO- AND EUKARYOTIC MICROORGANISMS

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Abstract. *Studies of representatives of the Actinobacteria phylum remain relevant as the largest and most promising source of new antibiotic compounds. The aim of the study was to investigate the antagonistic activity of actinobacteria isolated from Haliclona sp. sponges against indicator strains of pro- and eukaryotic microorganisms. The antagonistic activity of 14 isolated strains of actinobacteria was determined by the block method after their preliminary cultivation on agarized medium Gauze 2. The antagonistic effect of actinobacteria was determined against 12 strains of indicator pro- and eukaryotic microorganisms. Almost all of the studied strains of isolated actinobacteria inhibited the growth of at least one strain of the indicator microorganism. The most sensitive to secondary metabolites of actinobacteria were indicator strains of all gram-positive bacteria, as well as Pseudomonas aeruginosa ATCC 27853 and Candida albicans ATCC 18804. Strains Actinobacteria Hal 2, Hal 4 and Hal 14 showed the best antagonistic activity against indicator microorganisms, which were selected for further studies.*

Keywords: *antagonistic activity, actinobacteria, Haliclona sp. sponges, indicator pro- and eukaryotic microorganisms.*

Introduction. Today, resistance to antibiotic substances and the emergence of multidrug-resistant microorganisms is a problem of global importance in both medicine and biology [4]. Various strategies are proposed to solve this problem, one of which is the isolation and screening of strains of microorganisms producing antimicrobial substances [3].

The main source of antibiotic substances with a diverse spectrum and mechanisms of action are representatives of the *Actinobacteria* phylum [1].

The aim of the study was to investigate the antagonistic activity of actinobacteria isolated from *Haliclona* sp. sponges against strains of indicator pro- and eukaryotic microorganisms.

Materials and methods. The object of the study was 14 strains of actinobacteria isolated from *Haliclona* sp. sponges collected in the waters of the Odesa Bay of the Black Sea in 2022.

The antagonistic activity of the isolated strains of actinobacteria was carried out by the block method after their preliminary cultivation on nutrient agarized medium Gause 2 at 30 °C for 12 days [2].

Activity was determined against indicator strains of gram-positive bacteria (*Staphylococcus aureus* ATCC 25923*, Micrococcus luteus* ATCC 4698*, Enterococcus faecalis* ATCC 29212*, Bacillus subtilis* ATCC 6633*, Kocuria rhizophila* DSM 348) and gram-negative bacteria (*Escherichia coli* ATCC 25922*, Proteus vulgaris* ATCC 6896*, Salmonella enterica* NCTC 6017*, Klebsiella pneumoniae* ATCC 10031*, Pseudomonas aeruginosa* ATCC 27853*, Pseudomonas putida* KT 2440), as well as the yeast-like fungus *Candida albicans* ATCC 18804. Daily cultures of indicator microorganisms were used, which were used to inoculate the semi-liquid LB medium. After that, blocks of actinobacteria were placed on inoculations of indicator microorganisms. Cultivated at the temperature optimal for each indicator strain. The results were recorded after 24 hours and 48 hours.

All studies were conducted in triplicate. The results were processed in Microsoft Office Exel-2010.

Results and discussion. The obtained data showed that all investigated strains, except *Actinobacteria* Hal 8, were active against at least one indicator microorganism. Sensitive to the tested strains of actinobacteria were the following indicator strains *B. subtilis, K. rhizophila, E. faecalis, M. luteus, S. aureus, P. aeruginosa* and *C. albicans*. The zones of growth inhibition of indicator microorganisms ranged from 13.2 ± 0.1 mm to 27.4 ± 0.2 mm and depended on the specific strain of both actinobacteria and the indicator strain.

In view of the obtained results, the indicator strains of gram-positive bacteria showed better sensitivity to the metabolites of the investigated strains of actinobacteria, which does not contradict the data of literary sources [2]. However, it is noteworthy that the sensitivity of strain *P. aeruginosa* ATCC 27853, which was inhibited by 10 studied strains of actinobacteria, was found. The greatest inhibitory effect was found in the *Actinobacteria* Hal 2 strain, under the influence of which the zone of no growth of *P. aeruginosa* ATCC 27853 was 27.4 ± 0.2 mm.

Actinobacteria Hal 2, Hal 4, Hal 5, Hal 6 showed antagonistic activity against the eukaryotic microorganism *C. albicans* ATCC 18804.

Among the tested strains of actinobacteria, there were those that inhibited the growth of more than 4 indicator strains. These are strains *Actinobacteria* Hal 2, Hal 4 and Hal 14, but the range of susceptibility of indicator strains to their metabolites was different.

Conclusions. Almost all of the studied strains of actinobacteria showed antagonistic activity to indicator strains of pro- and eukaryotic microorganisms. The most sensitive to secondary metabolites of actinobacteria were indicator strains of all gram-positive bacteria, as well as *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 18804. The best antagonistic activity against indicator microorganisms was shown by strains *Actinobacteria* Hal 2, Hal 4 and Hal 14, which inhibited the growth of a large number of indicator microorganisms with significant the zones of no growth. Strains *Actinobacteria* Hal 2, Hal 4 and Hal 14 were selected for further studies of the activity of their secondary metabolites.

Машкова А., Страшнова І.

АНТАГОНІСТИЧНИЙ ВПЛИВ АКТИНОБАКТЕРІЙ, ВИДІЛЕНИХ ІЗ ГУБОК *HALICLONA* SP. НА ІНДИКАТОРНІ ШТАМИ ПРО- ТА ЕУКАРІОТИЧНИХ МІКРООРГАНІЗМІВ

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Анотація. *Дослідження представників філуму Actinobacteria не втрачають своєї актуальності як найбільш велике і перспективне джерело нових антибіотичних сполук. Метою роботи було дослідити антагоністичну активність актинобактерій, виділених з губок Haliclona sp., до індикаторних штамів про- та еукаріотичних мікроорганізмів. Антагоністичну активність 14 виділених штамів актинобактерій визначали методом блоків після попереднього їх культивування на агаризованому середовищі Гаузе 2. Антагоністичний вплив актинобактерій визначали до 12 штамів індикаторних про- та еукаріотичних мікроорганізмів. Майже усі досліджені штами пригнічували ріст хоча б одного штаму індикаторного мікроорганізму. Найчутливішими до вторинних метаболітів актинобактерій були індикаторні штами всіх грампозитивних бактерій, а також Pseudomonas aeruginosa ATCC 27853 та Candida albicans ATCC 18804. Найкращу антагоністичну активність щодо індикаторних мікроорганізмів проявили штами Actinobacteria Hal 2, Hal 4 та Hal 14, які відібрані для подальших досліджень.*

Ключові словаs: *антагоністична активність, актинобактерії, губки Haliclona sp., індикаторні про- та еукаріотичні мікроорганізми.*

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CORRELATION ANALYSIS OF INDICATORS OF ANTAGONISTIC ACTIVITY OF *STREPTOMYCES SP.* ONU 64

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Abstract. *A study of the antagonistic activity of actinomycete strains isolated from the bottom sediments of the Black Sea was conducted. Strain Streptomyces sp. ONU 64 showed the maximum antagonistic activity against conditionally pathogenic indicator strains. Correlation analysis showed the existence of a linear relationship between the manifestation of antagonistic activity of this strain and the composition of nutrient media used for preliminary cultivation.*

Keywords: *antagonistic activity, correlation analysis.*

Introduction. The search for previously unknown microbial strains is an effective approach for obtaining new biologically active substances [1]. However, in addition, there is a problem of obtaining the maximum amount of the active substances, that is, optimizing the cultivation conditions of the producer strain and the technology of obtaining the active substances. The solution of this scientific and practical task is implemented using statistical data processing [2, 3]. A "natural" measurement associations between variables in biological systems is the correlation coefficient. The purpose of this work was carried out in the process of study the antagonistic activity of *Streptomyces sp.* ONU 64, isolated from the bottom sediments of the Black Sea, correlation analysis of the dependences of the level of antagonistic activity and the composition of nutrient media used for preliminary cultivation of the strain. The revealed correlational dependences are planned to be used in the future for mathematical modeling and optimization of the conditions of cultivation of the strain.

Materials and methods. To study the antagonistic activity of strain *Streptomyces sp.* ONU 64, it was grown superficially on agar nutrient media of varying composition. Antagonistic activity was determined on LB medium (0.7% agar) using the block method. To carry out statistical analysis, we used the R 4.3.3 program and additional packages "corrplot", "RColorBrewer", "cluster" and "fastcluster" [Principal Component …., Introduction to Color Palettes…., fastcluster: Fast Hierarchical…., An Introduction to corrplot] [4, 5].

Results and discussion. It was shown that the manifestation of antagonistic activity of the strain *Streptomyces sp.* ONU 64 depends on many factors and, above all, is determined by the antagonist strain, the indicator strain, and the composition of the medium used.

The studied strain showed a high level of antagonistic activity against the indicator strains (Table 1). The maximum manifestation of antagonistic activity was recorded when using Gause 2, SCA and 79 media.

Table 1

Antagonistic activity of *Streptomyces sp.* ONU 64, after cultivation on different media, against indicator strains

Nutrient media	Growth inhibition zone, mm				
	Bacillus subtilis ATCC 6633	*Escherichia coli* ATCC 25922	*Pseudomonas putida KT 2440*	*Candida albicans* ATCC 18804	*Kucoria rhizophila* DSM 348
media 10	0,0±0,0	0,0±0,0	0,0±0,0	8.0±0.9	0,0±0,0
media 15	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0	0,0±0,0
media Gause 2	0,0±0,0	8.0±0.4	7,0±0.8	10.0±0.9	7.5±0,3
media Gause 1	6.0±0.9	0,0±0,0	0,0±0,0	10.0±0.7	6.5±0,8
media SCA	5.0±0.2	6.0±0.3	3,0±0.5	0,0±0,0	4.5±0,4
media 79	7.5±0.2	9.0±0.5	8,0±0.5	8.0±0.5	10.0±0,9

A correlation analysis between indicators of the antagonistic activity of the strain *Streptomyces sp*. ONU 64 against indicator strains and components of the nutrient medium was conducted. It was shown that the presence of a nitrogen source in the medium in the form of $(NH_4)_2SO_4$ (r=0.42) and Hottinger's broth (r=0.48), peptone (r=0.46) and casein (r=0.37) contributes to inhibition of the growth of the *Bacillus subtilis* ATCC 6633 strain. Ammonium nitrate (NH_4NO_3), yeast extract and calcium carbonate ($CaCO_3$), on the contrary, reduced the antagonistic activity of this strain against *B. subtilis* ATCC 6633 (Fig. 1).

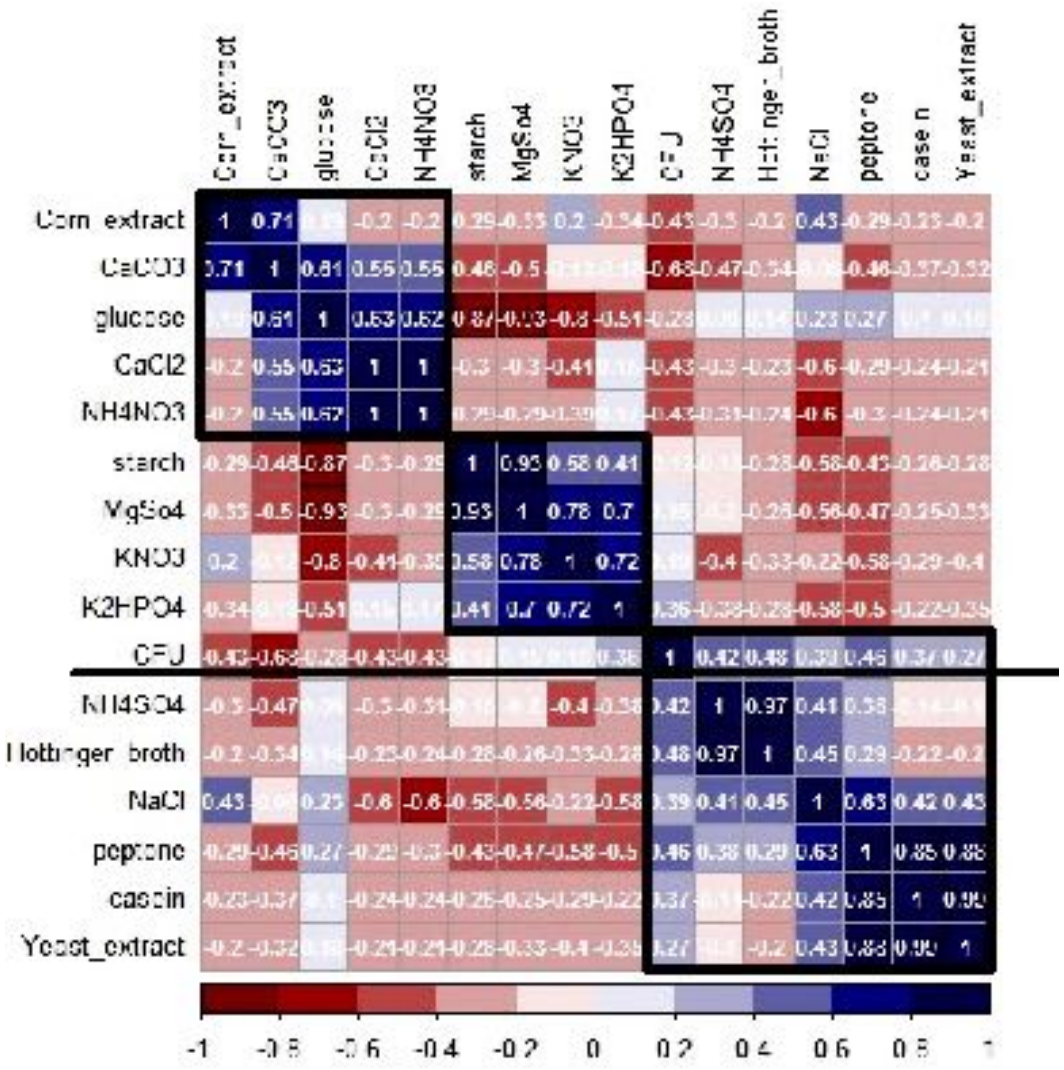

Fig. 1. Graphic representation of the correlation analysis between the composition of the nutrient medium and the indicator of the antagonistic activity of the strain *Streptomyces sp.* ONU 64 against *Bacillus subtilis* ATCC 6633 (CFU – indicator of antagonistic activity)

The manifestation of the antagonistic activity of the strain S*treptomyces sp.* ONU 64 against *Candida albicans* ATCC 18804, *Escherichia coli* ATCC 25922, *Pseudomonas putida* KT 2440 and *Kucoria rhizophila* DSM 348 was positively influenced by the following factors - potassium phosphate (r=0.41), water-soluble starch (r=0.51) and magnesium sulfate (r= 0.52) (Fig. 2).

However, organic extracts (corn extract, yeast extract) and casein, which act as sources of nitrogen, proteins and amino acids, inhibited the antimicrobial activity of S*treptomyces sp.* ONU 64 against these indicator strains (Fig. 2).

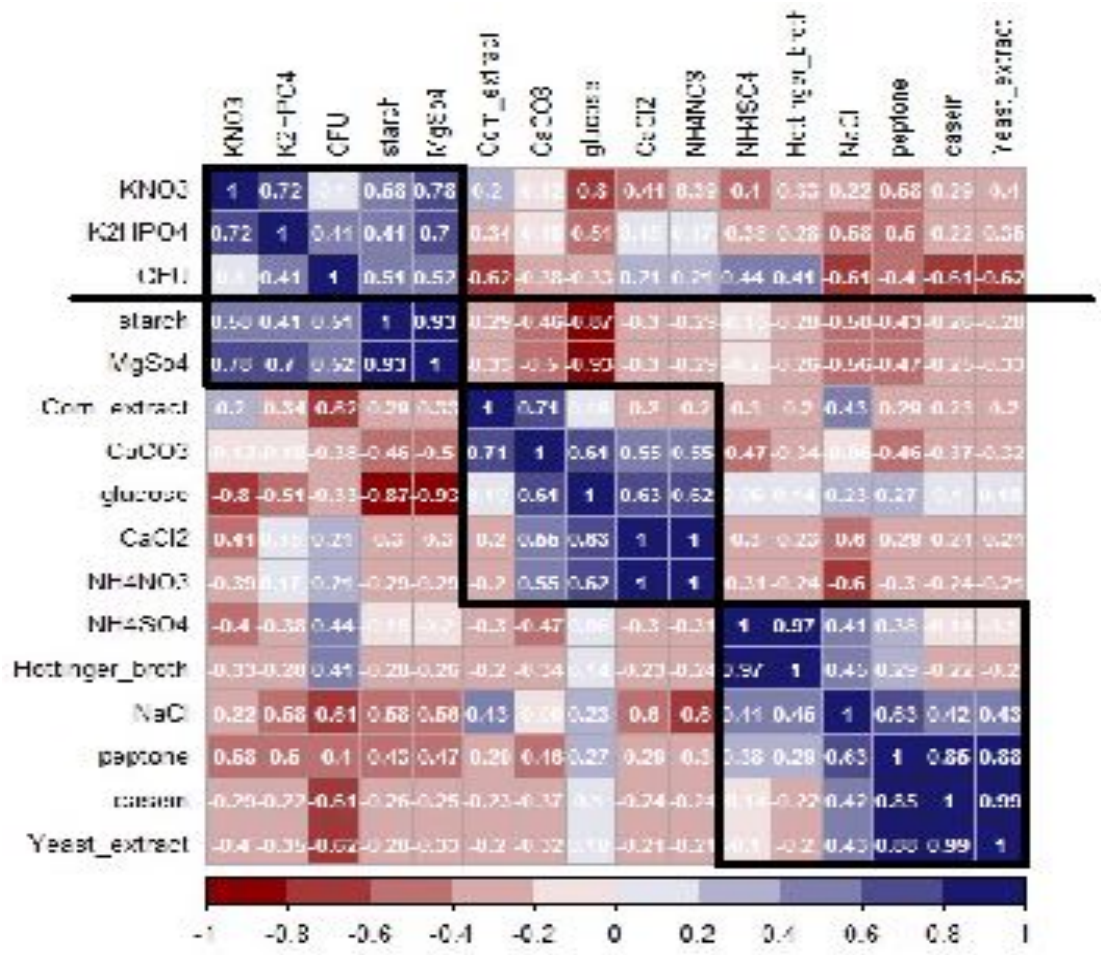

Fig. 2. Graphic representation of the correlation analysis between the composition of the nutrient medium and the indicator of the antagonistic activity of the strain *Streptomyces sp*. ONU 64 against *Candida albicans* ATCC 18804 (CFU – indicator of antagonistic activity)

Conclusions

1. Strain *Streptomyces sp.* ONU 64 showed high antagonistic activity against all opportunistic strains of the indicators. Its activity against *Candida albicans* ATCC 18804 should be noted in particular

2. The highest level of antagonistic activity was registered with the previous cultivation of the strain *Streptomyces sp.* ONU 64 on Gauze 2, SCA and 79 nutrient media.

3. Correlation analysis showed the linear dependence between the presence of combinations of nitrogen sources in the nutrient media and the antagonistic activity of the strain. The presence of glucose, corn extract, and yeast extract more often inhibits the antagonistic activity of *Streptomyces sp.* ONU 64.

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КОРЕЛЯЦІЙНИЙ АНАЛІЗ ПОКАЗНИКІВ АНТАГОНІСТИЧНОЇ АКТИВНОСТІ ШТАМУ *STREPTOMYCES SP.* ONU 64

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Анотація. *Проведено дослідження антагоністичної активності штамів актиноміцетів, ізольованих з донних відкладень Чорного моря. Штам 64 проявив максимальну антагоністичну активність по відношенню до умовно-патогенних штамів індикаторів. Кореляційний аналіз показав наявність лінійної залежності між проявом антагоністичної активності цього штаму та складом поживних середовищ, використаних для попереднього культивування.*

Ключові слова: *антагоністична активність, кореляційний аналіз.*

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STUDY OF THE COMPATIBILITY OF *TRICHODERMA* AND *BACILLUS* STRAINS FOR THE DEVELOPMENT OF A BIOTECHNOLOGICAL DRUG

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Abstract. *A study of the antimicrobial activity of Trichoderma strains against Bacillus strains to determine the possibility of their compatibility in a biotechnological preparation was conducted. It was found that Trichoderma strains inhibited the growth of Bacillus strains with antimicrobial activity that were isolated from environmental objects, however, towards commercial strains (B. amyloliquefaciens QST 713 and B. subtilis M-22 VIZR), Trichoderma strains were neutral. Based on the obtained results, a combination of strains for the development of a new drug to protect plants from phytopathogenic fungi is proposed.*

Keywords: *antagonistic activity, strains compatibility, consortium, biotechnological preparation.*

Introduction. To create a modern biotechnological drug, a consortium is most often used. Consortium is a microbial association of two or more microorganisms, which can be archaea, fungi, bacteria, viruses or algae [7].

A combination of *Trichoderma spp*. and *Bacillus spp*. is one of the most commonly used for biological control of phytopathogens [2, 4, 5]. However, in this case, direct or indirect interactions between different biological agents may occur, which may lead to a negative or positive effect on the effectiveness of the biopreparation [6].

Therefore, we investigated the antagonistic activity of *Trichoderma* strains against different strains of the genus *Bacillus* obtained from different sources.

Materials and methods. To study the antagonistic activity of strains of *Trichoderma* against strains of microorganisms, some *Bacillus* strains obtained from different sources were used. *Bacillus megaterium* LBX.001, *Bacillus circulans* LBX-003, *Bacillus subtilis* LBX-288, *Trichoderma viride* LBX-174 (working name *Trichoderma viride* No. 8) and *Trichoderma harzianum* LBX-181 (working name *Trichoderma harzianum* No. 9) from the museum of enterprise «Scientific production association "Agrobioinovatika"»; *Bacillus*

megaterium MV B-7168 from the preparation "Biophosphorin"; *Bacillus amyloliquefaciens* QST 713 from the fungicide "Serenada ASO SC" ; *Bacillus subtilis* M-22 VIZR from the drug "Ghamair"; *Bacillus subtilis* ONU 559 from collection of the Department of Microbiology, Virology and Microbiology of Odesa I.I. Mechnikov National University.

Bacillus strains were cultivated on MPA medium. *Trichoderma* strains were cultivated on Sabouraud's and MPA media.

To evaluate the antagonistic activity of micromycetes, the method of mixed (counter) cultures was used [3]. Petri plates with Sabouro and MPA nutrient media were inoculated with microorganisms at the opposite poles: *T. viride* No. 8 and *T. harzianum* No. 9 against *Bacillus* strains.

The results were calculated on the 5th day of cultivation at a temperature of 28 °C.

Results and discussion. According to the results shown in Table 1, we can see that the tested *Trichoderma* strains showed high antagonistic activity against *B. megaterium* LBX.001 and *B. circulans* LBX-003 strains. It should be noted that when MPA medium was used, the level of antagonistic activity of *Trichoderma* strains was somewhat lower (Table 1).

Table 1

Antagonistic activity of *T. viride* No. 8 and *T. harzianum* No. 9 strains against *Bacillus* strains

Test strains	Medium MPA		Medium Sabouro	
	T. viride № 8	*T. harzianum* № 9	*T. viride* № 8	*T. harzianum* № 9
B. megaterium	++	++	+++	+++
B. subtilis М-22 ВИЗР	n	n	n	n
B. subtilis ONU 559	++	++	+++	+++
B. amyloliquefaciens QST 713	n	n	n	n
B. megaterium МВ В-7168	n	++	+++	+++
B. subtilis LBX-288	n	++	+++	+++
B. circulans LBX-003	++	++	++	++

Note: n – neutralism; ++ weak antagonism; +++ strong antagonism

A similar pattern was observed when studying the effect of *Trichoderma* strains on *B. subtilis* LBX-288 (Table 1).

The strain *B. subtilis* ONU 559, isolated from the bottom sediments of the Black Sea, showed high antimicrobial activity against conditionally pathogenic microorganisms [1]. However, in co-cultivation with strains of *T. viride* No. 8 and *T. harzianum* No. 9, the antagonistic effect of fungi against *B. subtilis* ONU 559 was recorded (Table 1).

Towards the *B. circulans* LBX-003 strain, the level of antagonistic activity of strains *T. viride* No. 8 and *T. harzianum* No. 9 was defined as "weak antagonistic activity" (Table 1).

Towards *B. amyloliquefaciens* QST 713 and *B. subtilis* M-22 VIZR strains, both *Trichoderma* strains demonstrated neutrality (Table 1).

The *B. megaterium* MV B-7168 strain is sensitive to the antagonistic activity of *T. viride* No. 8 and *T. harzianum* No. 9 strains under the conditions of their co-cultivation on Sabouraud's medium (Table 1).

When conducting this series of experiments, we also investigated the presence of mutual antagonistic activity of *Bacillus* strains. The obtained results are shown in the table 2.

Table 2

Mutual antagonistic activity of *Bacillus* strains

Strains							**Marking**: n - neutralism + - antagonism ++ - antagonism with admiration // - bilateral antagonism **Strains** 1. *B. megaterium* LBX.001 2. *B. subtilis* M-22 ВИЗР 3. *B. subtilis* ONU 559 *4. B. amyloliquefaciens* QST 713 *5. B. megaterium* MB B-7168 6. *B. subtilis* LBX-288
	1	2	3	4	5	6	
1		++	н	++	н	н	
2	++		н	++	н	н	
3	н	н		н	н	н	
4	++	+	н		н	//	
5	н	н	н	н		н	
6	н	н	н	//	н		

As we can see, according to the above results, the strain *B. subtilis* ONU 559 was neutral to most strains, as were the strains *B. megaterium* MV B-7168 and *B. subtilis* LBX-288 (Table 2). The strain *B. amyloliquefaciens* QST 713 showed bilateral antagonism when it was co-cultivated with the strain *B. subtilis* LBX-288. The strain B. *subtilis* M-22 VIZR showed antagonistic activity against the strain *B. subtilis* ONU 559 and antagonism with capture against the strain *B. megaterium* LBX.001 (Table 2).

Conclusions

1. *T. viride* No. 8 and *T. harzianum* No. 9 strains showed strong antagonistic activity against microorganisms of the genus *Bacillus*. Strains isolated from natural sources (*B. subtilis* ONU 559, *B. megaterium* LBX.001, *B. subtilis* LBX-288 and *B. circulans* LBX-003) were not resistant to the antimicrobial activity of *Trichoderma* strains, unlike commercial strains (*B. amyloliquefaciens* QST 713 and *B. subtilis* M-22 VIZR).

2. The proposed consortium for the creation of a new biotechnological drug is *T. viride* No. 8 or *T. ha*rzianum No. 9 + *B. amyloliquefaciens* QST 713.

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ДОСЛІДЖЕННЯ СУМІСНОСТІ ШТАМІВ *TRICHODERMA* ТА *BACILLUS* ДЛЯ РОЗРОБКИ БІОТЕХНОЛОГІЧНОГО ПРЕПАРАТУ

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Анотація. *Проведено дослідження антимікробної активності штамів Trichoderma по відношенню до штамів Bacillus з метою визначення можливості їх сумісності в біотехнологічному препараті. Було встановлено, що штами триходерми пригнічували ріст штамів Bacillus з антимікробною активністю, які були ізольовані з об'єктів навколишнього середовища, однак по відношенню до комерційних штамів (B. amyloliquefaciens QST 713 та B. subtilis M-22 ВИЗР) штами триходерми були нейтральними. На основі отриманих результатів запропоновано комбінацію штамів для розробки нового препарату для захисту рослин від фітопатогенних грибів.*

Ключові слова: *антагоністична активність, сумісність штамів, консорціум, біотехнологічний препарат.*

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Наукове видання

XIX Міжнародна Літня Школа
Молекулярна біологія, біотехнологія та біомедицина

СУЧАСНІ ПРОБЛЕМИ БІОЛОГІЇ, БІОТЕХНОЛОГІЇ ТА БІОМЕДИЦИНИ

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