MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
ODESSA NATIONAL I. I. MECHNIKOV UNIVERSITY

MODERN PROBLEMS OF MICROBIOLOGY AND BIOTECHNOLOGY

International conference of young scientists

Abstracts

20th - 23rd June 2018, Odesa

ODESA
ONU
2018

UDC [579+60] (063)
M78

M78 **Modern** problems of Microbiology and Biotechnology: materials of young scientists international conference 20-23 june 2018. / Ed. board : M. B. Galkin, G. V. Yamborko, N. V. Limanska, O. Yu. Zinchenko – Odesa : «Odesa I. I. Mecnikov National University», 2018. – 130 p.
ISBN 978-617-689-267-0

This collection contains the materials of the young scientists international conference «Modern problems of Microbiology and Biotechnology». The materials reflect the content of the conference reports, which outline the results and prospects of research in various fields of microbiology and biotechnology, in which young scientists, PhD students, and students.

The authors of the abstracts are responsible for the accuracy of the materials and text.

UDC [579+60] (063)

ISBN 978-617-689-267-0

UDC 579.64:632.4+632.93

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EFFECT OF *LACTOBACILLUS PLANTARUM* ON AGENT OF DAMPING-OFF OF PINE SEEDLINGS

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Effect of some *Lactobacillus* strains and their combinations on agents of fusariosis of pine *Fusarium sp.* 17 has been studied. Bacteria of *L. plantarum* species could be studied as perspective microorganisms for improving germination of Scots pine seeds and protection of seedlings under the certain condition of soil treatment.

Key words: *Lactobacillus plantarum, Fusarium, damping-off, micromycetes, survival, antagonists.*

Introduction

In natural environment Scots pine trees often suffer from various stresses of biotic and abiotic nature. The most dangerous biotic stressors during all ontogenesis of these plants are *Fusarium, Alternaria, Botrytis, Pythium, Rhizoctonia, Phytophtora*. Damping-off of pine seedlings causes 33,0-40,0 % death in young plants in nurseries of forest cultures of Ukraine and other countries around the world [*Cherkis, 2015*].

One of the most effective biological methods of struggling against soil micromycetes – agents of root decays, is application of antagonistic bacteria and preparations based on them. Among perspective antagonists of phytopathogenic fungi bacteria of *Lactobacillus* genus can be mentioned out. Lactobacilli strains are characterized by high antagonistic activities including activity against agents of fusariousis [*Okorski, 2014*]. Lactobacilli have GRAS status ("Generally Recognised as Safe"), which means that they are absolutely non-dangerous for human and animal

health [*Gordon, 2015*]. No evidences of influence of lactobacilli on agent of damping-off of pine seedlings was found yet.

Aim of this work was to study the effect of some *Lactobacillus plantarum* strains and their combinations on fusariosis agent *Fusarium sp.* 17.

Materials and Methods

Fungi were isolated from damped pine seedlings by prints methods when a diseased seedling was laid down the dish with Saburo medium and left incubated at 25-28°C until the mycelium appeared.

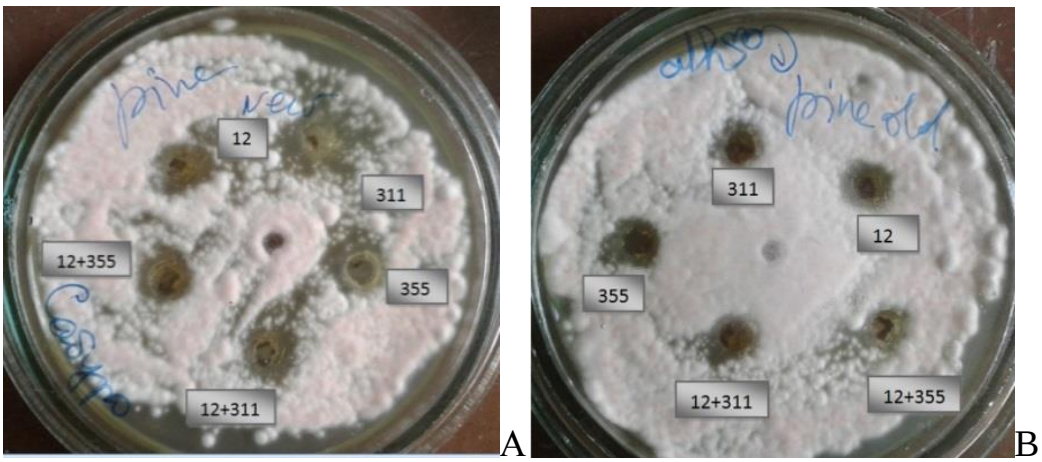
Bacteria of *L. plantarum* ONU 12, ONU 311, ONU 355 from collection of Department of Microbiology, Virology and Biotechnology of ONU and their consortia were cultivated in MRS medium at 37°C overnight and during 3 days [*De Man, 1960*].

The effect of the cultures and cell-free supernatants obtained after centrifugation at 10000g for 20 min, on micromycetes growth was studied by well-diffusion method. In freshly inoculated lawns of *Fusarium* sp. 17 well were cut. Culture of lactobacilli or cell-free supernatant were poured into the wells. Petry's dishes were incubated for 7-10 days until mycelium of a fungus appeared [*Ahangar, 2011*].

Results and Discussion

Morphological features of a mycelium (pinkish white, well developed, on some parts – dipped into the substrate, stroma coloured in wine-red colour) and that of conidia (microconidia are elipsoid-like, macroconidia – spindle-like, with 3 septa, with slightly formed stalk) allowed us to identify micromycetes as that belonging to *Fusarium* genus [*Bilai, 1977*]. Strain was named as *Fusarium* sp. 17.

The study of effect of lactobacilli culture on *Fusarium* sp. 17 growth showed that overnight cultures *L. plantarum* ONU 12, ONU 311, ONU 355 and their consortia caused zones of inhibition of mycelium growth (Fig. 1, А). Three-days cultures were less effective (Fig. 1, Б).



A B

Fig. 1. Zones of inhibition of *Fusarium sp.* 17 growth on Saburo medium under the influence of *Lactobacillus plantarum* cultures (central well – negative control without addition of lactobacilli; A – influence of overnight cultures, B – effect of 3-days cultures).

Cell-free cultural supernatant of lactobacilli caused less effect and less inhibition zones (not more than 2 mm). Bacteria of *L. plantarum* ONU 311 strains inhibited fusariosis agent better than other strains (inhibition zone 5 – 6 mm).

Conclusion

Strain *L. plantarum* ONU 311 showed the most effective antagonism against damping-off agent *Fusarium* sp. 17 as compared with other strains and their consortia. *L. plantarum* ONU 311 strain is a perspective agent of biological control of fusariosis. Further investigations on more cultures and more pathogenic strains should be carried out.

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UDC 582.282.23.043

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ANTIMICROBIAL PROPERTIES OF SOME PORPHYRIN COMPOUNDS AGAINST CATHETER RELATED INFECTIONS

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Summary

Central venous access is commonly used in clinical practice for a wide spectrum of procedures. The main complications limiting use of central and peripheral catheters include catheter-related infections. The epidemic of microbial resistance to widely-used antibiotic groups seriously complicates treatment of CRI

even in complex with systemic antibiotic therapy. In this paper we propose to consider porphyrin compounds as alternative antimicrobial agents for antimicrobial lock therapy. It was discovered antimicrobial action of porphyrin compounds against *S. aureus* and *E. coli* that was significantly enhanced in composition with EDTA as additional component of antimicrobial lock solution.

Key words: porphyrins, catheter-related infections, lock therapy, biofilm.

It is difficult to imagine modern medicine without permanent intravenous approach. There are various categories of patients whose lives depend on manipulations that require placing central or peripheral catheters. Despite of benefits use of intravenous catheters is closely related with several adverse effects. The most important one is development of the catheter-related infection (CRI) which becomes possible after catheter contamination. Two ways of catheter contamination are possible; the first one is extrinsic when catheter becomes contaminated from medical worker's hands or infusion of infected fluids. The second one is intrinsic, in this case contamination occurs with pathogens from the skin of the patient or pathogens that spreading from a distant focuses of chronic infection within patient's organism by hematogenous way. The most common pathogens that cause CRI are coagulase-negative microorganisms, *S. aureus*, C. *albicans, P. aeruginosa, E. coli* [6].

Biofilm formation occurs after microorganisms' interaction with catheter. This process is almost inevitable and depends on several factors such as material of a catheter that can promote more active adhesion of bacteria, rate of infusion and properties of infused fluids. Bacterial attachment to catheter surfaces occurs by interaction of MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) with fibronectin, fibrinogen, collagen, and heparin that cover introduced catheter immediately after installation [3].

Biofilm, for its part, represent a form of adaptive resistance resulting in significant reduction of antibiotic susceptibility. It becomes possible due to biofilm's structure, especially matrix, which diminishes diffusion of antimicrobial agent through biofilm and binds with them. Persisters as part of biofilm composition also

contribute to biofilms multiresistance [1]. Thus, development of the new antimicrobial agents that could be effective in antimicrobial lock therapy composition is the question of current interest.

Antimicrobial lock therapy (ALT) – method for catheter lumen sterilizing that involves injection of high-concentrated antimicrobial agent into the catheter lumen for extended periods of time. The antimicrobial lock solution consists of two components, antimicrobial agent that often combined with an additional component, in particular anticoagulant such as heparin.

The purpose of our research is a characterization of antimicrobial properties of porphyrin compounds as potential agents for antimicrobial lock solution. The biofilm was obtained by *S. aureus* ONU 536 and *E. coli* ONU 458 that were cultivated in Peptone Glucose Water during 24 and 48 hrs at 37 ˚C with adding of porphyrins and EDTA in polysterene microtiter plate. The Biofilm growth intensity was tested by measuring of optical density by automatic reader «μQuant» BioTek (USA).

Porphyrins – heterocyclic compounds that take part in biochemical processes in living organisms but there are a lot of their synthetic analogous. There are two classes of porphyrins, natural (NP) and synthetic (SP), difference based on their structure and origin. Synthetic porphyrins are derived from the tetrapyridylporphyrin skeleton (SP), which has pyridyl groups at each of the four *meso*-positions and hydrogens on all the pyrrole positions [4]. The studied porphyrins (protoporphyrin IX, hemin, tetrapyridylporphyrin SP, mono-nonyl-SP, tetra-nonyl-SP, nonyl-SP-Zn) were tested in different concentrations: 0.1, 1, 10 μM.

It was discovered that regarding *S. aureus* the most effective porphyrin compound is mono-nonyl-SP at concentration 10 μM. NPs also demonstrate antimicrobial activity but less potent. As to *E. coli* cultures, mono-nonyl-SP and nonyl-SP-Zn demonstrate the most potent antimicrobial influence at concentration 10 μM. NPs don’t express antimicrobial activity, they even stimulate growth of biofilm as you can see on diagram.

Antimicrobial properties of porphyrins are based on their similarity with heme. Heme is a source of iron, cofactor which takes part in electrons transferring, reactions

of peroxidase and oxidase catalysis and photons absorption [1]. In Gram-negative and Gram-positive microorganisms heme capture occurs via active transport, heme binds with TonB- ExbB- and ExbD-dependent receptor on bacterial surface. Porphyrins exploit the same path. They enter the cell and affect bacterial heme-catalysed reactions preventing their normal function in the cell metabolism. Development of resistance is very unlikely, rare bacterial mutants that become resistant to porphyrins via loss of heme-uptake systems would be less virulent. This would minimize the impact of resistance since such mutants should survive less well in body fluids and tissues. Furthermore, porphyrins can also penetrate the outer membrane independently of the heme transporters due to their hydrophobic nature and their preferential association with lipid membranes [5].

Thus, we selected two potential porphyrin compounds that we tested in composition with EDTA for the next stage of our research. We observed that antimicrobial action of porphyrins in composition with EDTA is more active compared to its separate action. Also, 48 hrs cultures become more sensitive to antimicrobial action. This synergistic effect can be explained by chelating abilities of EDTA. The affinity of EDTA toward metal ions (in particular divalent ions) determines the breakdown of a biofilm. Divalent cations such as calcium and magnesium cross-link with the polymer strands and provide greater binding force in a developed biofilm [2]. Also, EDTA has anticoagulant properties that allow substituting heparin as additional component in ALT [4].

In conclusion it should be noted, that mono-nonyl-SP and nonyl-SP-Zn was selected as the most prospective porphyrin compounds. Both demonstrate significant inhibition of biofilm growth at 24 and 48 hrs cultures. In composition with EDTA as additional compound their action was enhanced. Because of antimicrobial properties of porphyrin compounds and synergism that they demonstrate in composition with EDTA, they are promising agents for further research.

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UDC 604:579.6:663.1:577

I. Bulanchuk, A. Tugai

INTERACTION OF HUMAN INTERFERON-B WITH MELANINS OF SOIL MICROMYCETES CLADOSPORIUM CLADOSPORIOIDES (FRESEN.) G.A. DE VRIES CULTIVATED UNDER THE INFLUENCE OF A NANOPARTICLE OF A COLLOIDAL IRON SOLUTION

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Abstract

We investigated the 3D fluorescence profiles of interferon with melanins isolated from *Cladosporium cladosporioides* and fluorescence fading spectra. The possibility of protein-ligand interactions is shown. The reduction of the dissociation constant of melanin with interferon was revealed as a result of an increase in the chemical affinity in both cases, when added to the culture medium of iron for the strain of 10 to 10 and for the strain 396 in 4 g, may indicate an increase in the level of

virulence under the action of iron, with the strain from the contaminated area is more dangerous. In all cases, on the 3D-profile, besides the main peak of interferon, there is a secondary peak of the interferon complex with melanins.

Keywords: melanins, 3D spectrofluorescence, interferon, *Cladosporium.*

Introduction

Melanins are produced by organisms in all biological kingdoms, including a wide range of pathogenic bacteria, fungi, and worms, serving as key factors in their virulence [1]. In mushrooms, melanin polymers are able to store iron and calcium, neutralize the active forms of oxygen produced by macrophages [2]. It is known that heterocyclic polymers, which include mushroom melanins, can actively bind to various proteins due to the displacement of the p-electron density, stacking interactions between the phenolic and indole groups of melanins with aromatic amino acids of the protein, donor-acceptor interactions with lysine, arginine, and histidine [3]. Interferon, in turn, is a key element of immune protection, so we studied the possibility of melanins to form complexes with interferon.

Nanoparticles of colloidal iron solution are actively used in agriculture and lead, as our previous studies have shown, to increase the biomass of the fungus grown on the medium with the addition of colloidal iron solution 3-4 times as compared to control (on a standard nutrient medium) and changes in the ratio of melanin production, which can serve as an additional factor of virulence [4].

Materials and methods

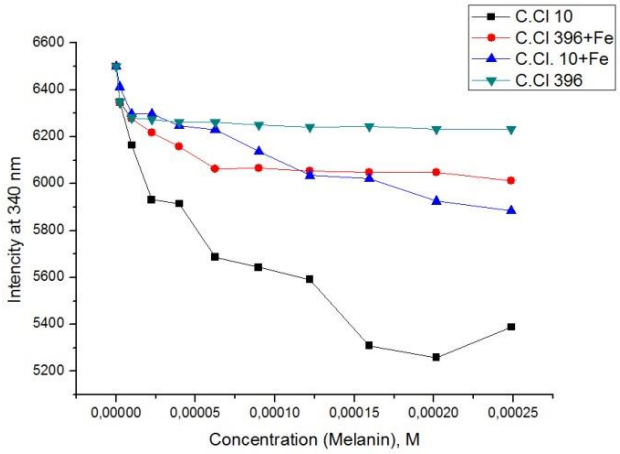
Cladosporium cladosporioides standard strain-producing strain 396 was selected as test objects. Cultivations were carried out at + 25 ° C on a liquid Chapek nutrient medium with the addition of the test solution. Studies of protein-ligand interaction were conducted on spectrofluorimeter Jasco FP8200 by quenching of fluorescnece and chainges in 3D-spectrofluorimetric profiles on interferon-β in 50 mM TRIS-HCL pH 7,7 in cuvette 10 mm [5].

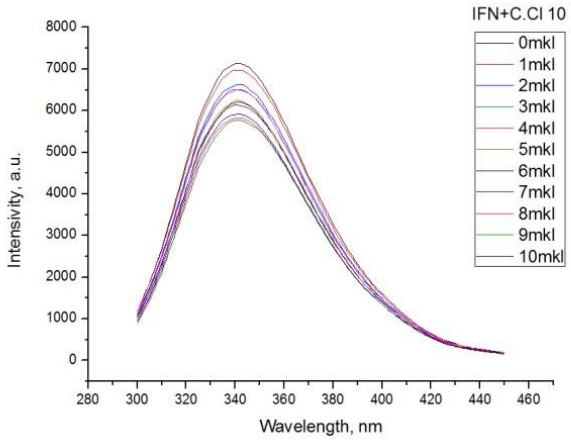
Results and discussion

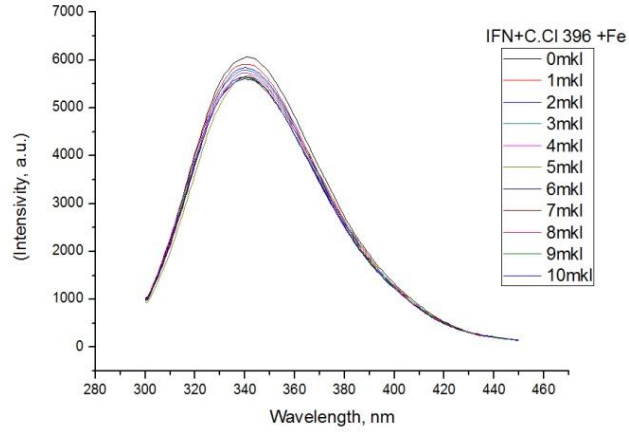
The conducted research showed that, when the *Cladosporium cladosporioides* strain 10 was processed by strain 10, the dependence of the fluorescence intensity becomes similar to the similar activity of the producer strain *C. Cl* 396 (Fig. 1. A), which may indicate the similarity of the melanin composition of the new polymers.

When analyzing the graphs of fading fluorescence, it was established (Fig. 1B) that the dissociation constant for interferon and melanin C. Cl 10 was Kd = 2.16E-5 ± 0.42E-5, and when the fungus was treated with a nanoparticle, the pigment allocated gave Kd = 2.25 E-6 ± 0.32E-7.

A







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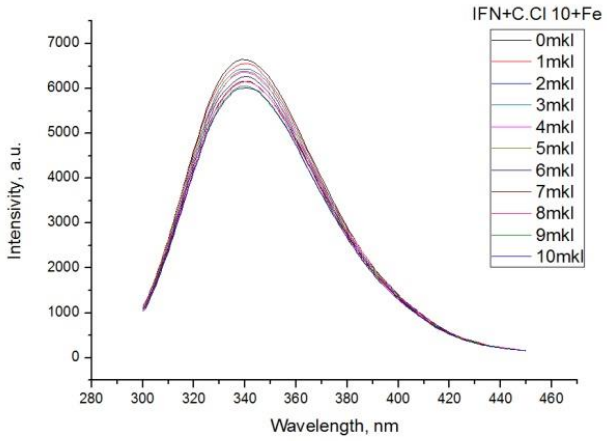

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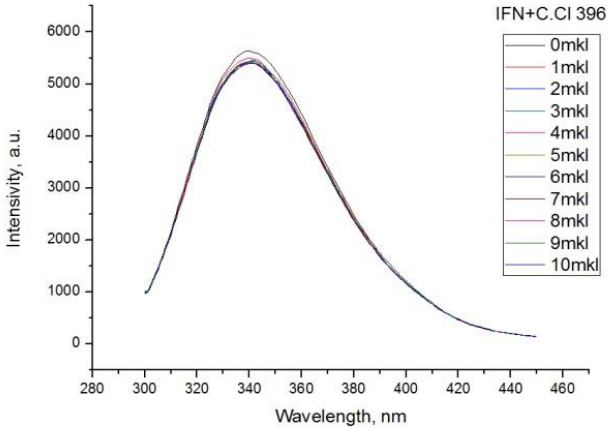

B

Fig. 1. Dependence of the fluorescence intensity at 340 nm, depending on the concentration of melanin (A) and quenching of fluorescence when titrated with melanins (B) *Cladosporium cladosporioides* (1- interferon and melanin C. Cl 10, 2- interferon and melanin C. Cl 396+Fe, 3- interferon and melanin C. Cl 10+Fe, 4- interferon and melanin C. Cl 396)

At the same time, for a strain 396 Kd = 4.5E-5 ± 0.75E-6, and for a strain of 396 under the action of colloidal iron Kd = 1.17E-5 ± 0.16E-6. Reducing the dissociation constant of melanin with interferon, due to the increase of chemical affinity in both cases, when added to the culture medium of iron for the strain 10 in 10 and for the strain 396 in 4 g, may indicate an increase in the level of virulence under the action of iron, with the strain from the contaminated the territory is more dangerous.

There is also a slight shift in the titration of 5-10 nm, which is evidenced by the change in the dipole moment of the protein and its partial denaturation. Means of analyzing the structure of melanins still remain extremely imperfect. However, we are suggesting that we use the 3D spectrofluorescence to "see" the complex of melanin with interferon (Figure 2).

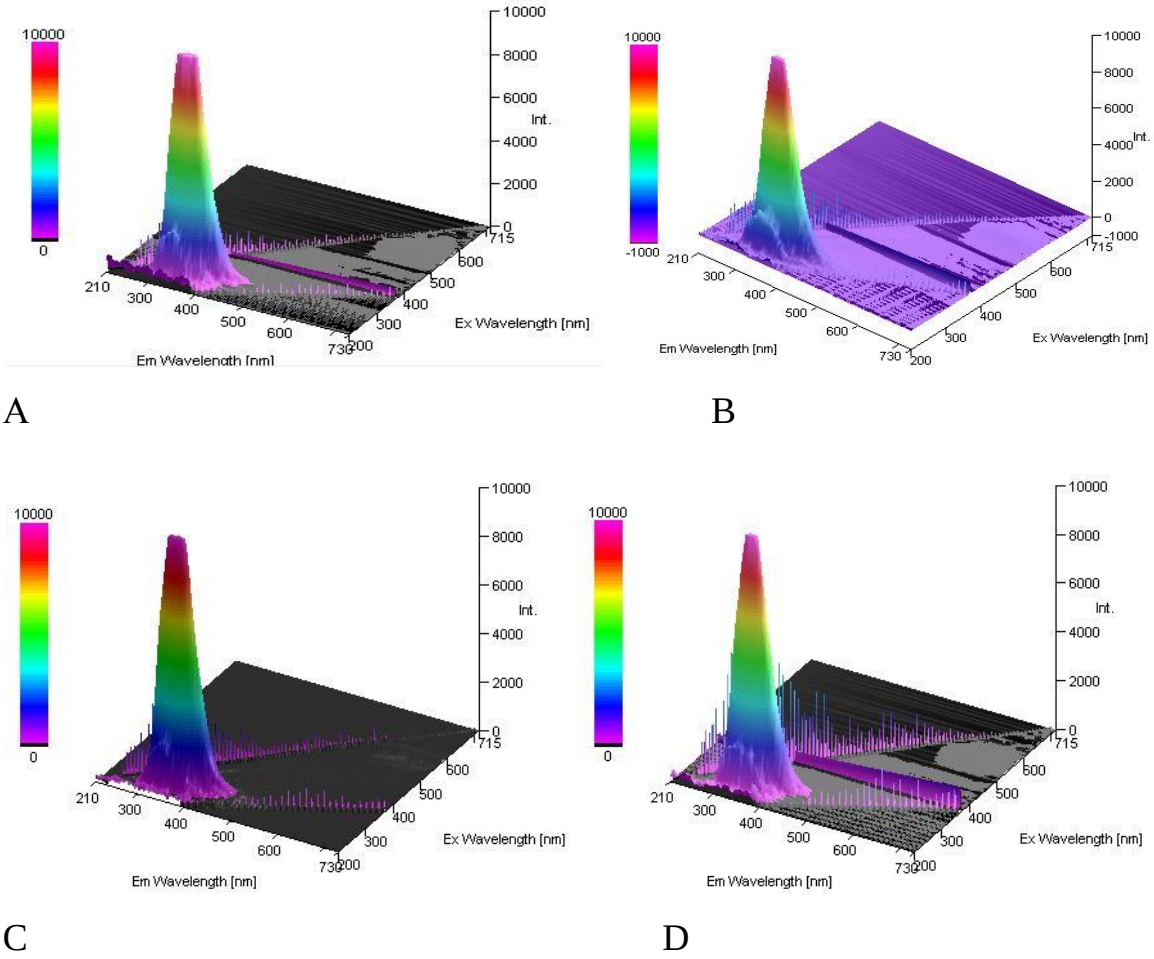

Fig. 2. 3D profiles of fluorescence of interferon in a mixture with melanins: A- C.Cl 10, B-C.Cl 396+Fe , C- C.Cl 10+Fe, D-C.Cl 396.

In all cases, besides the main peak of interferon, there is a secondary peak of the interferon complex with melanins (Table 1). Melanins increase the emission and absorption energy in the region of 240 nm by means of pi-electron transitions in carboxyl and amide groups and reduce the emission and absorption energy in the region of 280 nm by means of the imidazole ring [5].

Table 1. Characterization of secondary 3D fluorescence peaks of the interferon-melanin mixture

C.Cl 10		C.Cl 396+Fe		C.Cl 10+Fe		C.Cl 396	
λex/λem	F,a.u.	λex/λem	F,a.u.	λex/λem	F,a.u.	λex/λem	F,a.u.
240/329	2247,01	240/334,5	1996,59	240/334,5	1922,93	240/334,5	1850,98

Conclusions

The addition of nano colloid iron to the culture medium leads to an increase in the virulence of *Cladosporium cladosporioides*. In this case strain of radioactively contaminated territory most actively interacts with interferon. The possibility of an indexation of the complex of interferon with melanins is shown.

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UDC 663.31.001.76 (043.3)

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THE USE OF WINE YEAST IN THE PRODUCTION OF RED WINES IN BIOTECHNOLOGY

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Abstract. The experiments conducted indicate that yeast *Saccharomyces vini* the studied race *siha aktivhefe 4* refer to glucosophilic yeasts of the genus *Saccharomce*s, fermenting the most significant fraction of sugar in grape juice. These yeasts end up fermentation, and many of them are involved in the formation of the aroma of young red wines. Yeast assimilate and ferment glucose, galactose, sucrose, maltose and raffinose (by 1/3), do not absorb and do not ferment dextrins, lactose, inulin, xylose, arabinose. Assimilate ethyl alcohol and glycerin, do not assimilate mannitol, dulcite, sorbitol. From organic acids, they absorb acetic and lactic acid, they do not absorb amber, apple, wine and lemon. In winemaking, yeast can play a negative role, causing turbidity of the finished wines.

Introduction

Winemaking is a complex biotechnological process of turning grape matter into wine, due to the vital activity of microorganisms. Therefore, in order to control the technological process in order to obtain high quality wines, knowledge of the biology and properties of the microorganisms of grape must and wine is required [1].

In the winemaking industry, yeast is distinguished between wild and pure yeast cultures. Yeast in winemaking refers to three families: *Saccharomycetaceae (Saccharomyces, Pichia, Hansenul, Sanidae, Zigosaccharomyces, Brettanomyces, Torulopsis, Rhodotorula), Schizosaccharomycetaceae (Schizosaccharomyces genus) and Saccharomycodaceae (genera accharomycodes and Hanseniaspora*).

The yeasts of the genus Saccharomyces are of the highest importance and spread in the wine-growing sector. Yeast races have an individual feature of alcohol-forming ability, sulfite-length, biosynthesis of volatile components and other products that determine the composition and organoleptic qualities of wine [2, 3]. Thus, the biological properties of yeast are important for obtaining high quality wine.

The purpose of our work was to study the biological characteristics of the race of wine yeasts *Saccharomyces vinisiiaaktivhefe 4*, which are used in the wine-making process of the full wines of Cabernet wines of the mark "French Boulevard" at the plant of the Private Joint-Stock Company "OdessawinProm".

Materials and methods

The experimental part of the work was carried out in the bacteriological laboratory of the "OdessawinProm" plant. Cultivated yeast on wort-agar and glucose-peptone agar with yeast extract. Characterized by the nature of growth on a dense medium - the size, color of the colonies.

When conducting a microscopic analysis of yeast race, the method of a dropped drop was used - a drop of a suspension of yeast cells was applied to the slide glass, which was covered with overhead glass from above. Under a microscope, morphology, cell size, dead and dead cells were detected.

When measuring the size of microorganisms, an ocular-micrometer was used. In order to assess the ability to use carbohydrates, the main background environment of the following composition (GLP) was prepared: peptone - 5,0; K2NRO4 1.0. 10% aqueous solutions of hydrocarbons and alcohols were prepared separately, sterilized by autoclaving at 0.5 atm. Sterile solutions were added to the base background in an amount such that the concentration in the medium was 1-2 g per 100 ml.

Formation of acids was recorded by changing the pH of the medium. To do this, an indicator of bromtimolblou was added to the medium (changing the color from yellow to blue in the range of pH 6.0-7.6).

The main background medium was poured into test tubes of 8-10 ml, lowered to the bottom of each test tube, the "float" was sterilized at 1.0 atm. The accumulation of acidic or alkaline metabolism products was evidenced by a change in the color of the indicator. The formation of gas was evidenced by the accumulation of it in the float.

Research results

As a result of microscopic studies by the method of the expanded drop, it has been found that yeast cells of the *Saccharomyces vinisihaaktivhefe 4* grape juice after 3 days of fermentation have an elliptic form. The size of cells (5-9) x (4-8) microns. They are located separately or in pairs, the kidneys for some time remain connected with the mother cell.

Spore formation occurs easily with the formation of asks, preferably with 2 or 4 oval spores. The nature of the sediment varies from loose to dense, depending on the stage of development of yeast. In an adult culture, there is never a noticeable film, but the glass of the test tube may remain a ring formed by yeast raised by foam during rapid fermentation.

On a hard wort-agar colony, the Siha aktivhefe 4 races were matte, grainy, chopped, white, or gray, moist, grainy, convex, folded, slightly cut.

Yeast races *sihaaktivhefe 4* digested 80% glucose, fructose, maltose, sucrose. The starch was not digested due to the lack of an enzyme. Such sugars as lactose, arabinose and raffinose were also virtually not fermented in the environment.

Conclusion

Experiments conducted indicate that yeast *Saccharomyces vini* the studied race *sihaaktivhefe 4* refer to glucosophilic yeasts of the genus *Saccharomce*s, fermenting the most significant fraction of sugar in grape juice.

These yeasts end up fermentation, and many of them are involved in the formation of the aroma of young red wines.

Succulents such as lactose, arabinose and raffinose, practically not fermented, remain in the vine at almost the initial concentration, which gives a well-shined lighted wine a harmonious taste.

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UDC 574.52

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GENOTOXICITY DETECTION IN THE WATER OF THE BLACK SEA WATER BY AMES TEST

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The Ames test has been extensively used for rapid evaluation of the action of chemical substances on the DNA molecule [2]. Several genetically modified strains have been produced which combine a low level of spontaneous mutation with an excellent response to mutagenesis induced by different classes of agents [3]. This test has been used to evaluate the mutagenicity of complex mixtures in the air, in rivers, lakes, sediments, industrial effluents, and in drinking water. Genotoxicity in water occurs due to the industrial and domestic wastes, to contamination by agricultural products or even to potentially reactive natural products [2, 3].

The use of the Ames test has been recommended for environmental studies by international organizations and is recognized as a primary test for the evaluation of

genotoxicity related to carcinogenic potential by the Federal Register, USA. The contamination of surface waters is an important chapter in the study of the genotoxicity of complex mixtures [2, 3].

The research aim was to investigate the genotoxic activity in the water of the Black Sea.

Materials and methods. Water samples from different sites along the coast of the Black See were subjected to the Ames test for the detection of possible mutagens. Strains *Salmonella typhimurium* TA 100 and *Salmonella typhimurium* TA 98 were used in the study. The procedure used in the experiment is set out in the Method of Comprehensive Toxicity Estimation [1].

Results. According to the results, it was showed that the majority of water samples isolated from the Black Sea in summer 2016 had a high level of toxic activity in the test system of *Salmonella typhimurium* TA 100. The highest toxicity was showed by the water isolated in the area of Kovalevskiy Dacha. The water from the tide wave line in this point lead to the death of more than 85,0 % of viable cells (fig. 1), which corresponds to the level of powerful toxic effects. It was this sample that caused mutagenic activity, which was 4,03 ± 0,075 times higher than the control values (fig. 2), which corresponds to the level of moderate mutagenic action. A sample of sea water taken from the coast in the area of Kovalevskiy Dacha caused the death of 77.0% of viable cells (fig. 1). The mutagenic activity of this sample corresponded to the level of "weak mutagenic effect" (1.1 ± 0.10 units, fig. 1).

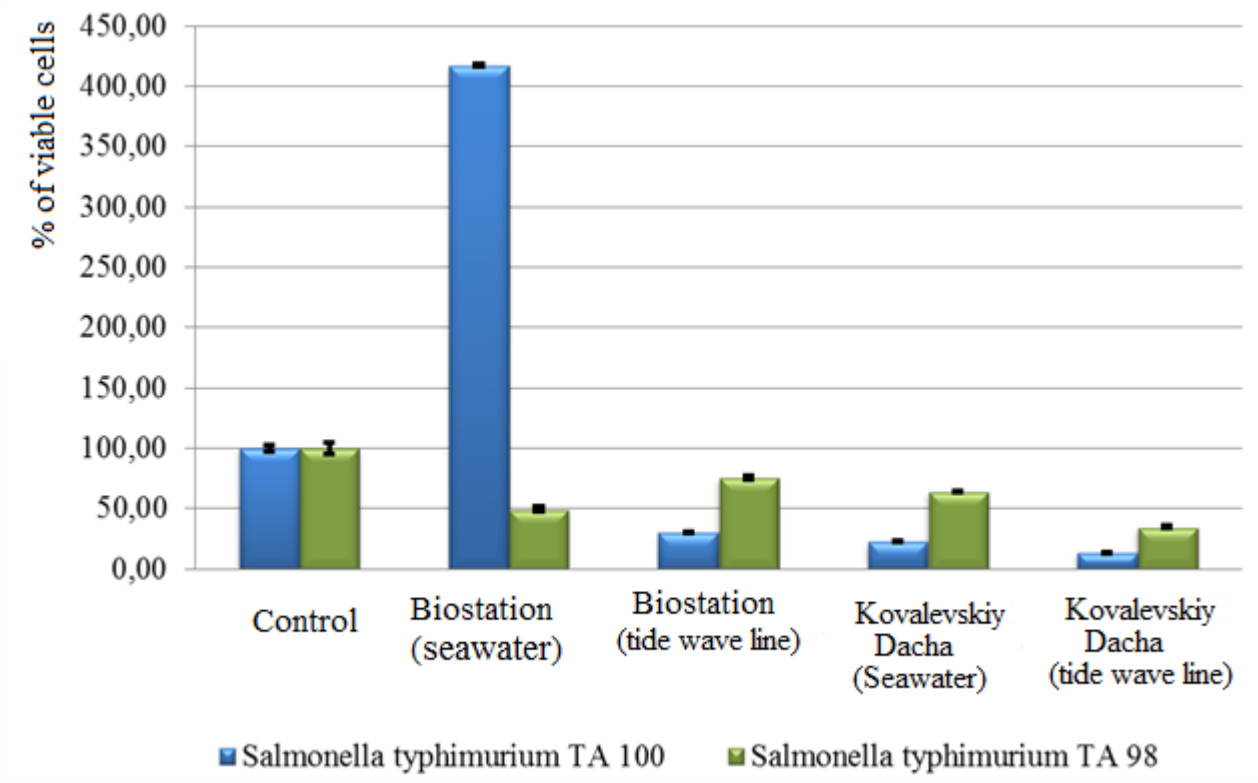

Fig. 1 Results of biotesting of sea water of the Black Sea using bacterial test systems of *Salmonella typhimurium* TA 100 and *Salmonella typhimurium* TA 98 (toxicity)

The results of biotesting of these water samples using the bacterial test system of *Salmonella typhimurium* TA 98 caused less loss of test strain cells (65.0 % of viable *Salmonella* cells, fig. 1). At the same time, a significant mutagenic activity was recorded, which was 6.03 ± 0.21 relative units compared to the control values (fig. 2). A sample of sea water taken from the coast near the Biological Station caused a strong mutagenic activity in the test system of *Salmonella typhimurium* TA 98 (5,73 ± 0,09 units) with a simultaneous high toxicity - the death of more than 50,0 % of viable cells.

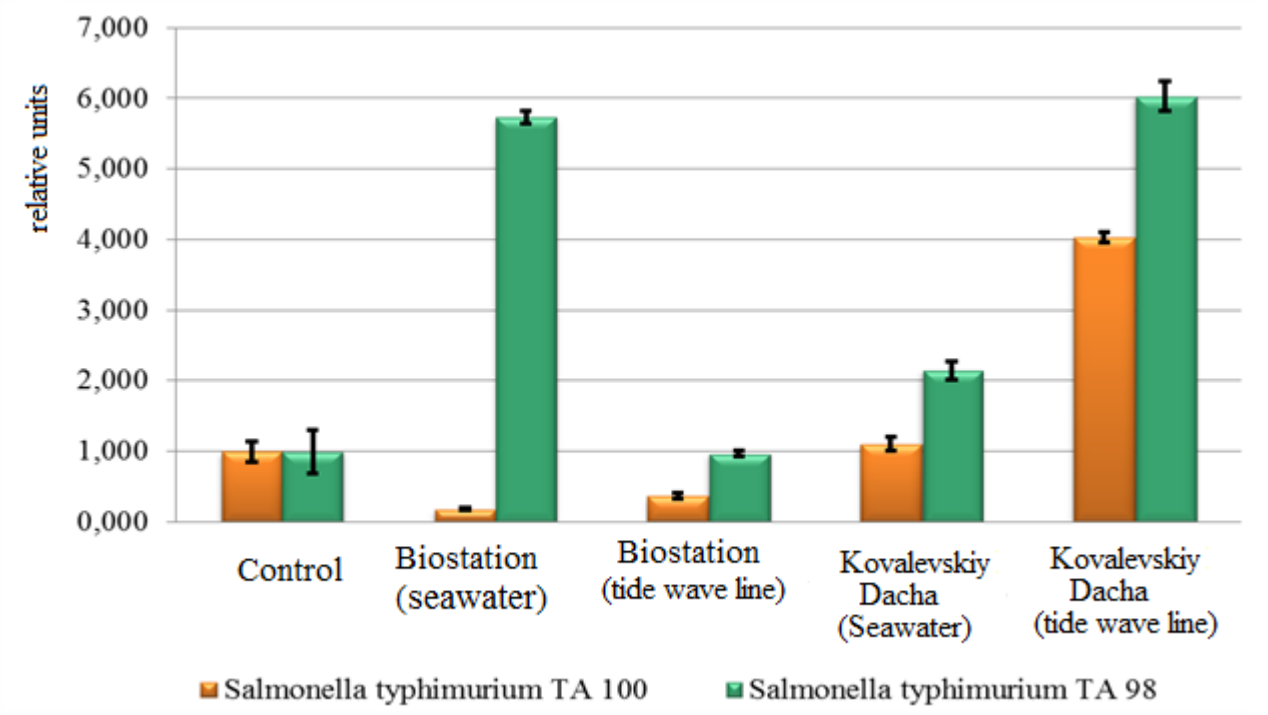

Fig. 2 Results of biotesting of sea water of the Black Sea using bacterial test systems of *Salmonella typhimurium* TA 100 and *Salmonella typhimurium* TA 98 (mutagenic activity)

Thus, the sea water of the Black Sea led to the negative biological responses in the *Salmonella typhimurium* TA 100 and *Salmonella typhimurium* TA 100 test systems. The most significant negative genotoxic parameters were recorded when biotesting water from the tide wave line near Kovalevskiy Dacha.

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UDC 531/534/: [57+61]

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EFFICIENCY OF PURIFICATION OF WATER FROM CYCLIC AROMATIC XENOBIOTICS WITH STRAINS *P. FLUORESCENS* ONU328 AND *P. MALTOPHILIA* ONU329

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Abstract. It has been experimentally established that non-pathogenic strains of bacteria of the genus *Pseudomonas,* identified by the fatty acid composition of their cellular lipids as *P. fluorescens* ONU328, *P. maltophilia* ONU329 (isolated from the marine environment), have oxidizing ability for cyclic aromatic xenobiotics (phenol, N-cetylpyridinium bromide). The high phenol-oxidizing ability of the investigated strains of microorganisms is proved. When water of bacterial cells in the amount of 7.5 × 105 CFU/ ml is introduced into phenol contaminated (at a concentration of 300 mg / l), the degree of water purification from phenol is 100% on day 18 when using as a biodestructor strain *P. maltophilia* ONU329 and 22 days - when using strain *P. fluorescens* ONU328. It has been experimentally confirmed that when the free cells of bacteria of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 strains in the amount of 5.5×10^4 CFU/ ml are introduced once into the contaminated water, the purification rate of water from N-cetylpyridinium bromide at a concentration of 20 mg / l reaches 50.7% for the 4th day and 55.7% for the 6th day, respectively. With the repeated introduction of fresh portions of microorganism-destructors or use of

higher starting doses (5.5×10^8 CFU/ ml), the water purification rate from N-CPB is 98.0-98.5%.

Keywords: purification of water, phenol, N-cetylpyridinium bromide, bacteria of the genus *Pseudomonas*

Introduction. Cyclic aromatic xenobiotics (phenolic and other difficult oxidation compounds) are toxic pollutants of the environment. Microbiological methods of water purification from cyclic aromatic xenobiotics, which also include water-soluble salts widespread N-cetylpyridinium limited. This is due to their high antimicrobial activity against fungi, gram-positive and gram-negative microorganisms. N-cetylpyridinium halides are cationic surfactants which have high surface activity and the disinfecting effect; are the basis of most disinfectants: chlorhexidine, a diocid. Diocid is a disinfectant combination agent based on a mixture of N-cetylpyridinium chloride or bromide (N-CPC or N-CPB) and ethanol merkuryl chloride (active mercury antiseptic) in the ratio (2:1). The widespread use of N-cetylpyridinium halides only as the basis of disinfectants leads to their accumulation in effluent production of pharmaceutical preparations, in effluent of medical institutions, etc. Getting into water bodies, these surface-active substances like surface-active substances of anionic and non-ionic nature, participate in the processes of redistribution and transformation of other pollutants, sometimes translating them into a more toxic form [1]. Information on the ability of microorganisms to decompose synthetic surface-active substances of cationic nature on the example of "biologically rigid" N-CPC are presented in [2]. The authors found that the ability to destroy surfactants varies widely among microorganisms, even among members of the same genus. For example, the degree of biodegradation of N-CPC at a concentration of 20 mg / l in the presence of strains of Staphylococcus warneri A and Staphylococcus warneri T is 94% and 45%, respectively, on the 5th day; and in the presence of *Bacillus anthracis, Bacillus licheniformis, Bacillus amyloliquefaciens* - respectively 84.8%, 60.7% and 52.3% (on the 6th day). When achieving high results on biodegradation of N-CPC, the following should be attributed to the shortcomings of the above-mentioned microbiological method: the

duration of the process of cultivation of the most biochemically active destructor strains (isolated from glass-fiber waste water) - from a week to a month with a small cell titer of bacterial strains (10^3- 10^4 CFU/ml) that were added to the surfactant solution; lack of information on the resistance of isolated destructor strains to high concentrations of other cyclic aromatic xenobiotics, including highly toxic phenol (phenol, like N-CPC, is fixed in pharmaceutical effluent, medical facilities); the pathogenicity of the most active strains-destructors has not been evaluated, which is necessary for their wide use in biotechnology of water purification from cyclic aromatic xenobiotics.

The aim of the work is to propose an efficient method of water purification from cyclic aromatic xenobiotics (for example phenol, "biologically rigid" cationic surfactant N-cetylpyridinium bromide) when using some non-pathogenic bacteria of the genus *Pseudomonas* as biodestructors.

Materials and methods. To conduct the study used two strains of bacteria of the genus *Pseudomonas* spp., Which have been previously isolated from marine environment and set of morphological, cultural, physiological and biochemical traits identified using classical bacteriological methods and test systems AER 50 START Medium (bioMerieux, France) are assigned to the species *P. fluorescens* ONU-328 and *P. maltophilia* ONU-329. Additionally, by fatty acid composition, whose spectra were obtained on a Agilent 7890 gas chromatograph (Agilent Technologies, USA), and decoded using a library database program RTSBA6 6.21 MIDI Sherlock, Investigated strains with a high index of similarity (Sim Index> 0,72) identified as *P fluorescens* ONU328, *P. maltophilia* ONU329.

Assessment of the KPAR and phenol-destructive capacity of free cells of strains *P. fluorescens* ONU328 and *P. maltophilia* ONU329 was performed according to the degree of water purification from cyclic aromatic xenobiotics (phenol, N-CPB)

$$\alpha = [(C_0\text{-}C) / C_0] \times 100\%,$$

where C_0 and C are the concentrations of a particular xenobiotic before and after treatment.

After the biodegradation process, all samples were analyzed for the residual concentration of N-cetylpyridinium bromide (N-CPB) in them. Determination of the residual concentration of N-CPB in control and test samples was carried out using an extraction-colorimetric method based on the interaction of N-CPB with methylorange to form a chloroform-soluble yellow complex [3]. The intensity of the color of the chloroform extract is proportional to the concentration of the methyl-a-cationic surfactant complex. Chloroform extracts (containing a certain amount of N-CPB) were photographed at $\lambda = 415$ nm with respect to pure chloroform.

The experiments were performed in triplicate. Statistical processing of the research results was carried out using the computer program "Microsoft Office Excel 2007".

Results and discussions. Our studies have shown that strains of *P. fluorescens* ONU328, *P. maltophilia* ONU329 are a promising basis for biotechnology biopreparations: they are non-pathogenic for humans and have a high oxidative potential for phenolic compounds and cationic surfactants of cyclic structure. The choice of these nonpathogenic strains of microorganisms as possible destructors of "biologically rigid" cationic surfactants of cyclic structure was due to the effective result of their oxidative action against high concentrations of highly toxic phenolic compounds, patented in [4].

The results of water purification from cyclic aromatic xenobiotics - N-cetylpyridinium bromide (N-CPB) in the presence of strains of *P. fluorescens* ONU328 and *P. maltophilia* ONU329 are presented in the table. The method was carried out as follows. The microorganisms were cultured on a shaker incubator New Brunswick Scientific Incubator Shaker INNOVA 43R in vials of 100 ml medium at 150 rpm for 24 hours at 30 °C. Sowing of nutrient medium was carried out by daily culture, grown on MPA in stationary conditions (thermostat). The seed volume was 1.0% of the volume of the medium. Non-pathogenic strains of *P. fluorescens* ONUU328, *P. maltophilia* ONU329 were suspended in the M-9 mineral medium and in an amount of $5.5 \times 10\ 4$ cfu / ml were introduced into a storage tank where the water purification process from the cationic surfactant was performed. A day later,

the concentration of N-cetylpyridinium bromide decreased from 20 mg / l to 11.2 ± 0.15 mg / l and 12.8 ± 0.45 mg / l when used as biodestructors of *P. fluorescens* ONU328 and *P. maltophilia* ONU329 respectively (the degree of water purification was 36-44%).

Table

Efficiency of microbiological water purification from a difficult-oxidizing cyclic surfactant of cationic type - N-cetylpyridinium bromide

Strain	Concentration of N-CPB after purification, mg / l	Degree of destruction of N-CPB,%
Exposition - 1 days		
P. fluorescens ONU328	11,2±0,15	44,0
P. maltophilia ONU329	12,8±0,45	36,0
Control	20,0±0,45	0,0
Exposition - 4 days		
P. fluorescens ONU328	9,9±0,20	50,7
P. maltophilia ONU329	10,5±0,15	47,5
Control	20,0±0,45	0,0
Exposition - 6 days		
P. fluorescens ONU328	9,7±0,15	50,6
P. maltophilia ONU329	8,9±0,12	55,7
Control	19,7±0,40	1,5

Note: * concentration of N-CPB in water - 20 mg/l; the concentration of bacterial cells with their one-time introduction into the treated water is 5.5×10^4 cells/ml.

It has been experimentally established that when the free cells of the strain of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 in an amount of 5.5×10^4 CFU/ ml are introduced once into the contaminated water, the purification rate of water from N-cetylpyridinium bromide at a concentration of 20 mg / l reaches 50.7% for the 4th day and 55.7% for the 6th day, respectively (table). If it is necessary to achieve a

higher degree of purification (98.0-98.5%) from N-CPB, fresh portions of microorganisms-destructors can be re-introduced or higher starting doses can be used. The residual concentration of N-CPB in water corresponds to the norm for traditional wastewater treatment plants with activated sludge, that is, the wastewater that has been purified by the microbiological method can be discharged into the municipal sewage system.

Conclusions

It has been experimentally established that the following conditions are optimal for the effective purification of water from phenol, N-cetylpyridinium bromide by microbiological method using the strains of *P. fluorescens* ONU328 or *P. maltophilia* ONU329 as cyclic aromatic xenobiotics destructors: 30 ^{0}C; the culture concentration is (7.5-5.5) × 10^5 CFU/ ml - and the degree of water purification from phenol (with an initial concentration of 300 mg / l) is 100% on the 18-22 day, and the N-CPB is 50.7 -55.7% for single-dose administration of bacterial cultures (from the initial concentration of cationic surfactant 20 mg / l) for 4-6 days. When the fresh portions of microorganism-destructors are re-introduced in the amount of 5.5 × 10^5 CFU/ ml or using their higher starting doses (5.5 × 108 cfu / ml), the water purification rate from N-CPB for a week is 98.0- 98.5%.

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UDC 531/534/: [57+61]

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MICROBIOLOGICAL METHOD OF PURIFICATION OF WATER FROM PHENOL ASSOCIATION OF NON-PATHOGENIC BACTERIA STRAPS OF THE GENUS *PSEUDOMONAS*

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Abstract

A microbiological method for purifying water from phenol has been developed, which consists in using phenol-bacterial association of strains of non-pathogenic bacteria of the genus *Pseudomonas: P. cepacia* ONU-327 and *P.* fluorescens ONU-328, taken in a volume ratio of 1: 1. When using a biopreparation on the basis of the association of the bacterial strains under study, the oxidation of phenol is accelerated 2.2 times as compared to the treatment of phenol-containing water with monocultures. Deep purification of water from phenol with a concentration of 300 mg/l occurs within 10 days with a single injection of bacterial cells in an amount of 7.5×10^5 CFU/ml, pH 7, a temperature of 28-30 °C. It has been established that the bacterial association of strains of *P. cepacia* ONU-327 and *P. fluorescens* ONU-328 is capable not only of effective destruction of phenol, but also possesses a high sorption-accumulating potential for ions of heavy metals [Pb (II), Zn (II) , Cu (II), Cr (V)], which reveals the prospects of its use in the biopreparation for the purification of multicomponent pharmacies, with the predominant content of phenolic contaminants.

Keywords: purification of water, phenol, association of destructors, *P. cepacia* ONU-327, *P. fluorescens* ONU-328

Introduction

Phenol is a toxic environmental pollutant. Its maximum permissible concentration (MPC) in the water of reservoirs used for fishery purposes is small and is 0.001 mg/l [1]. Therefore, the problem of finding an effective method for purifying water from phenol is topical.

To date, the microbiological method of water purification from phenolic and other cyclic aromatic compounds, based on the use of the potential of heterotrophic biochemically active microorganisms, capable of consuming a wide range of organic compounds, including aromatic xenobiotics as a substrate for growth and development, is the most environmentally friendly, reliable , in a non-volatile way in comparison with the existing physicochemical methods; does not create secondary hazardous waste and does not require the use of chemical reagents, additional oxidants.

There is information about the mineralization of phenol to simple compounds during its biodegradation with microorganisms *Aspergillus niger* [2], the Indian strain Staphylococcus aureus isolated from Amla Khadi, Ankleshwar [3], halophilic fungi (*Aspergillus, Pencillium, Fusarium*) isolated from sediments along the Gulf of Suez and sediments of the Red Sea [4].

At achievement of high results on water purification from phenol the main disadvantage of the above-mentioned microbiological methods is the use of microorganisms-destructors possessing pathogenic or conditionally pathogenic properties, and therefore are not recommended for use in biotechnologies for cleaning the environment from phenol.

The aim of the work is to offer an association of non-pathogenic strains of bacteria of the genus *Pseudomonas* with increased biochemical activity against phenol.

Materials and methods

Two biochemically active non-pathogenic strains of microorganisms were used as the objects of the study. By fatty acid composition, whose spectra were obtained on a Agilent 7890 gas chromatograph and decrypted using the library database program RTSBA6 6.21 MIDI Sherlock, Investigated strains with a high index of similarity (Sim Index ≥ 0,72) identified as *Pseudomonas cepacia* (isolated from soils) and *Pseudomonas fluorescens* (isolated from the marine environment). Strains *Pseudomonas* spp. stored in the collection of microorganisms of the Department of Microbiology, Virology and Biotechnology of Odessa I.I. Mechnikov National University: *Pseudomonas ceracia* ONU-327, *Pseudomonas fluorescens* ONU-328.

The concentration of phenol in water samples was determined by a photometric method based on the formation of colored phenol compounds with 4-aminoantipyrine in the presence of potassium hexacyanoferrate (III) at pH 10.0. The experiments were performed in five replicates. The results are processed using MS Excel 2003.

Results and discussions

The results of microbiological purification of water from phenol in the presence of individual strains of microorganisms *P. cepacia* ONU-327, *P. fluorescens* ONU-328 and their associations obtained at 30 °C are shown in fig. 1.

The effectiveness of the microbiological method of water purification from phenol has been experimentally confirmed. In laboratory conditions it has been established that the strains of microorganisms used are resistant to high concentrations of phenol (300 mg/l) and have a phenol-oxidizing ability, especially in association.

The proposed method is that the water containing phenol is purified by bacterial association of *P. cepacia* ONU-327 strains and *P. fluorescens* ONU-328 (1: 1 by volume), the bacteria are cultured for 24 hours at 30 ° C for MPA, then the bacterial cells are suspended in M-9 medium containing up to 300 mg / l of phenol and kept for 10 days (fig. 1). With the introduction of contaminated water into the strain *P. cepacia* ONU-327 in an amount of 7.5×10^5 CFU/ml, the degree of water purification from phenol on day 10 reached ~ 45%. The use of strain *P. fluorescens*

ONU-328 increased the efficiency of water dephenolization up to 78% on the 10th day (fig. 1).

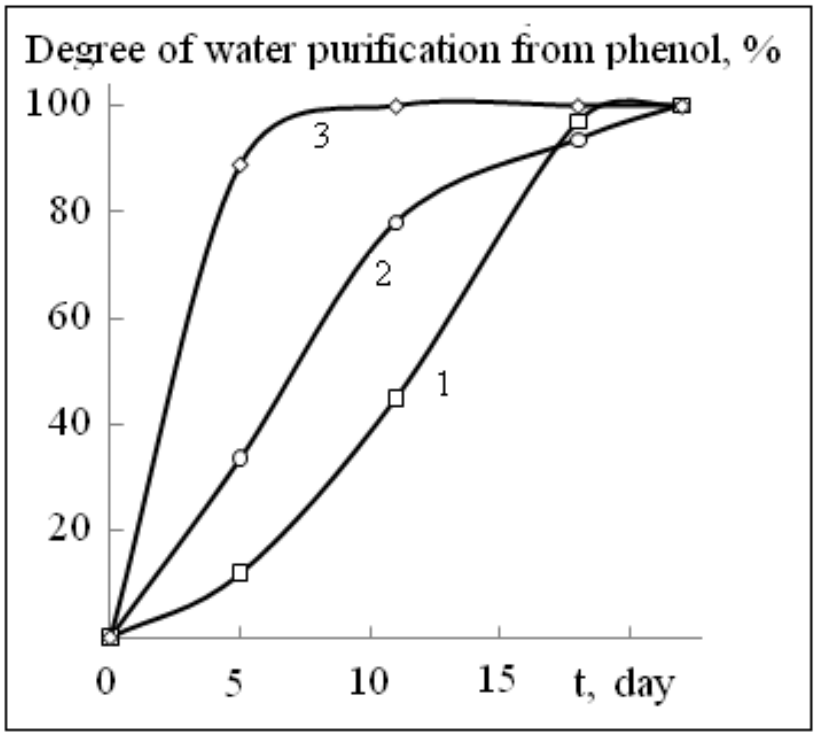

Fig 1. Degree of water purification from phenol (%) for a time (t, day) in the presence of strains of *P. cepacia* ONU-327 (1); *P. fluorescens* ONU-328 (2) and their associations of 1:1 by volume (3). Note: *initial concentration of phenol is 300 mg/l; the concentration of bacterial cells is 7.5 × 105 CFU/ml

It has been experimentally confirmed that the use of bacterial association of strains of *P. cepacia* ONU-327 and *P. fluorescens* ONU-328 (1: 1 by volume) for the same period (10 days) promotes deep purification of water from phenol - by 100%. The proposed method compared with [5, 6] allows to accelerate the process of water dephenolization 2.2 times.

In order to develop recommendations for the use of a method for purifying effluents from the production of pharmaceutical preparations that contain a large amount of phenolic contaminants, as well as heavy metal ions, the biotechnological properties of the strains studied and their associations with respect to inorganic pollutants were additionally tested. It has been established that strains of microorganisms possess a high sorption-accumulating ability with respect to metal

ions in solution in cationic form Pb (II), Cu (II), Zn (II); the synergistic effect on Cr (VI) is observed with the use of immobilized bacterial cells in the composition of biofloquial cells of the association of strains of microorganisms *P. cepacia* ONU-327, *P. fluorescens* ONU-328 (taken in a volume ratio of 1: 1). Thus, the bacterial association of strains *P. cepacia* ONU-327, *P. fluorescens* ONU-328 has a wide spectrum of biotechnological action: high destructive potential for phenolic compounds and sorption-accumulating for ions of heavy metals, which reveals the prospects of using bacterial association of microorganisms in the composition biopreparation for the purification of multi-component effluents, with a predominant content of phenolic contaminants.

Conclusions

Microbiological method for purifying water from phenol has been developed, which consists in using phenol-bacterial association of strains of non-pathogenic bacteria of the genus *Pseudomonas*: *P. cepacia* ONU-327 and *P. fluorescens* ONU-328, taken in a volume ratio of 1: 1. When using a biopreparation on the basis of the association of the bacterial strains under study, the oxidation of phenol is accelerated 2.2 times as compared to the treatment of phenol-containing water with monocultures. Deep purification of water from phenol with a concentration of 300 mg/l occurs within 10 days with a one-time injection of bacterial cells in an amount of 7.5×10^5 CFU/ml, pH 7, a temperature of 28-30 °C. The proposed method is suitable for widespread use in biotechnology of effluent treatment of pharmaceutical preparations containing, in addition to phenolic compounds and ions of heavy metals, due to the high phenol-oxidizing capacity of the bacterial association and its storage-accumulating effect on heavy metal ions.

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UDC 579.222

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THE FORMATION OF NODULES ON THE ROOTS OF SOYBEAN PLANTS UNDER THE ACTION OF A BIOLOGICAL PRODUCT ECOVITAL

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The purpose of the work: was to determine the effectiveness of the formation of tubers on the roots of soybean plants with the help of the drug Ecovital. **Materials and methods.** The study used soy plants Annushka. Inoculated seeds on the day of sowing with a complex microbial preparation Ecovital Bacterial load was 10^7 cells/ seed. Repeat experiment 4-time. Investigation of symbiotic activity under the condition of seed treatment Ecovital. **Results**. Soybean culture studies show that the

use of the drug Ekovital positively affects the formation of bulbous bacteria. The largest number of tuber bacteria formed the phase of pouring seeds - 47.1 pounds per plant.

***Key words*:** nodules, soybean, Ecovital

One of the decisive factors for the development of plants is the level of phosphorus availability. However, due to the low solubility of soil phosphorus compounds, cultivated plants do not receive a sufficient amount of this element [1]. Therefore, the optimization of phosphorus nutrition of agricultural crops plays a significant role in increasing their productivity. The provision of plants with phosphorus has an extremely important value for their growth, development and yield formation [2].

The effectiveness of phosphatability bacteria in crop cultivation technologies is largely determined by their ability to absorb in the root zone of plants. Basically, the survival of bacteria in the root zone of plants depends on their ability to colonize a certain area of the root system and successfully compete with it with other microorganisms [7].

Therefore, the activity and orientation of plant-microbial interactions can only be influenced by the study of bacterial competitiveness when introduced into the root zone of plants, their influence on the functioning of the existing microbial group, and the action of the environment conditions on its activity.

The ontogenesis of plants occurs in their close interaction with microorganisms of the soil, which inhabit the rhizosphere and form associations "microorganisms - the root system of plants" [4]. In the process of life, this system is influenced by various external biotic and abiotic factors, including unfavorable to living organisms. Therefore, increasing the sustainability of ecosystems to stress conditions, strengthening the protective functions of organisms with the help of certain biological methods could significantly reduce the impact or prevent the action of negative factors. Thus, the research of many authors established the multi-lateral action of rhizospheric microorganisms that are part of the bio-inoculants [3-6].

Successful cultivation of any agricultural crop should take into account both the aspects of the economic efficiency of production and the methods of realization of the produced products, as well as the rational use of production means for creating the optimal conditions for the functioning of agrocenoses. Thus, the growing technology should include a complex of successive operations aimed at obtaining high yields taking into account the biological characteristics of the plants during the development phases. Recently, in many countries, despite the wide use of agrochemicals in the cultivation of crops, priority it given to the use of microbial drugs.

The deficiency of vegetable protein, the orientation of agriculture to environmentally appropriate production, as well as the high cost of mineral and organic fertilizers cause increased interest in legumes. Crop data is an inexhaustible source of soil enrichment with nitrogen compounds due to fixation of nitrogen by bottle bacteria in symbiosis with plants, and therefore have an important agricultural value. Their cultivation can reduce the cost of crop production due to the inclusion in the process of agricultural production of atmospheric nitrogen, improve the phytosanitary state of crops and significantly increase the productivity of arable land [2].

Since the crop area in Ukraine grows annually, crops are grown in new places where it has never been cultivated. In these conditions it is necessary to ensure the presence of active strains of tuber bacteria in the soil due to pre-sowing inoculation of soy. Currently, the biological product "Ecovital" is widely used for this purpose. It consists of complementary strains of nitrogen fixing rhizobia (*Rhizobium* or *Bradyrhizobium*) and a strain of phosphate mobbing bacteria *Bacillus megaterium*. The drug causes a positive effect on the soil fertility

Materials and methods

The objects of research were soybean herbs of Annaushka. The seeds were inoculated on the day of sowing with a complex microbial preparation product Ekovital based on tubers (*Rhizobium* or *Bradyrhizobium*) and phosphate mobisation (*Bacillus megaterium*) bacteria. The bacterial load was 10^7 cells/ seed. In the control

variant, the seeds were not treated in any way. In addition to controlling and treating seeds, Ekovital also had a variant of seed treatment with sterile water. The seeds were cultivated in pots with soil for 30 days.

The research was conducted on the basis of the Biotechnological Research and Training Center in the laboratory. Repeat experiment four times. Investigation of symbiotic activity under the condition of seed treatment product Ecovital. In all phases of organogenesis of soybean plants, an increase in the number of tubers in variants with the use of the product Ecovital was noted. Statistical processing of the results was performed using the Microsoft Office Excel 2007 computer program.

Results and their discussion

From the results of the experiment shows that the greatest number of nodules on soybean roots was obtained when using the product Ecovital.

Table.

The number of nodules formed on the roots of soybeans, depending on the seed treatment before planting

Version	Number of tubers, pieces / plant		
	Phase flowering	phase of formation beans	the infusion phase seed
Control	26,8 ± 1,3	34,7 ± 1,7	37,0 ± 1,9
Seed treatment with water	28,5 ± 1,4	37,4 ± 1,9	41,5 ± 2
Processing of seed Ecovital	31,7 ± 1,6	41,9 ± 2,1	47,1 ± 2,3

Also it should be noted that the number of nodules in the treatment of seeds with sterile water was more than in the control. Soybean plants whose seeds were treated with sterile water had more nodules on the roots in comparison with the control: in the flowering phase - by 6%, in the bean formation phase - by 17%, in the seed pouring phase - by 12%. The soybean seeds that was processed by the product

Ecovital had the best results. In comparison with the control of plants that were treated with Ecovital, there were more than just nodules on the roots: in the flowering phase – by 18%, in the phase of bean formation – by 20%, in the phase of seed pouring – by 27%.

Conclusions. A sufficient amount of nutrients during the growth and development of soy plants is a prerequisite for effective nitrogen fixation. The results of studies on soybean culture show that the use of the product Ecovital positively affects the formation of bulbous bacteria. These results show the effectiveness and practical importance of the application of product Ecovital to obtain a greater number of nodules on soybean roots.

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UDC 579.2+579.64

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THE SECRETION OF SIDEROPHORES BY SOME STRAINS OF *PANTOEA AGGLOMERANS*

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Pantoea agglomerans is distributed in nature as a commensal, epiphyte, and endophyte of plants and animals. The strains of *P. agglomerans* are sensitive to polyvalent virulent phages, such as phages of plant pathogen *Erwinia amylovora*.

The *P. agglomerans* strains are the most promising among the biocontrol agents, since they can be antagonists to phytopathogens and they have antibacterial and antifungal activity, they are capable of competition in the colonization of plants.

The identification and study of antibacterial properties of *P. agglomerans* metabolites can become one of the methods for solving urgent agriculture problems. It is known that the strains of *P. agglomerans* produce a large amount of biologically active substances. Among them there are also siderophores.

Siderophores are low-molecular substances that chelate ions that are released by microorganisms and plants with deficiency of iron ions in the environment. Some studies have shown that the production of siderophores by bacteria, that are stimulised the plant growth was the most effective mechanism in phytopathogen control [3]. The relationship between siderophores and virulence of microorganisms is proved, and approaches for their clinical application are being developed[1].

The plant strain *Bacillus megaterium* 484 was used as a control for the presence of siderophores.

To isolate the bacterial siderophores, a daily culture was prepared in 5 ml of LB medium and incubated at 28 ° C for 24 hours with aeration. Chromium azurol S (CAS) 60.5 mg was dissolved in 50 ml of H2O and mixed with 10 ml of solution (1 mM FeCl3 6 H2O in 10 mM HCl). NDTMA solution 72.9 mg was dissolved in 40 ml

of H2O. A solution of chromium azurol was added at constant instillation to a solution of NDTMA. The resulting solution was autoclaved at 0.5 atm for 30 min. The total volume of the solution was 100 ml. To 900 ml of sterile solution was added 900 ml of LB medium at pH 6.8. The resulting non-hot solution was poured into sterile Petri dishes and waited for complete hardening. After that, 5 µl of 24 hours of culture was dripped in the center of a Petri dish and left to dry for 5-10 minutes in a laminar box. Cultivation was carried out at a temperature of 28 ° C for 5 - 7 days [2].

It is shown in the literature that bacteria which can produce siderophores absorb iron ions of the medium and form yellow or orange zones around the colonies. The control dish with the medium had a bright blue color (Fig. 1 (B)). The strain of *B. megaterium* 484 was used as a control, its colonies formed a yellow zone (iron utilization zone) around them, while the other environment had a brightly colored blue color (Fig. 1 (A)). Such a characteristic yellow zone is formed precisely at the expense of secretion with the membrane siderophores, which are inherent *B. megaterium*.

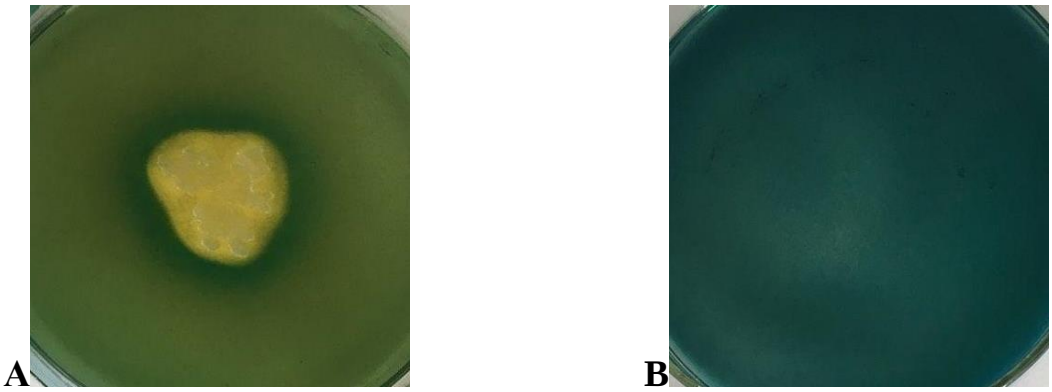

Fig. 1. The production of siderophores: A) the control strain *B. megaterium* 484; B) the control medium with iron ions (Fe^{3+})

The *P. agglomerans* strain №9№7(0)2 was characterized by the smallest zone of iron utilization around the colony in comparison with other strains. The largest zone of iron utilization around the colonies was noted in 3 strains of *P. agglomerans:*

g157(1)2, g157(2)3 and *№2* (fig. 2 (A, B and C). The other studied strains were characterized by the same zones of iron utilization.

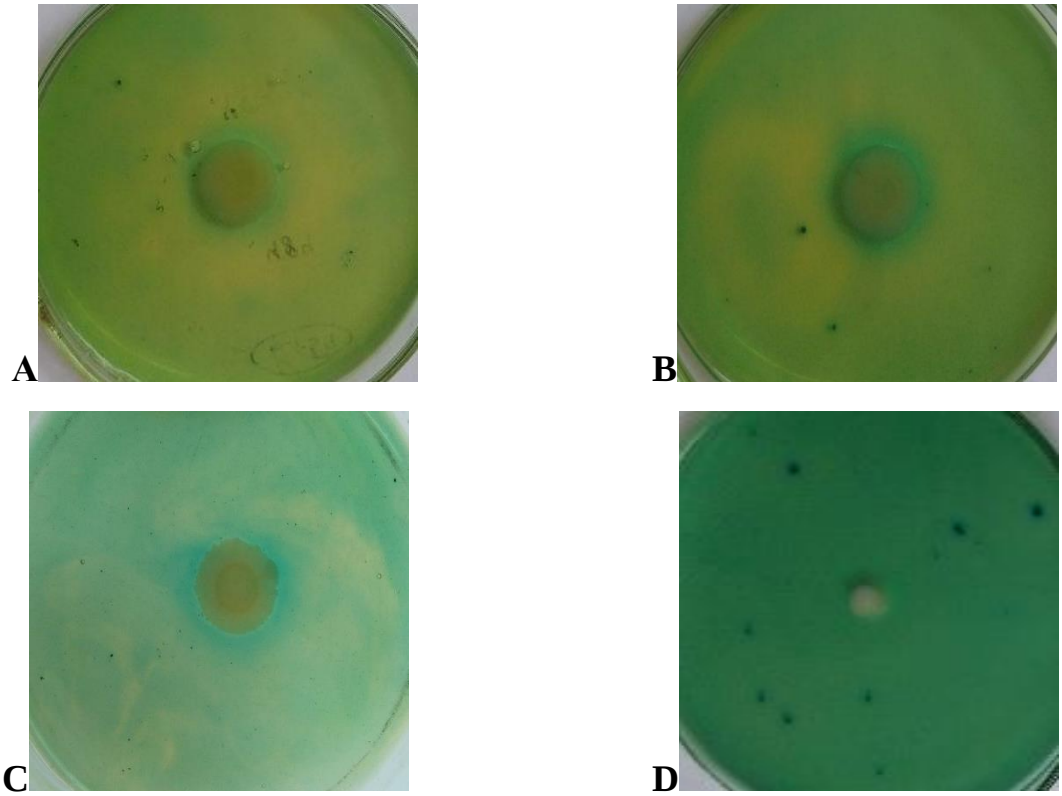

Fig. 2. The production of siderophores by strains of *P. agglomerans:* A) *P. agglomerans* g157(1)2; *B*) *P. agglomerans* g157(2)3; C) *P. agglomerans №2*; D) *P. agglomerans* №9№7(o)2.

The blue aureole was formed around the colonies of *P. agglomerans* and the other selective medium had a yellow color. Probably the siderophores formed by the *P. agglomerans* strains are not bound to the membrane and are secreted into the environment.

Nowadays, there are many studies that have shown positive properties of *P. agglomerans.* These properties also include the ability to produce siderophores. Certain strains isolated from plants positively influenced the germination of wheat seeds and inhibited the growth of fungal microphlora. Based on the work results, it is possible to use *P. agglomerans* in agriculture in order to increase yields.

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UDC 579.2

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MARKER LIPIDS IDENTIFICATION FOR THE DETECTION OF RESISTANT AND ANTIBIOTICSENSITIVE *STREPTOCOCCUS PNEUMONIAE*

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Streptococcus pneumoniae is an important pathogen that causes respiratory-tract infections, sepsis, meningitis and pneumonia. *S. pneumonia* commonly causes diseases in the youngest and oldest sections of the population and patients with immunodeficienciesin both more and less developed countries [1].

Autolysis is one of the virulence factors that is induced by specific enzyme LytA during stationary phase of growth. Autolysis occurs due to drug treatment or nutritional starvation and not shown in logarithmic phase. Autolysis facilitates spread of toxins in infected organism [2].

The aim of this study was to find differences in lipid profiles of drug resistant and non-resistant *Streptococcus pneumoniae* and a possible target for a new antibacterial compound 2A.

The experimental part of the work performed in Chemistry Department of Umeå University in Sweden in Swedish Metabolomics Center. Two strains of *S. pneumoniae* were used in this study: wild type (WT) T4 strain and strain 852. T4 strain is sensitive to the 2A and lyse in presence of 2A Strain 852, to the contrary, is resistant to 2A compound and does not lyse. Compound 2A is suggested as a new antibacterial agent that triggers the autolysis of pathogen Streptococcus pneumoniae. We hypothesized that the target metabolites will be present in the sensitive wild type but absent in the resistant mutant. To directly identify extracted lipids we used ultra-high performance liquid chromatography coupled to triple quadrupole time-of-flight tandem mass spectrometry (UHPLC–Qq-TOF-MS/MS).

The analysis of chromatograms (raw data – Fig. 2) was done in Profinder, Agilent. Two approaches were used: targeted and untargeted data analysis. Targeted analysis is a mode of operation when specific library is used to find certain groups of compounds based mainly on their known mass, retention time and adducts. This mode provides identification and quantification of compounds. The shortcoming of this method is that only compounds that exsist in the library can be found. Contrary, the untargeted type of analysis quantifies basically all metabolites in the sample but the identification of the compound is not provided. Therefore untargeted analysis is followed by identification of the compounds through available databases. The aim of the data analysis was to find the differences in metabolite levels for 4 experimental conditions.

Both targeted and untargeted analyses show the difference between mutant and wild type and the difference between antibiotic and solvent (Fig. 1).

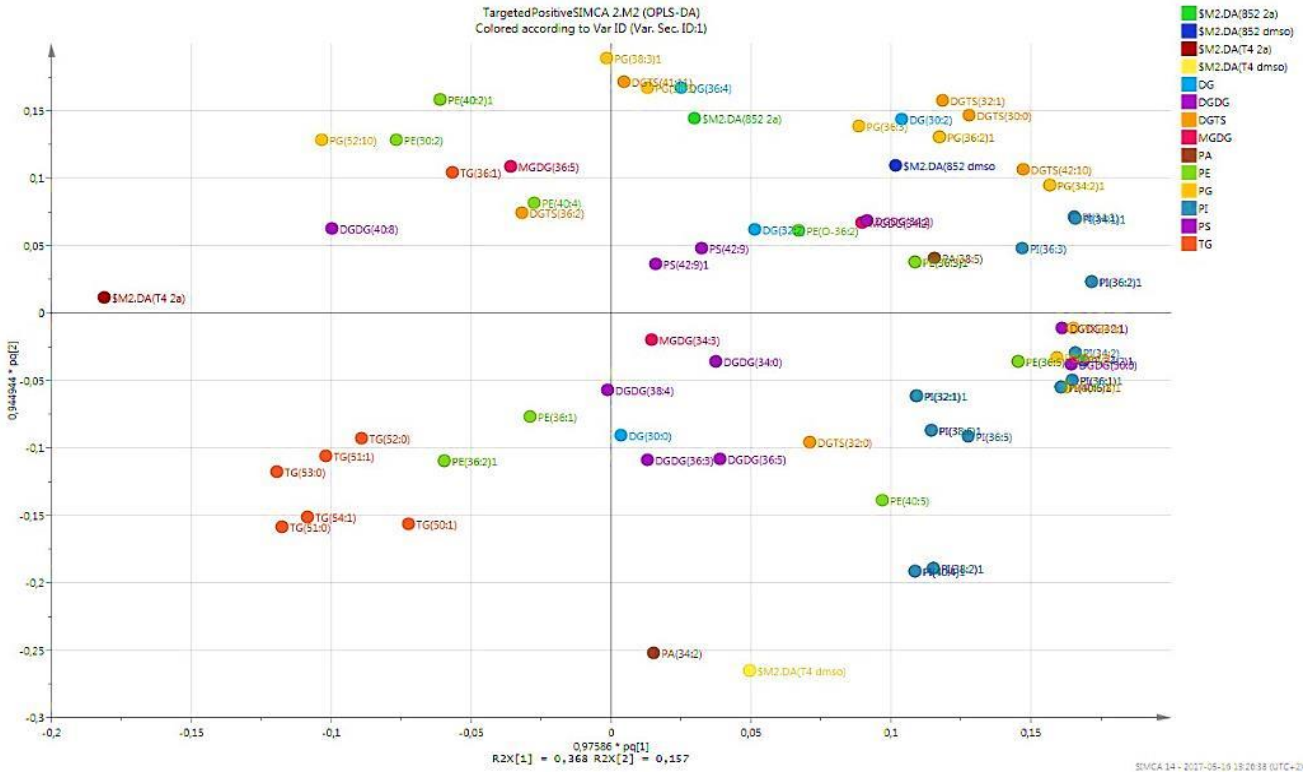

Fig. 1. OPLS-DA Loading plot for Targeted Lipidomics.

Triradylglycerols were increased in wild type. Diacylglyceryl-trimethylhomoserines were increased in mutant strain. Phosphatidylinositols were increased in absence of 2A in both strains. Table 1 shows differences between of presence and absence of significant metabolites in experimental conditions. Importantly, only a small group of metabolites was substantially increased in T4 strain grown in presence of 2A but not in control. These compounds are suggested as potential target for 2a binding.

Table 1

The presence of major groups of metabolites in *S. pneumoniae T4* and *S. pneumoniae 852* in the presence and absence of an antibiotic compound

Metabolites	***S. Pneumoniae 852* in presence of 2A**	***S. Pneumoniae T4***	***S. Pneumoniae T4* in presence of 2A**	***S. Pneumoniae T4***
Diacylglyceryl-trimethylhomoserine	+	+	-	-
Glycerophosphates	-	-	-	+
Triradylglycerols	-	-	+	+
Glycosyldiradylglycerols	+	-	+	-
Glycerophosphoethanolamines	+	+	+	+
Phosphatidylinositol	-	+	-	+
Monogalactosyldiacylglycerol	+	+	+	+

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UDC 552.57:579.6

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STUDY OF THE ABILITY OF THE STRAIN *ACIDITHIOBACILLUS FERROOXIDANS COAL 17* TO COAL DESULFURIZATION

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Volatile sulfur compounds, such as SO_2, H_2S, SO_3, belong to the group of the most toxic substances that are constant and noxious pollutants of the atmosphere. Sulfur dioxide is mainly formed as a product of combustion of fossil fuels containing sulfur. According to the statistics 50 to 70 million tons of sulfur is emitted to the atmosphere in the world each year [4, 5].

Most often coals contain pyritic and organic sulfur. As a result of oxidation these coals may also include elemental and sulfate sulfur. The predominant impurity of coal is inorganic sulfur, which can be removed by physical methods. The remaining organic sulfur can be eliminated by chemical methods, which are very expensive, or by heat processing using the temperature at which sulfur bonds break up. Heat processing also allows the removal of pyritic sulfur [4, 5].

Recently, a new solution for the challenge of the elimination of organic and inorganic sulfur from coal has been presented. It is based on the use of bacteria. The relevant processes run in ambient temperature and under ambient pressure, which makes this approach attractive and profitable. The method is based on the ability of autotrophic bacteria to oxidize the reduced forms of sulfur and to transform them into soluble, easy-to-wash-out compounds. The most frequently used species of such bacteria are *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Acidithiobacillus acidophilus* [4, 5].

Bacterial activity depends on the specific characteristics of applied species of microorganisms, coal type and content, available surface area of the substrate, temperature, pH value, and the quantity of bacteria per unit of coal mass. The time

for biodesulfurization varies from a few months, for coal extracted in dumps, to a few days when the refined form of coal is stirred in the medium ensuring bacterial growth.

The research is aimed to study the ability of pure culture of *Acidithiobacillus ferrooxidans Coal 17* to desulfurization of coal from mine "Chervonogradska" (Chervonograd, Lviv region, Ukraine).

Materials and methods. In our study, we used strain *Acidithiobacillus ferrooxidans Coal 17.* The wide range of pH values (2-5) in which these bacteria are active, their exceptional tolerance to heavy metals, low temperatures they require and the fact they are autotropic make it possible to use them for a large industrial processes. The additional advantage of the proposed approach is the formation of ferric sulfate that is an active oxidant of many sulfide minerals and allows extraction of many metals including rare ones. The indication of biological desulfurization of coal from the mine "Chervonogradska" using the strain *Acidithiobacillus ferrooxidans Coal 17* was the object of the study. The experiments were carried out using the media that were recommended for cultivation of acidophilic bacteria (table 1) [1, 2, 3]. The initial content of sulfur in coal was 2.6-2.8 %%. The results of desulfurization were defined as a significant decrease in the percentage of sulfur content in coal compared to the initial level.

Table1

Composition of nutrient media

Component	**Lethen medium**	**9K medium**	**Modified 9K medium**
$(NH_4)_2SO_4$	0,15	3,0	0,2
KCl	0,0	0,1	0,1
KH_2PO_4	0,1	0,5	0,1
$MgSO_4 \cdot 7H_2O$	0,5	0,5	0,4
$Ca(NO_3)_2 \cdot 4H_2O$	0,01	0,1	0,0
Yeast extract	0,0	0,0	0,2

In addition to these cultural media, $FeSO4\times7H_2O$ was used as an energy source in a concentration of 44.5 g/L (to Lethen and 9K media), 25.0 g/L (to modified 9K medium), and 15.0 g/L (to Lethen medium). Also, the solid/liquid phase ratio (S: L) had been varied from 1: 5 to 1:10. Investigation of the intensity of the bacterial desulfurization process was carried out at pH ≤ 2,0 and at a temperature of 30,0 ± 2,0 °C during 7 days under standard conditions.

Results. The obtained results indicate that the maximum quantity of *Acidithiobacillus ferrooxidans Coal 17* (7.3 ± 0.9 × 105 CFU/ml) was reported when using the Lethen medium which contained 44.5 g/L of ferrous sulfate and with the solid/liquid phase ratio of 1:10 (table 2). Similarly, pretty high levels of *Acidithiobacillus ferrooxidans Coal 17* (6.8 ± 0.5 × 105 CFU/ml, 5.3 ± 0.23 × 105 CFU/ml and 5.3 ± 0.4 × 105 CFU/ml) were registered when using 9K and modified 9K media (test indices Var4, Var5, Var6).

Table 2

Indicators of the desulfurization process effectiveness when using known cultural media

Test indices	Combinations of nutrients, energy sources and phase ratios	Measured indicators	
		CFU/ml	S(%)
Var1	Lethen medium, S: L = 1: 5, $FeSO_4\times7H_2O$ = 15,0 g/dm^3	$1,5 \pm 0,4\times10^5$	1,85
Var2	Lethen medium, S: L = 1: 5, $FeSO_4\times7H_2O$ = 44,5 g/dm^3	$1,9 \pm 0,07\times10^5$	1,9
Var3	Lethen medium, S: L = 1: 10, $FeSO_4\times7H_2O$ = 44,5 g/dm^3	$7,3 \pm 0,9\times10^5$	1,7
Var4	9K medium, S: L = 1: 5, $FeSO_4\times7H_2O$ = 44,5 g/dm^3	$5,3 \pm 0,4\times10^5$	1,99
Var5	9K medium, S: L = 1: 10, $FeSO_4\times7H_2O$ = 44,5 g/dm^3	$5,3 \pm 0,23\times10^5$	1,95
Var6	Modifie 9K medium, S: L = 1: 5, $FeSO_4\times7H_2O$ = 25,0 g/dm^3	$6,8 \pm 0,5\times10^5$	1,4

However, after 7 days of cultivation, the quantity of the microorganisms declined significantly in all variants. For instance, for the variant of the experiment which was showing the maximum figures before (Var3), the decrease in the number was more than 2 orders of magnitude (5,3 ± 0,2 × 103 CFU/ml). For Var4, Var5 and

Var6 a significant decrease was also observed, too (5,0 ± 0,03 × 103 CFU/ml, 4,8 ± 0,03 × 104 CFU/ml and 3,2 ± 0, 1 × 104 CFU/ml, respectively).

According to the results of the study, it can be said that strain *Acidithiobacillus ferrooxidans Coal 17* shows the ability to desulfurization of coal and it can be used to develop the industrial technology of biodesulfurization. It may be that additional steps of coal cleaning would be required after the bioitechnological processing, but the usage of such technology has significant positive economical impact to the overall process of coal treatment.

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UDC 582.282.23:547.992:549.28

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SCREENING OF PIGMENT-SYNTHESIZING YEASTS – BIOINDICATORS OF HEAVY METALS

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Influence of heavy metals on the intensity of pigments accumulation by yeasts cells has been investigated. Screening of yeasts - bioindicators of metal ions (in particular, $Cr_2O_7^{2-}$, Cd^{2+}, Ag^{+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) was carried out. For water quality bioindication the most informative, as indicator microorganisms, are *Rhodotorula rubra* RA-10, *Rh. aurantiaca* Y-1193, *Rh. aurantiaca* Y-1195 and *Rh. glutinis* Y-1335 yeasts, which have the highest concentration range between loss of pigment and growth inhibition (from 25 to 90 %).

Key words: pigments, yeasts, bioindication, heavy metals.

Introduction

Recent bioindicative studies are associated with the use of decomposers-prokaryotes as bioindicators, namely, pigment-synthesizing bactria [1]. We were first who screened for pigment-synthesizing yeast-bioindicators of heavy metal ions I and II classes of danger.

Materials and methods of research

Collectible 18-days yeasts culture *Rhodotorula* was seeded by solid nutrient medium on Sabouraud set congeal, which was prepared on the base of the water with certain heavy metal (HM) salt concentrations.

The studies used salts of HM, such as: $CuCl_2 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, $Cu(NO_3)_2$, $ZnCl_2$, $ZnSO_4 \cdot 7H_2O$, $Zn(CH_3COO)_2 \cdot 2H_2O$, $CdCl_2$, $Ni(NO_3)_2 \cdot 6H_2O$, $NiSO_4 \cdot 7H_2O$ (used in the experiment with *Rh. rubra* RA-10), $K_2Cr_2O_7$, $AgNO_3$.

Yeasts incubated in the thermostat under the temperature 28°C, results were fixed by visual observation on 3rd day and comparison of the experimental samples with the control was carried out.

For the calculation of the color intensity difference between experimental and control samples (dE), the Petri dishes with yeasts colonies were photographed, photos were loaded in the program Adobe Photoshop, indexes of the color model channels (Lab), and then the difference of the pigment color intensity was calculated in the program CIEDE 2000 (in standard units) [Pat. 49812 Ukraine]. We have also developed a method for identifying pigment-synthesizing microorganisms with using the above-mentioned program package [Pat. 117350 Ukraine].

To determine the concentration of carotenoids in biomass of yeast *Rh. mucilaginosa* Y-1394 was used the well-known spectrophotometric method (without prior separation) [2].

Results and their discussion

Found that the yeasts *Rhodotorula* lost the ability to accumulate pigments from certain concentrations of the HM ions, and between the loss of pigments and growth blocking, a certain concentration interval was observed, which significantly changed for each metal and yeast strain. To confirm the effectiveness of the widely used scale for the visual assessment of pigment accumulation of microorganisms [1, 3] the research was conducted on the spectrophotometric determination of the quantitative content of carotenoids in yeast *Rh. mucilaginosa* Y-1394, which synthesize several types of pigments (table 1).

Comparing the visual assessment of the yeasts pigment accumulation and the concentration of carotenoids was found to have a strong correlation (p) = 1,0. Dependence of signs is statistically significant ($p<0,05$). At the concentration of $Cr_2O_7^{2-}$10,4 mg/dm^3 (dE = 13,7 ± 0,04 conventional units), the continuous growth of moderately pigmented colonies (++) *Rh. mucilaginosa* Y-1394 was observed. The concentration of carotenoids (β-carotene, torolulin and torularodine) was 2-fold lower compared to control. Under the concentration $Cr_2O_7^{2-}$ 10,4 mg/dm^3 (dE=13,7±0,04 conventional units) the continuous growth of moderately pigmented colonies (++)

Rh. mucilaginosa Y-1394 was noted. The concentration of carotenoids (β-carotene, torolulin and torularodine) was 2-fold lower compared to control.

Under the concentration of $Cr_2O_7^{2-}$ 20,7 mg/dm^3 (dE=24,3±0,23 conventional units) there was a continuous growth of non-pigmented colonies of *Rh. mucilaginosa* Y-1394 on the 3rd day (the concentration of β-carotene was 10,3 times lower than in the control), which on the 9th completely restored the ability to pigment accumulation. However, $Cr_2O_7^{2-}$ ions showed the most toxic effect on the intensity of yeasts pigmentation of *Rh. glutinis* Y-1333. Culture *Rh. rubra* RA-10 on the contrary, it turned out to be 5 times more stable than dichromate.

Table 1

Productivity of carotin-containing yeast *Rh. mucilaginosa* Y-1394 for the action of $Cr_2O_7^{2-}$

Ions concentration $Cr_2O_7^{2-}$ mg/dm^3	Visual assessment of yeasts pigment accumulation	β- yeast, mg/cm^3	Torolulin, mg/cm^3	Torularodine, mg/cm^3
Control (without metal)	P * ++++ (100 %)	0,195±0,001	0,079±0,0013	0,311±0,0052
5,2	+++ (75 %)	0,144±0,0023	0,058±0,002	0,252±0,0018
10,4	++ (50 %)	0,098±0,0011	0,0385±0,0006	0,149±0,0031
15,5	+ (25 %)	0,045±0,003	0,0191±0,0012	0,075±0,002
20,7	- (0 %)	0,019±0,00041	0,0097±0,000	0,028±0,0004

Remarks: * pigment genesis: ++++ – intensive, +++ – good, ++ – moderate, + – weak, - – absent.

Cultures *Rh. rubra* RA-10 and *Rh. mucilaginosa* Y-1394 were relatively resistant to nickel ions. On yeasts *Rh. aurantiaca* Y-1193 nikel ions caused 2,5 times more toxic effect than on *Rh. mucilaginosa* Y-1394.

As for the action of Cadmium ions, yeast *Rh. aurantiaca* Y-1195, *Rh. glutinis* Y-1333, *Rh. glutinis* Y-1335 and *Rh. mucilaginosa* Y-1394 lost the ability to form carotene at concentrations of Cd^{2+} 200; 200; 300 and 900 mg/dm^3, respectively.

Concentration intervals (CI) between the loss of pigment and growth retardation for the actions of the Argentum ions were noted for yeast *Rh. rubra* RA-10, *Rh. aurantiaca* Y-1193 and *Rh. aurantiaca* Y-1195 and were 10; 40 та 30 %, respectively.

Between of the studied metal anions, the most toxic effect on yeast cells caused $Cu(NO_3)_2$. Growth of the *Rh. aurantiaca* Y-1193 and *Rh. glutinis* Y-1335 was blocked under the concentration of Cu^{2+} 200 and 300 mg/dm^3, respectively (dE=19,7±0,03 and 20,0±0,56 conventional units). The yeasts *Rh. glutinis* Y-1335 were much more stable with respect to all anions of Zinc and Copper than *Rh. aurantiaca* Y-1193. Perhaps this is due to the fact that the yeasts *Rh. glutinis* synthesize a wider range of carotenoids compared to *Rh. aurantiaca*, which perform a protective function in the cell. Thus, the visual observation of the change in the brightness of the pigments due to the effects of various concentrations of the ions of the HM is a reliable and informative express method of indicating contaminants.

Conclusions. Thus, yeasts *Rh. rubra* RA-10, *Rh. aurantiaca* Y-1193, *Rh. aurantiaca* Y-1195 and *Rh. glutinis* Y-1335 should be recommended for bioindication research under conditions of water pollution with metal salts (Al, Cr, Ni, Cu, Zn, Ag, Cd), as they have almost every compound CI between the loss of pigments and growth retardation (from 25 to 90 %).

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UDC 579.64+579.69

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COMPLEX INVESTIGATIONS OF PLANT MICROBIOTA REPRESENTATIVES

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Summary. Phytopathogenic bacteria - crown gall agents - were isolated from grape plants. Antagonistic bacteria - also plant dwellers - were used to struggle these pathogens. *Lactobacillus plantarum* effectively inhibited crown gall agents and survived in soil at least for 45 days. Lactobacilli could interfere pathogenesis of the disease on a step of pathogen attachment. Together with *Bacillus megaterium*, lactobacilli showed stimulation effect on plant growth.

Key words: *Lactobacillus plantarum, Agrobacterium tumefaciens*, antagonism, survival

Complex investigations of microbiota of plants are carried out in Department of Microbiology, Virology and Biotechnology of Odessa National I.I. Mechnikov University. On the first stage of our research we isolate microorganisms from their natural environment - from plant vessels or plant surfaces. Our latest investigations included isolation of bacteria - representatives of endophytic microbiota of grape. We found out that the amount of microorganisms inhabiting plant vessels reached from $(1,5\pm0,2)x10^2$ to $(6,1\pm0,7)x10^4$ CFU/g.

Bacteria formed colonies of various morphology on nutrient media - round, slimy, with smooth edge, colorless or pinkish or white. 15,4 % of microorganisms from grape vessels were represented by yeasts, 38,5 % - by gram-positive bacteria and 46,1 % - by gram-negative bacteria. Using polymerase chain reaction assay, we found out the presence of pathogenic *Agrobacterium vitis* - crown gall agent - in 7,4 % of the tested grape plants cultivated on the south of Ukraine.

Next stage of our investigations included the application of strong bacterial antagonists in protection of plants against pathogenic agrobacteria. First, we used *Lactobacillus plantarum* strains because of their ability to inhibit a wide range of microorganisms [1, 2, 3].

We inoculated soil with lactobacilli to find out if they can survive in soil and still possess their antagonistic abilities. If a primary concentration of *L. plantarum* suspension 1,2 x 10^9 - 1,6 x 10^9 CFU/g was added to soil, lactobacilli survived at least 45 days in amount of 18±1 - 37±5 CFU/g in soil without plants and 37±2 - 720±20 CFU/g in soil of plant rhizosphere.

Lactobacilli isolated from soil after their adding there, had the same antagonistic activities as the museum strains. Thus, they inhibited 94 % of plant microbiota representatives, 57 % - gram-negative, 37 % - gram-positive. Only 6 % of microorganisms isolated from plants were resistant to antagonistic action of lactobacilli.

All studied museum strains of *L. plantarum* inhibited tumour formation in 93,3 - 96,7% of carrot explants. Using strain of *Agrobacterium tumefaciens* possessing GFP plasmid, we could find out that lactobacilli interfered crown gall pathogenesis on a step of attachment.

When test plants were treated with lactobacilli, less population of pathogen survived on plant surface. Moreover, agrobacteria could not penetrate the vessels of plants.

Lactobacilli also showed stimulating activity increasing plants growth in hydroponics and in soil. They could improve germination of treated seeds in 5 - 25%.

It was possible to create a consortium containing *L. plantarum* and *Bacillus megaterium*, which formed biofilms with well developed matrix on wheat seeds.

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UDC P57; L612,8; P615

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REDISTRIBUTION OF CELL ADHESIVE PROTEINS IN THE BRAIN OF RATS UNDER CONDITION OF DRUG-INDUCED HEPATOTOXICITY

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Abstract

Tuberculosis remains a global major health problem, especially in the developing countries. Tuberculosis is cause of death among curable infectious diseases; it is an airborne infectious disease that killed millions of people worldwide. Among antituberculosis drugs, rifampicin (rifampin) is the most common antibiotic,

however it has well-known hepatotoxicity that can affect the brain function. The given work covers the investigation of the effect of rifampicin and isoniazid induced hepatitis on adhesive proteins in various parts of the brain (hippocampus, cerebellum, thalamus, and the cortex) in experimental model with rats. Neural cell adhesion molecule (NCAM) plays key role in these processes, taking part in the regulation of the migration and differentiation as well as in the mechanism of regulating the membrane potential, which determines the excitability of these cells, and interacting with the factors to enhance the receptors of the growth factors. The obtained results demonstrate that the level of membrane NCAM was increased just in the thalamus of rats with drug induced hepatotoxicity. Significant elevation of membrane NCAM in thalamus may be caused changing of cell-cell adhesion in this brain region to prevent synaptic value under endotoxication.

Introduction. Antitubercular drug-induced hepatotoxicity is the most challenging clinical problems [1]. Rifampicin is a common antibiotic that is used during anti-tuberculosis therapies and has well-known hepatotoxicity. Although this treatment regimen has been highly effective, treatment-related adverse effects including hepatotoxicity [3, 4], skin reactions, gastrointestinal and neurological disorders account for significant morbidity leading to reduced effectiveness of therapy [2, 3]. Microecological violations of various organs and body systems are triggers for development of metabolic diseases and related pathological processes that is poorly understood [5]. The significant pathological changes in the liver can provoke the noticeable changes in various organs, including various parts of the brain. Furthermore, over the last decade, a lot of information has been gathered on the antibiotics impact to the CNS. However, regarding the influence of antibiotics, particularly rifampicin, on different areas of the brain under hepatotoxicity there is still not a clear.

Therefore, the aim of this work was to determine the influence of antibiotics rifampicin and isoniazid on adhesive proteins in various parts of the brain under conditions of hepatotoxicity in an experimental model with rats.

Materials and methods. Twelve female Wistar rats at the age of 1 month (150-155 g) were used in the present study. The care and use of animals was conducted in accordance with the principles outlined in the current Guide of the Care and Use of Experimental Animals in accordance with the ethical standards and was approved by Local Ethics Review Committee on Animal Experiments in Dnipropetrovsk Medical Academy (Dnipro, Ukraine). The rats were divided into two groups (n=6): the control group (I), the experimental group (II). To develop the model of drug induced hepatitis, we used rifampicin in the dose of 86 mg/kg and isoniazid 50 mg/kg intragastrally three times a week for 28 days [6]. The animals of the control group received a similar volume of solvent and distilled water. The animals were decapitated at the end of experiment under anesthesia. The parts of the brain (cerebellum, visual cortex, hippocampus and thalamus) were isolated and used for extraction of membrane proteins by differential ultracentrifugation.

The neural cell adhesion molecules concentration in the different brain areas was determined with ELISA using monospecific polyclonal antibodies against the NCAM (Abcam, UK). Results were measured with reader Anthos 2010 (Finland) at 492 nm. The specific protein level was expressed as micrograms of protein per 100 mg of tissue. The total protein content was estimated according to Bradford microassay [7]. Statistical analysis was performed with one-way analysis of variance (ANOVA). Values with $P< 0.05$ were considered statistically significant.

Results and discussion. The development of drug-induced hepatotoxicity leads to significant decrease of the level of the total protein in membrane fraction obtained from hippocampus (Fig.1).

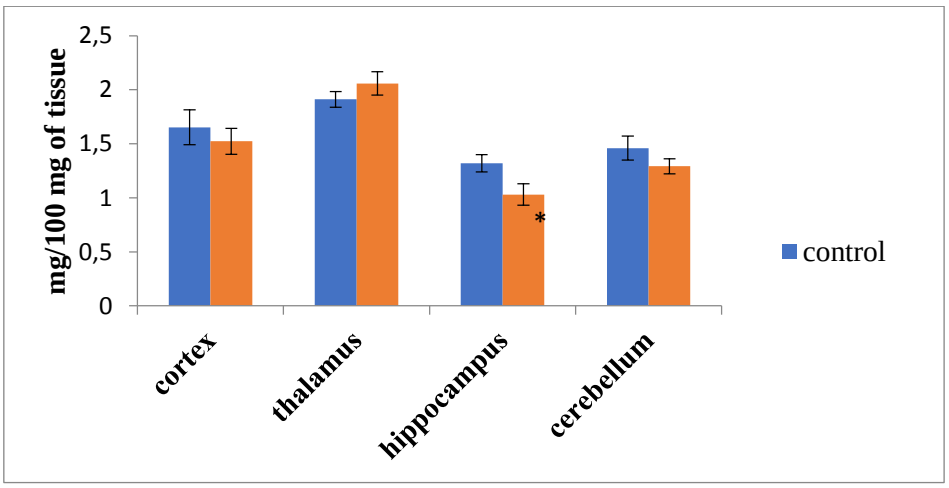

Fig. 1. The content of the total membrane proteins in the rat brain.

*** – significant changes with respect to the control, P<0.05.**

The level of total proteins of the membrane fraction from hippocampus of control animals was 1,32±0,08 mg/100 mg of tissue and it was reduced to 1,03±0,1 mg/100 mg of tissue under drug induced hepatotoxicity (P<0,05). In the control cerebellum it was 1,46±0,1 mg/100 mg of tissue and decreased to 1,29±0,07 mg/100 mg of tissue after antibiotics treatment.

Drug induced hepatotoxicity lead to increase of the membrane NCAM content just in the thalamus relatively to the control group up to 131 % (Fig. 2).

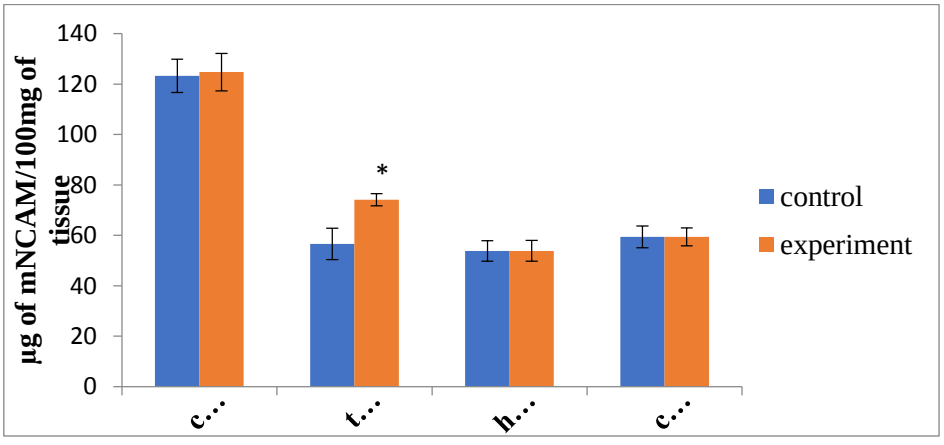

Fig. 2. The content of the membrane form of the neuronal cell adhesion molecule in the rat brain. * - significant changes with respect to the control, P<0.05.

Any changes of NCAM level in the other studied brain areas was noted.

Development and the maintenance of the morphology of the neurons are the immense importance for the normal development and function of the brain. NCAM plays key role in these processes, taking part in the regulation of the migration and differentiation, takes part in the mechanism of regulating the membrane potential, which determines the excitability of these cells, interacts with the factors to enhance the receptors of the growth factors.

Obtained data allow us to conclude that thalamus is the part of the brain responsible for sensory sensitivity is sensitive to the drug induced hepatotoxicity according used protocol. Significant redistribution of membrane form of NCAM in thalamus may be caused changing of cell-cell adhesion in this brain region to prevent synaptic value. In our previous study the same tendency was noted for rats with experimental chronic hepatitis. Probably it is compensatory mechanism for providing of synaptic plasticity.

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UDC 636.087.3:579.8-026.81

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EFFECT OF BACTERIA *BACILLUS MEGATERIUM* OHU 500 ON THE GROWTH AND DEVELOPMENT OF PLANTS

The ability of the *Bacillus megaterium* strain ONU 500 is shown, which synthesizes phytohormones that stimulate the growth and development of plants. In experiments in vitro, the research strain increases the germination of tomato seeds by 10-16% and of cucumbers by 7-20%. In in vitro experiments, treatment with a 3% and 5% suspension of the *B. megaterium* ONU 500 strain leads to an increase in the weight of the aboveground and underground part of tomato sprouts by 98% and 125%, and 78% and 204%, respectively. When determining the effect of *B. megaterium* ONU 500 in tomato sprouts, growth characteristics are observed.

Key words: Bacillus megaterium ONU 500, growth stimulating properties, biofilm, antagonist.

Among the most widely studied and introduced to use in biomethod are biological agents, which are based on bacteria of the genus *Bacillus*. They actively colonize the root system of plants and, producing phytohormones, act as antagonists

of a wide range of harmful phytopathogens. Such physiological signs as: the ability to form spores, a multilayered cell wall, the secretion of peptide antibiotics and extracellular enzymes-contribute to the ubiquitous spread, survival and fertility of crops. PGPR-strains of bacteria stimulate the growth and development of plants not only through the formation of biologically active substances, but also due to the ability to nitrogen fixation, improve hydrogen and mineral nutrition of plants, prevent or reduce the growth of phytopathogens, thanks to the ability to synthesize substances of bactericidal and fungicidal action [1, 3].

Materials and methods

To study the effect of *B. megaterium ONU 500* strain on some growth characteristics of plants, tomato seeds of Zlatovlaska variety and Racibor cucumbers were used. In the first variant of the experiment, the surface of the seeds was sterilized by submerging for 2 minutes. in a 25% hydrogen peroxide solution and 70% ethanol and washed in three sterile waters, the sterilized portion of the seeds was spread on the surface of the LB agar medium to test the sterilization efficiency. In the second - the seeds were not sterilized. To establish the effect of the research strain on plant formation, the sterilized seeds were soaked in a certain concentration (1%, 2%, 3%, 4%, 5%) of the *B. megaterium ONU 500* night culture suspension for 5 hours, Controls - for 5 hours in sterile water. The wetted seeds were transferred to sterile moist chambers and allowed to germinate at room temperature (about 25 ° C). The studies were conducted in three replicates: tomatoes (50 pieces) and cucumber (20 pieces, due to the large seed size) with three independent experiments. The results were taken into account on days 3, 7 and 12. Germination energy was determined after 3 days, germination - after 7 days, and on day 12 - weighing of aboveground and underground parts of sprouts to determine the effect of strain *B. megaterium ONU 500* on the formation of germs [2].

When determining the effect of strain *B. megaterium ONU 500* in tomato sprouts in vivo, non-sterile tomato seeds of Zlatovylaska variety (50 pieces Repeat) were used, which was soaked for 5 hours. in different concentrations (1%, 2%, 3%,

4%, 5%) of the working solution of the night culture *B. megaterium ONU 500* - experience and in sterile water - control. Then they were planted in pots with soil and put on germination at room temperature (about 25 ° C). The effect of *B.megaterium ONU 500* strain was observed for 3 weeks.

When determining the effect of *B. megaterium ONU 500* in vegetative tomato plants (*Lycopersicon esculentum Mill.*) Bacteria were cultured for 24 hours at 28 ° C in LB medium to a concentration of 108 cfu / ml. The daily culture was plated in LB liquid medium and cultured for 18 hours at 28 ° C (180 rpm on the Innova 43R NewBrunswick shaker incubator) until the culture reached a stationary growth phase. Bacteria were separated by centrifugation at 9000 g for 15 min. The next stage of the experiment was the soaking of non-sterile tomato seeds of "Goldilocks" variety in 2% aqueous suspension of *B. megaterium ONU 500* night culture and in sterile tap water for 5 hours. In each experimental group there were 150 pcs. seeds that were sown in the soil and grown to the stage of three real leaves. After that, each group of the previous treatment was divided into three subgroups and transplanted seedlings into pots with radical watering of 80 ml. The plants were treated with a 2% daily suspension of *B. megaterium ONU 500* bacteria. As a control, the soaked seeds were used in tap water and watered plants. Each experimental variant was performed in triplicate, in each repetition, 10 plants were planted. The action of *B. megaterium ONU 500* in vegetative plants was observed for 4 weeks [5].

Results and its discussion

B. megaterium ONU 500, according to previous studies, is characterized by significant antagonistic activity against the phytopathogenic bacteria of the genera *Erwinia, Ralstonia i Rhizobium*. Therefore, the creation of drugs based on this strain of bacilli or their metabolites is a promising approach to the treatment of bacterial, fungal and mixed infections [4].

When determining the effect of *B. megaterium ONU 500* strain on the germination capacity of in vitro vegetables, non-sterile seeds of tomatoes and cucumbers were used, which was soaked in various concentrations of the *B. megaterium ONU 500* suspension at 5:00. The choice of precisely the non-sterile

seeds for this experiment is explained by the simulation of the real process of seed preparation prior to disembarkation in agriculture. However, the autochthonous microbiota that is present on the seed surface can affect the interaction of the research strain and the plant, germinate. Therefore, after selecting the optimal concentration, the effect of the research strain on sterile vegetable seeds was also tested.

When studying the effect of different concentrations of *B. megaterium ONU 500* suspension in non-sterile seeds of tomatoes and cucumbers, the maximum stimulating activity was established for a 5% suspension in both vegetable crops. Compared to the control (seeds soaked in water), the stimulating activity of a 5% suspension of *B. megaterium ONU 500* strain was 16% for tomato seeds and 20% for cucumber seeds.

When checking the action of a 5% suspension of *B. megaterium ONU 500* strain on germination of sterile and non-sterile tomato seeds, no differences in germination were found. The similarity of sterile seeds was 99%, and that of non-sterile seeds - 96%. At the same time, the germination of sterile tomato seeds in water treatment was at the same level (97%) with this index for a 5% suspension of the *B. megaterium ONU 500* strain and exceeded the similarity index of non-sterile seeds when treated with water by 17%.

When determining the effect of strain *B. megaterium ONU 500* on the growth characteristics of vegetable cultures in vitro, the more active effect of the test strain was observed on tomato sprouts. So for them, the weight of the underground part increased from 58% to 204% with respect to control, and the upper one - from 18% to 98% for different concentrations of the *B. megaterium ONU 500* suspension. For tomato seeds, the best result was observed when processing 3% and 5% bacterial culture *B. megaterium ONU 500* Thus, in comparison with water, the weight increase of the aboveground and underground parts of the shoots when treated with a 3% suspension was 98% and 125%, respectively, and when treated with 5% suspension, 78% and 204%.

In cucumber, 2% -4% treatment of *B. megaterium ONU 500* seeds resulted in a reduction in the weight of the aboveground and underground part of the shoots from

13% to 25%. When processing seeds 1% and 5% with a suspension of *B. megaterium ONU 500*, the weight of both parts of the shoots increased. The maximum value of the stimulating action was established for a 5% suspension of antagonistic bacillus strain - 30% for the aerial part and 44% for the underground part of the shoots. A 1% suspension of the research strain had a slight stimulating effect on the aerial part (4% - the difference is not reliable relative to the control) and the underground part (22%).

When determining the effect of strain *B. megaterium ONU 500* on the growth characteristics of tomatoes in vivo, when determining the optimal concentration of bacterial solution, the best results were established for tomatoes from seeds soaked in 2% and 3% aqueous suspension of *B.megaterium ONU 500*. In these Plants were observed above the aerial part, better germination of seeds, larger leaves that did not have manifestations of chlorosis. The worst results show tomato seeds soaked in H2O and 1% suspension of bacilli: the sprouts were weak and lagged behind in development.

It is interesting that, in contrast to the in vitro experiments, 4% and 5% of the *B. megaterium ONU 500* suspension in vivo had no pronounced stimulating effect, but rather inhibited the development of germs. Such a phenomenon can be explained by the fact that in an in vitro experiment the bacteria of the *B. megaterium ONU 500* strain can only be rozmozhnyuyutsyutsya due to those substances allocated Roline. In turn, they synthesize phytohormones in certain concentrations that stimulate the growth and development of plants. In experiments in vivo, the bacilli receive nutrients from the plant and from the soil, which is one of the best environments for their development. Thus, the number of cells of a research strain may increase or a more intensive synthesis of phytohormones in a growth-friendly medium may occur. And increasing the concentration of phytohormones leads, on the contrary, to the inhibition of plants.

When determining the effect of a complex preparation based on *B. megaterium ONU 500* strain on vegetative tomato plants (*L. esculentum Mill*.), It was found that an increase in weight with respect to control was observed for all research options. Inoculation of the seeds and subcutaneous replenishment with a *B. megaterium ONU*

500 strain gave the greatest increase in the total plant mass by 280% compared to the control variant. The weight of the control variant was 11.82 g. In the experimental seed samples, soaked in water, and the roots were processed by the method of root feeding of a suspension of *B. megaterium ONU 500* bacteria, the growth of the plant mass was 163% if the seeds were soaked in a suspension of *B. megaterium ONU 500*, and the roots were watered with sterile tap water, an increase in mass was observed at 170%. The maximum values by 280% increase in weight were demonstrated by treatment of seeds and roots with *B. megaterium ONU 500* strain, which indicates a significant increase in stimulating effect.

So, it can be concluded that the strain *B. megaterium ONU 500* is characterized by growth stimulating activity with respect to selected vegetable crops. The interaction of the autochthonous microbiota and the antagonistic strain *B. megaterium ONU 500* does not lead to a decrease or increase in the germination of seeds. At the same time, an increase in the germinating capacity during sterilization of seeds can be associated with a change in the permeability of the cell wall after treatment with hydrogen peroxide and alcohol, and a faster water penetration into the seeds. The results obtained are promising, as an increase in the growth and weight of plants leads to the strengthening of plant immunity, which will make it possible to use this strain as a biopreparation.

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UDC 577:004.94

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INTERACTION 2’-5’ OLIGORIBOADENILATES TO DNA-METHYLTRANSPHERASE ECORI *IN VITRO* AND *IN SILICO*

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Abstract. DNA methylation is a key epigenetic mechanism that controls not only the expression of genes, but also a large number of processes at the molecular, cellular, and organism levels. The objectives of this work were, Conducting a molecular modeling of the interaction of oligonucleotides with DNA-methyltransferase EcoRI and Investigating the interaction of oligonucleotides with a model DNA-methyl transferase in vitro. It is shown that the most effective binding of oligonucleotides to methyltransferase occurs with 3 and 5 monomeric aptamers. Modeling methods are supposed to be low toxicity and influence on a number of functions of an organism.

Keywords: methyltransferase, oligonucleotides, EcoRI.

Introduction

Disturbance in the balance of methylation /demethylation of DNA, which in turn serves as a key event in epigenetic deregulation in carcinogenesis, was studied. At the moment, a number of inhibitors of DNA-methyltransferases are known, but their mutagenic and toxic effects are a significant disadvantage of these compounds. Natural and synthetic oligoadenylates, which can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction with regulatory domains, can be used as safe analogues [1]. We studied the interaction of oligoadenylates with methyltransferase EcoRI by molecular typing, the effect of oligoadenylates on the model recombinant EcoRI, and the effects of certain oligoadenylates on the expression of genes were modeled and their toxicity was predicted.

Materials and methods

By way of computer software, the interaction of different oligoadenylates and their analogs with the methyltransferase of EsoRI in a rigid conformation was investigated. For the study, we used 2'-5 'and 3'-5' oligoadenylates in length from 1 to 10 monomers, both ribonucleotides and deoxyribonucleotides. For this purpose, protonation of the basic and deprotonation of acid radicals in the methyltransferase EcoRI and oligoribonucleotides was carried out. Docking simulations were run at AutoDock Vina. All calculations were carried out on flexible ligands and a modeled fixed-structure DNA-methyltransferase. The studies in vitro were performed on the native DNA phage λ c1857s7 by the method of the methyl sensitivity restriction method [2].

For the prediction of the spectra of individual oligoadenylates, the effect of gene expression was carried out in the DIGEP Way2Drug program with the subsequent cluster analysis of the results obtained in the String service. Also, their hypothetical dose of LD50 was provided by various methods of introduction with the help of the GUSAR program [3].

Results and discussion

However, a significant disadvantage of these compounds is their probable mutational and toxic effects. At present, a number of relatively safe competitive and noncompetitive C5 DNA-methyltransferase oligonucleotide inhibitors are known [4].

Therefore, we have focused on studying the effects of oligonucleotides, namely, 2'-5 'oligoadenylates on the activity of DNA-methyltransferases. These compounds have a broad spectrum of biological activity, including immunomodulatory, antiviral, antitumor, capable of binding and regulating the activity of signaling cascade proteins by changing the conformation and modulation of activity. The ability of oligoadenylates to regulate apoptosis and the proliferation process is of interest in the study of the effect of 2'-5 'oligonucleotides on DNA methylation processes [5].

The highest levels of affinity for methyltransferase were characterized by 3 and 5 member oligoadenylates, which is believed to be due to incomplete interaction specificity, high conformational mobility, and interaction due to the displacement of the p-electron density. In favor of our assumptions is a robot [6].

The binding energies for 3 and 5 monomeric oligonucleotides. It should be noted that the predominantly most affinity is characterized by the natural forms of oligonucleotides and codicepin derivates. To predict the spectra of individual oligoadenylates exposure to genes expression was conducted in the DIGEP program Way2Drug with the subsequent cluster analysis of the results obtained in the String service. Also, their hypothetical dose of LD_{50} was foreseen with different methods of administration with the help of the GUSAR program [3].

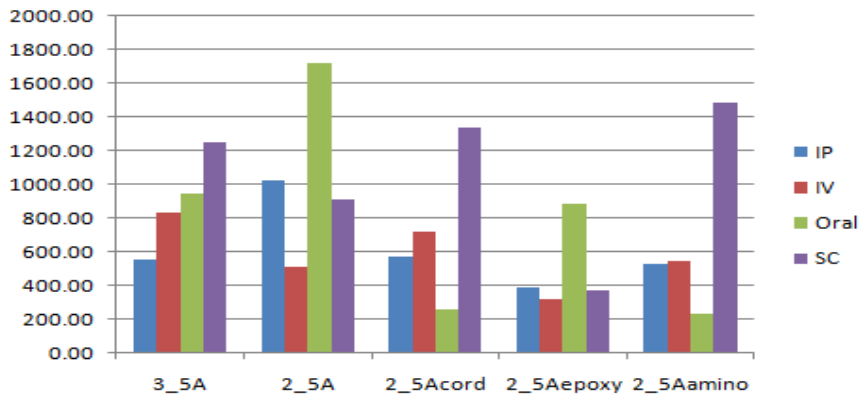

Fig. 1. Dosage prediction of LD50 (mg/kg) on rats in the GUSAR software environment of individual oligoadenylates Note: IR - intraperitoneal injection, IV - intravenous, Oral - oral, SC - subcutaneous.

The analysis in the GUSAR program in showed that oligoriboedenylates are classified as Grade 4-5 (slightly or almost non-toxic) according to the classification of the OECD Project with a dose of LD50 in the range of 200-800 mg/kg (fig. 1).

It should be noted that the safest oligonucleotide among the presented serves 2'-5'A_3 epoxy and the earliest methods of administration - intraperitoneal and intravenous.

The study of individual oligonucleotides in the DIGEP and String program in showed that oligoriboadenylate activates the metabolites of glutathione metabolism (MGST1, RRM2, RRM2B), steroid biosynthesis (SQLE, SC5DL), C5 sterol desaturase (FADS1, SC5DL), oxidoreductase activity (MGST1, RRM2, RRM2B, SQLE, SC5DL, FADS1, CYP2E1), activation of phagocytosis and antioxidant system (IL23A, S100A9). The study of individual oligonucleotides in the DIGEP and String program in Fig. 6 showed that oligoriboadenylates inhibit a number of genes, among which the DNMT1 gene is to be isolated. Protein DNMT1 in violation of the balance of methylation causes hypermethylation of promoter regions of oncosuppressors.

In the course of the research, it was found that total RNA does not have any modifying effect on the methylation process involving the model EsoRI methyltransferase. At the same time, the complex of yeast RNA with D-mannitol and 3'-5'A_3 showed an inhibition effect only at a concentration of 0.37 μM. Since it is known that 2'-5' threeadenylate is highly effective in binding to enzyme domains, we have investigated this particular group of oligonucleotides. It has been shown that 2'-5'A_3epoxy effectively inhibits the activity of the EsoRI methyltransferase in the range 18-55 μM. For this trimmer, it is known and proven to be able to simulate enzymatic activity by binding to enzymes and changing their conformation, as demonstrated by the protein kinase example. We have found that oligonucleotide 2'-5'A_3epoxy effectively inhibits the activity of the EsoRI methyltransferase in the range 18-55 μM in Fig. 2. Triggers of oligonucleotides are known to be most optimal for binding to the active center of the enzyme. Previous studies have shown that 2'-5'A_3 epoxy is characterized by highly effective binding and is most closely located in the active

center of protein kinase C [7]. The binding of an oligonucleotide to a protein depends on the type of modification, while the epoxy derivative trimer of adenine is best suited. The binding also depends on the number of adenine monomers, and, as shown by the previous results on protein kinase C, the trimers are the most preferred.

When using 2'-5'A_3-NH_2, modulating activity was observed only at a dose of 37 μM. At the same time, 2'-5'A_3cord demonstrated its activity in the range of 0.37 - 74 μM. It can be assumed that the effect of inhibition of the enzyme EcoRI 2'-5'A_3 cord oligoadenylate in broad range concentrations can be explained by the synergistic effect of the enzyme conformation and the stacking interaction with the alteration of the secondary structure of the DNA that interferes with the methylation.

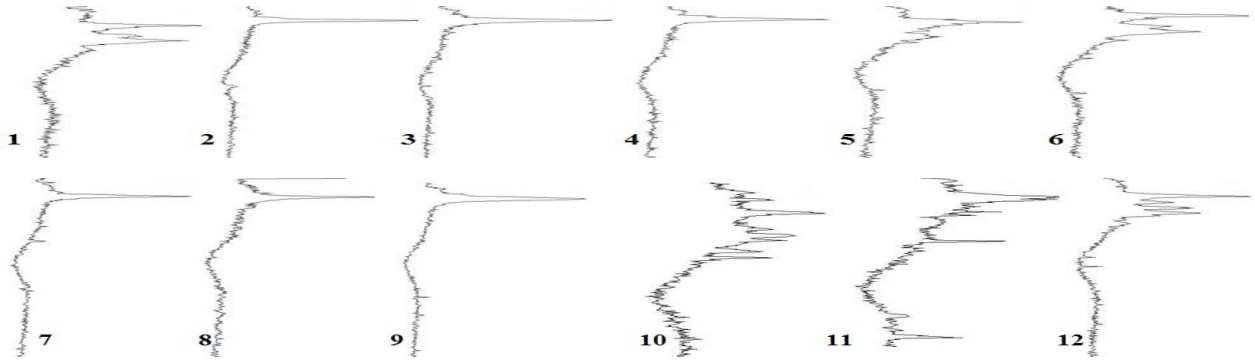

Fig. 2. Electrophoretic DNA fragmentation of phage λ c1857 S7 treated with methytransferase with the addition of natural and synthetic oligonucleotides

Note: 1 marker (Phage DNA λ processed by ESRI restriction enzyme); 2- control (ESRI methytransferase + ESRRI restriction enzyme); 3 – 0.37 мкМ RNA;4 – 3,7 мкМ RNA; 5- 0.37 мкМ Nuclex;6 – 3,7 мкМ Nuclex; 7 - 0.37 мкМ 3'-5'A_3; 8 – 3,7 мкМ 3'-5'A_3; 9- 0.37 мкМ 2'-5'A_3-NH_2;10 – 3,7 мкМ 2'-5'A_3-NH_2; 11- 0.37 мкМ 2'-5'A_3 cord;12 – 3,7 мкМ 2'-5'A_3 cord

Conclusions. It is shown that the most effective binding of oligonucleotides to methyltransferase occurs with 3 and 5 monomeric aptamers. Toxicity analysis showed that oligoriboadenylates have a dose of LD50 in the range of 200-1500 mg/kg. The study of individual oligonucleotides in the DIGEP program showed their ability to activate the antioxidant and immune system with the simultaneous inhibition of methyltransferase activity. The electrophoretic distribution showed the

ability of oligonucleotides to inhibit the methyltransferase reaction in a wide range of micromolar concentrations.

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UDC 616.379-008.64

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INFLUENCE OF DIFFERENT KINDS OF ANTIOXIDANTS ON THE CHARACTERISTICS OF ASTROCYTES UNDER HYPERGLYCEMIA CONDITIONS

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Abstract

We investigated the neurochemical activity of the white rats nervous system under conditions of experimental hyperglycemia and antioxidant therapy with melatonin, vitamin E. Significant increase of lipid peroxidation products lever and a decrease of reduced glutathione (GSH) lever in brain structures of rats under experimental hyperglycemia was determined. We identified significant increase of the lever and polypeptide composition of glial fibrillary acidic protein (GFAP) in brain structures of rats with experimental hyperglycemia. Antioxidant therapy

promotes to a significant increase the GSH levels, decrease the levels of lipid peroxidation products and the level of GFAP and its fragments in the brain structures of rats with experimental hyperglycemia.

Keywords: HYPERGLYCEMIA, CENTRAL NERVOUS SYSTEM, GFAP, ANTIOXIDANTS, OXIDATIVE STRESS.

Introduction

Diabetes is a growing global problem. According to the International Federation of Diabetes, as of 2017, there are approximately 424.9 million patients with diabetes. Hyperglycemia is one of the main causes of diabetic complications, regardless of its type. The increasing complications of the central nervous system (CNS) are the result of chronic hyperglycemia. They are associated with disorders of the central metabolic pathways of glucose utilization and development of oxidative stress [3], accompanied by the formation of free radicals (reactive oxygen species, ROS) and free radical oxidation products. ROS and their oxidation products damage cell components. It causes the disorders of cell function [2]. Normally, antioxidant enzymes (such as superoxide dismutase, catalase, glutathione peroxidase) in the nervous system cells eliminate ROS and products of free radical oxidation, but metabolic disorders cause inhibition of these enzymes [1].

Therefore, the oxidative stress, that arises in the tissues, causes structural and functional changes in the cells of astrocytes and neurons, and these dysfunctions lead to behavioral and cognitive impairment in patients with diabetes.

Materials and methods

Animals have been kept in plastic cages under temperature conditions of 20-23 ºC, in standard conditions with periodicity of the day: light – 12 hours, night – 12 hours. We have investigated biochemical parameters of prooxidant and antioxidant system, and GFAP levers in the cerebellum, hippocampus and cortex of the cerebral hemispheres of the Wistar rats. The induction of diabetes was performed by a single intraperitoneal administration of streptozotocin (STZ) at a dose of 50 mg / kg.

Animals were divided into several groups: control (n = 6); rats with streptozotocin diabetes (STZD; n = 10); STZD + melatonin 10 mg / kg (n = 6); STZD + vitamin E (50 mg / kg; n = 6). The rats were decapitated for further studies at 45^{th} days after induction of diabetes.

The blood glucose levels were determined using glucose oxidase method (Trinder, 1969). The products of lipid peroxidation were determined using thiobarbituric acid reactive substances (TBARS) assay (Esteraauer & Cheeseman, 1990). The GSH levels were determined using Ellman's method. The levers of GFAP we investigated using modified Lowry`s assay (Miller, 1951) and Bradford`s method (1968). Equal quantities of GFAP were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer to nitrocellulose membranes (Laemmli, 1970). The blots were then incubated with primary antibodies against GFAP (Santa Cruz Biotechnology Inc., USA) at 1:2 000 dilutions. After 1 h incubation, the blots were rinsed with TBS-Tween (25 mM Tris-HCl, 0.1% Tween-20). The blots were then incubated for 1 hour with a secondary antibody, a goat anti-rabbit Ig peroxidase conjugate (Sigma, USA). Blots were visualized using diaminobenzidine and H_2O_2 as substrates. The relative amounts of immunoreactive bands on Western blots were quantified in arbitrary units by scanning blots using a computerized software program (LabWorks 4.0; UVP Inc., UK). All data are presented as mean ± standard error of the mean (SEM). Differences in the levels of GSH, end products of LPO, and GFAP between groups were analyzed using analysis of variance (ANOVA). When ANOVA showed a significant effect on the group on any of dependent variables, the Student’s t-test was used for posting multiple comparisons. No significance difference was defined as $P > 0.05$.

Results and discussion

Diabetic rats receiving melatonin had significantly decreased levers of LPO end products by 22% in the hippocampus, by 24% in the cortex and in the cerebellum in comparison with a group of diabetic control. Melatonin therapy showed the highest result in terms of the content of the enzyme cofactor of glutathione peroxidase. The

GSH levers was increased by 45% in the hippocampus, by 43% in the cortex, and 45% in the cerebellum in comparison with a group of diabetic control. According to the results of immunoblotting, the least degraded polypeptides were found in the brain of the rats receiving melatonin. There was no significant difference in LPO and products levers compared to animals receiving vitamin E. Course injections of vitamin E effectively inhibited the development of oxidative stress and excessive astrogliosis in the brain STZD of rats. Introduction of vitamin E to diabetic rats has contributed to a statistically significant decrease in the level of LPO products: 21% in the hippocampus, 28% in the cortex inc. and 23% – in the cerebellum in comparison with the STZD group. Injections of vitamin E caused an increase in the level of GSH by 39% in the hippocampus, by 32% in the cortex, and 41% in the cerebellum in comparison with a group of diabetic control.

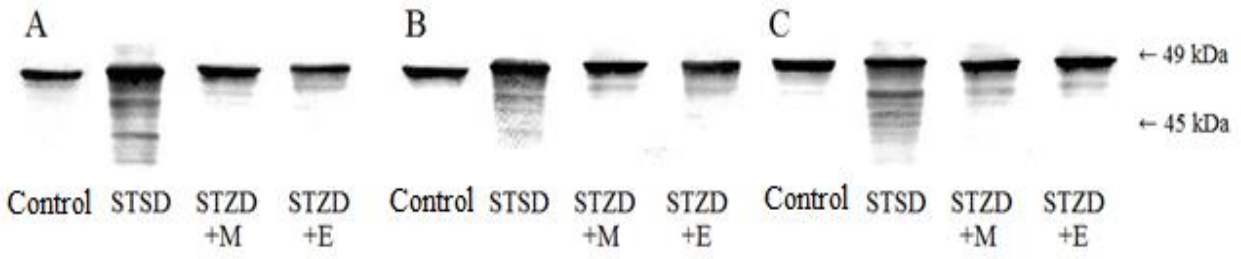

Fig. 1. The GFAP fractions in hippocampus (A), cortex (B), and cerebellum (C). STZD + M is "STZD + melatonin 10 mg/kg" group. STZD + M is "STZD + vitamin E 50 mg/kg" group

Nevertheless, it is quite difficult to uniquely determine which antioxidant is most effective because there is no enough information about optimal doses for melatonin, vitamin E. Alternatively, there is perspective path to use combinations of antioxidants with different mechanisms of action.

Conclusions

In conclusion, melatonin and vitamin E can significantly increase the GSH levels, decrease the lipid peroxidation and the level of GFAP and its fragments in the brain structures of rats with experimental hyperglycemia. It is evident that antioxidant

therapy has huge potential as an effective anti-diabetic drug. Further research is necessary to investigate the influence of combinations of antioxidants on characteristics of astrocytes under hyperglycemia conditions.

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UDC 579.64

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BIOLOGICAL PROPERTIES OF BACTERIA OF THE GENUS *BACILLUS* – AROMATIC COMPOUNDS DESTRUCTORS

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Introduction

Pollution of the environment with aromatic hydrocarbons is a worldwide problem. In the process of destroying phenols in the environment, microorganisms play a major role. Microbiological detoxification of aromatic compounds is one of the most promising methods of environmental purification, during which the aromatic ring cleaves and the formation of non-toxic compounds, carbon dioxide and water occurs. Bacteria of the genus *Bacillus* are widespread in soils and reservoirs, play an important role in the processes of mineralization of organic compounds.

The aim of the work is to study biological properties of bacteria of the genus *Bacillus* - aromatic compounds destroyers

Materials and methods

The experimental part of the work was performed at the Scientific and Training Biotechnological Center of the Odesa Mechnykov University . The material for the study was strain *B. Megaterium* ONU 330, isolated from seawater. In this work, the classical bacteriological methods were used. The investigation of phenol-destructive activity was carried out at temperature of 18 and 30 ° C on a liquid mineral medium of M 9 of the following composition: Na2HPO4- 6.78 g / L, KH2PO4- 3 g / l, NH4Cl-1 g / l, NaCl- 0.5 g / l with a concentration of phenol 300 mg / l. A photometric method using 4-amino-antipyrine (at a wavelength of 540 nm) was used to determine the residual concentration of phenol.

The results of research

As a result of the research, it was found that strain *B. megaterium,* isolated from seawater, is represented by Gram-positive long rods with rounded ends of the size of 0.629 x 1050 micrometers. In smears, cells are combined into small chains (2-3 cells) with an average length of about 3.5 micrometers, the spore is located in the middle of the cell. The cultural and biochemical properties of the strain are presented in Table 1.

Table 1.

Cultural and biochemical properties of strain *B. megaterium*

The presence	mobility	catalase	lecithinase	hemolysis	mannit	starch	urea	Growth			
								on broth	on milk	on agar	on gelatin

+	+	–	–	–	–	+	+	clouding of the medium, the film does not form, the precipitate is insignificant	peptonization	colonies of mucous membranes, yellowish - white, darken in the presence of tyrosine in the medium	slow funnel -shaped dilution

The ability of microorganisms to degrade phenol in different temperature regimes has been experimentally confirmed. Degree of degradation of phenol at temperature of 4 ° C throughout the duration of exposure (5 - 40 days) varied from 18.7 ± 0.1% to 90.0 ± 2.5% (Table 2).

Table 2.

Phenolic-oxidation activity of strain *B. megaterium* (%)

Strain	t, °C	Exposition, days					
		5	11	18	22	34	40
B. megaterium	30°C	50.6±4.3	75.8±4.9	80.0±5.2	95.0±5.5	100±0	100±0
	18°C	22.4±2	26.7±2.5	53.8±9.8	80.2±4.0	97.0±8.1	100±0
	4°C	18.7±0.1	26.7±1.5	52.4±0.5	73.8±1.3	88.0±1.2	90.0±2.5

At temperature of 18 ° C the increase in destructive activity was recorded to 100 ± 0%.

Conclusions

1. According to the results of the study of biological properties the species belonging to the investigated strain to *B. megaterium* have been confirmed.

2. Strain *B. megaterium* is a destructor of phenol. which exhibits biochemical activity in a wide range of temperatures.

3. The collection culture of *B. megaterium* is resistant to phenolic loading conditions,which allows it to be recommended for application in biotechnologies for water purification from phenol.

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UDC P57; L612,8; P615

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DISTRIBUTION OF CALCIUM-BINDING PROTEIN S100B IN THE RAT BRAIN UNDER EXPERIMENTAL STRESS

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Abstract

The main biochemical mechanism for protecting the body in the presence of the influence of stress factors is the induction of calcium-dependent regulatory systems. In this paper, the content of calcium-binding protein S100b in the brain of rats was determined under conditions of prolonged and complicated water-immobilization stress and the impact of exogenous alpha-ketoglutarate as a

neuroprotector. Quantitative determination of the S100b protein was performed using competitive ELISA. The tendency to increase production of S100b in the hippocampus and thalamus in the early post-stress period and a significant increase of this protein within 14 days of stress was determined. The impact of exogenous alpha-ketoglutarate in drinking water for 14 days after stress was established.

Key words: water-immobilization stress, S100b protein, alpha-ketoglutarate.

Introduction

The initial response to stress leads to an increase in the concentration of Ca^{2+} in the cell, which is one of the regulatory mechanisms of intracellular processes activation of metabolic changes. Significant increase in the content of free calcium ions occurs in two ways: the first one is – the release of Ca^{2+} from the bones and an increase in its content in the blood under the influence of stress-induced increase in parathyroid hormone levels in blood, which contributes to an increase in the entry of the specified cation into the cells of the organs responsible for adaptation; the second mechanism is associated with the release of catecholamines and other hormones that activate the introduction of Ca^{2+} into the cell, increase in it intracellular concentration, induction of protein kinases, and as a consequence – the activation of calcium-dependent intracellular processes.

Calcium-binding protein of astrocytes S100b is involved in Ca^{2+}-dependent regulation of various intracellular processes, such as calcium homeostasis, phosphorylation of proteins, enzyme activity, cell proliferation and differentiation, cytoskeleton component dynamics, structural organization of membranes, protection against oxidative damage of cells under different conditions of pathological states [1-2]. The content of S100b protein in the central and peripheral nervous system significantly exceeds its concentration in other tissues and amounts to 2.8 μg/mg (0.1-0.5 % of the total protein). In the brain, the S100b protein is approximately 104 times more than in any other part of the body. The largest amount of this protein, about 85-90 % of their total content in the nerve tissue, is concentrated in astrocytes [3-5].

The main aim was to study the distribution of calcium-binding protein S100b protein in different areas of the rat brain, which primarily react to stress factors, and determine the neuroprotective impact of exogenous alpha-ketoglutarate after water-immobilization stress.

Materials and methods

In this work, the brain of 24 adult Wistar rat weighting 180-220 g was used, which simulated for complicated and prolonged water-immobilization stress for 3 days [6]. All activities with the animals were conducted in accordance with the "Regulations on the use of animals in biomedical experiments" [7]. During experiment, animals were tested in an open field due to Buresh in order to analyze their behavioral reactions. After the experiment, the animals were decapitated under a slight anesthetic and their brains were removed. For further research, cerebellum, visual cortex, hippocampus and thalamus were used. Extraction of protein fractions from the rat brain was performed by ultracentrifugation of the brain homogenate in a hypotonic buffer of 25 mM tris, pH 7.4 with a cocktail of inhibitors for endogenous proteinase (Roshe, Germany)/

The quantitative determination of S100b in fractions derived from differ brain areas was carried out according to the standard method of competitive solid-phase enzyme-linked immunosorbent assay using monospecific antibodies and purely purified S100b as a calibrator (Sigma, USA).

Results and discussion

According to the results, the amount of S100b in the cerebellum, visual cortex and hippocampus did not significantly differ from the control animals immediately after the completion of the water-immobilization stress. In thalamus, unlike other brain areas, a significant increase of studied protein was recorded (Fig. 1).

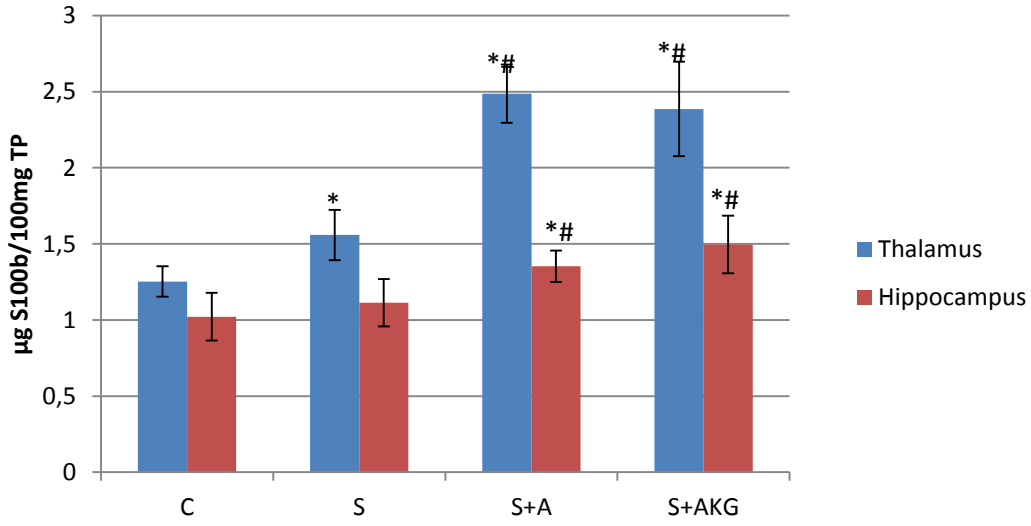

Fig. 1. The content of S100b in hippocampus and thalamus of rats
S – control animals, S – animals an hour after the completion of the water immobilization stress, S+A – 14 days of physiological adaptation after stress, S+AKG – 2 % of exogenous alpha ketoglutarate in drinking water for 14 days after stress, n = 6, * – P <0.05 compare to the control; # – P <0.05 compare to the group of stressed animals.

Thalamus is the brain area that is most responsive to changes in the environment, because the nuclei of this structure analyze auditory, visual, and tactile signals. Thalamus is the so-called information concentrator. Through it there is a relay between different subcortical sites. The structure of the thalamus includes the reticular formation of the brain, which is responsible for enhancing the reflexes. The obtained data indicate that fastest activation of calcium-dependent mechanisms of regulation of nerve cells under stress conditions occurs in thalamus. Studies of the distant stress effect have shown increased activation of calcium-binding protein S100b production not only in thalamus but also in the hippocampus, the brain area of the limbic system responsible for memory and training. One of the tasks of this experiment was to investigate the effectiveness of exogenous alpha ketoglutarate, presented by SGPlus (Sweden). It is believed that AKG, as a natural intermediate metabolite, is a key substrate for many enzymes to activate adaptive systems under stress and pathological conditions. However, the results suggest that an increased pool of S100b remains after stress even in the case of an additional AKG in drinking water. However, other experimental parameters (behavioral reactions of animals, a

common pool of cytosolic proteins in the experimental brain regions of rats and the level of other neurospecific proteins) have been significantly improved to the control.

Conclusions

Water-immobilized stress induces an increase in S100b levels in the thalamus and hippocampus; the use of 2% of exogenous alpha ketoglutarate in drinking water for 14 days after stress improves behavioral reactions of animals but does not lead to normalization of the content of S100b protein in the brain. Whether the increased content of S100b in thalamus and hippocampus is the protective mechanism (or adaptive) under stress requires further detailed analysis.

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UDC 579.64

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BIOLOGICAL PROPERTIES OF NITROGEN-FIXING BACTERIA ISOLATED FROM RHIZOSPHERE

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Summary. Nirogen-fixing bacteria were isolated from rhizosphere of grape and characterized by the main biological properties: morphology, ability to grow on nutrient media, fatty-acid analysis. Strain was identified as a candidate to *Azotobacter* sp.

Key words: nitrogen-fixing microorganisms, fatty-acid profiles, rhizosphere

Introduction

Nitrogen-fixing bacteria are very important for agriculture and biotechnology. *Azotobacter spp.* are the most known microorganisms able to fix nitrogen from atmosphere and thus supplying nitrogen-containing compounds to plants. Biotechnology of improving of plant growth relies on biological preparations containing beneficial microorganisms. Biopreparations based on nitrogen-fixing bacteria are very promising and include easy steps of application.

Aim. To isolate nitrogen-fixing microorganisms from rhizosphere of plants.

Materials and Methods

Samples were picked from the upper 15 cm layer of soil and used for isolation of nitrogen fixing bacteria. Ashby medium without nitrogen source was used for the

experiment. Only nitrogen-fixing bacteria could grow on Ashby medium. Cultural characteristics were observed after incubation at 25-30°C for 24-48 hours. Bacteria from slimy colonies were characterized using the criteria of Bergey's Manual of Systematic Bacteriology. Identification by fatty-acid analysis with gas chromatography was carried out.

Results and Discussion

4 strains of nitrogen-fixing microorganisms were isolated on Ashby medium. They formed white slimy mucous colonies. Gram staining indicated the presence of thin Gram-negative rods (Fig. 1). One strain was taken to further analysis.

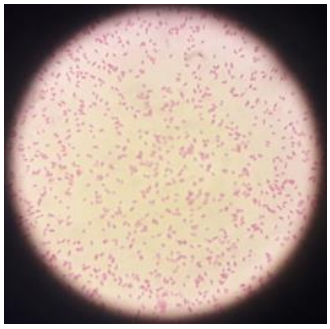

Fig. 1. Gram-negative cells of nitrogen-fixing bacteria isolated from grapevine rhizosphere.

The study of fatty acid profile carried out by gas chromatography (Agilent, USA) allows to determine the composition of cells fatty acid (Table 1).

Table 1

Fatty acid composition of a nitrogen-fixing bacterium isolated from grapevine rhizosphere

Peak Name	Percent
12:0/ Dodecanoicacid	8.55
13:0/ Tridecanoicacid	0.59
14:0/ Tetradecanoicacid	6.67
15:0 anteiso/12-Methyltetradecanoic acid	0.36
Sum in Feature 2	20.24
Sum in Feature 3	22.72
16:1 w5c/ (11Z)-11-Hexadecenoic acid	0.39
16:0/ Hexadecanoicacid	16.89
15:0 iso 3OH/ 3-Hydroxy-13-Methyltetradecanoic acid	3.97
17:1 w8c/(9Z)-9-Heptadecenoic acid	0.70
17:0 cyclo/ cis-9,10-Methylene-Hexadecanoic acid	6.18

17:0/Heptadecanoic acid	1.14
Sum in Feature 8	11.27
18:1 2OH/ 2-Hydroxyoctadecanoicacid	0.33
Summed Feature 2	20.24
Summed Feature 3	22.74
Summed Feature 8	11.27

Biological properties allowed us to suggest that the isolated strain belongs to *Azotobacter* sp.

Conclusion

The nitrogen-fixing microorganism candidatus to *Azotobacter* genus was isolated from rhizosphere of grape. This strain will be studied further for precise identification and for application in biotechnology of stimulation of plant growth.

UDC 582.282.23.045

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THE MECHANISMS OF MICROBIAL INTERACTION DURING THE MULTISPECIES ASSOCIATION FORMATION

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Summary

The *Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* interaction characteristics during the multispecies biofilm formation were studied. At all formation stages the cultures of a microbial multispecies association influenced each other, manifesting an antagonistic form of interactions. The antagonism degree of the cultured microorganisms was not the same in different media.

Key words: *Candida albicans, Staphylococcus aureus*, *Pseudomonas aeruginosa*, multispecies biofilm, antagonism.

Interspecies interactions are essential for the persistence and development of any kind of complex community, and microbial biofilms are no exception [1].

Multispecies biofilms are structured and spatially defined communities that have received much attention due to their omnipresence *in vivo* [2].

Species residing in these complex bacterial communities usually interact both intra- and interspecifically. Such interactions are considered to not only be fundamental in shaping overall biomass and the spatial distribution of cells residing in multispecies biofilms, but also to result in coordinated regulation of gene expression in the different species. Historically, the studying of interspecies interactions has focused on growth-inhibitory interactions, but a variety of phenotypic outcomes other than antibiosis are possible, including alterations in developmental processes such as sporulation and biofilm formation or secondary metabolite production [2].

These communal interactions often lead to emergent biofilm characteristics, such as enhanced antibiotic and immune system tolerance, and other stresses, which have been provide benefits to all biofilm members [3].

Closing the gap between visual observation of biofilms and biological processes inside of team may become crucial for resolving biofilm related problems, which is of utmost importance to environmental, industrial, and clinical implications.

The aim of the work was the study of interaction during the multispecies association formation by *Candida albicans* ATCC 18804, *Pseudomonas aeruginosa* ATCC 15692 and *Staphylococcus aureus* ATCC 25923.

The work was carried out at Biotechnological Research and Training Center.

The studied microorganisms were cultured in Sabouraud and Spider media. Interaction of microorganisms during the formation of biofilm was determined during co-cultivation (24 and 48 hrs) in polystyrene plates using the described method [4]. In the work the culture interaction forms with different initial ratio of their cells were determined: 10:1, 1:1 and 1:10 CFU/ml.

In the work the gradual formation of monobiofilms in both nutrient media was noted. This process consisted of some stages. In stages I – II, planktonic bacteria

initiate attachment to an abiotic surface. Stage III corresponds to microcolony formation and stage IV – to biofilm maturation.

To determine the microorganism interaction form during the multispecies biofilm creation co-cultivation of *C. albicans* and *P. aeruginosa, C. albicans* and *S. aureus* combinations was carried out. Then obtained results were compared with the sum of monospecies ones that was 100 %.

During the biofilm formation process all studied cultures influenced each other, manifesting an antagonistic form of interactions.

The antagonism degree of the cultured microorganisms in the studied media was not the same. In Sabouraud nutrient medium the microbial interaction level caused decreasing in the biofilm cell number in 2.9 times for *C. albicans* and *P. aeruginosa* co-cultivation and 2.0 times for *C. albicans* and *S. aureus* compared with the appreciated monocultures.

In Spider nutrient medium cell number was decreased in 3.0 times in the case of *C. albicans – P. aeruginosa* combination and more than 2.0 times for *C. albicans – S. aureus* one.

Thus, it was found that at all formation stages the cultures of a microbial multispecies association influenced each other, manifesting an antagonistic form of interactions.

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UDC 579.222:579.841.1

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BIOFILM FORMATION OF *PSEUDOMONAS AERUGINOSA* PA01 ΔWSPF1 STRAIN WITH HIGH C-DI-GMP LEVEL

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Bis-(3-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is in the spotlight of the scientists as the result of last achievement of microbial genomics and great interests in microbial communities [6, 8]. Depends on concentration of this regulator bacteria shifts its life-form from motile to sessile (biofilm formation) [7]. Aim was to determine swarming motility and biofilm formation abilities in *P. aeruginosa* PA01 ΔwspF1, with high c-di-GMP level.

K e y w o r d s: cyclic-di-GMP, *P. aeruginosa* PA01, *P. aeruginosa* PA01 ΔwspF1, adhesion, biofilm, swarming motility.

Cytoplasmic c-di-GMP is a bacterial secondary messenger, that regulate numerous of physiological processes: cell-to-cell communication, biofilm formation, motility, virulence, etc. [1-3]. It is found that c-di-GMP affects all stages of the biofilm formation process in *Pseudomonas aeruginosa* from the beginning of adhesion to biofilm decay. This compound regulate biosynthesis of matrix components, quorum sensing signal molecules, biosurfactants. [4, 7]. The aim of this work was studding a features of *P. aeruginosa* PA01 ΔwspF1 swarming motility and biofilm formation in fact that this strain characterize with high level of c-di-CMP in its cells.

Materials and methods

In this work two strains of *P. aeruginosa* were used. *P. aeruginosa* PA01 - the wild type strain was obtained from the collection of Odessa Mechnikov National

University microbiology, virology and biotechnology department. PA01 ΔwspF1 with high level of c-di-GMP was kindly provided by Dr. Olena Rzhepishevskaya from University of Umea, Sweden. The methods of research were described in another article [9].

Results and discussion

In this study we focused attention on comparison of hidrophobicity, z-potential, motility, and exopolysaccharides and biosurfactants secretion in two *P. aeruginosa* strains – PA01 (wild type), and PA01 ΔwspF1. According to literature data its known that in *P. aeruginosa* PA01 intracellular content of this messenger equals 3.5 fmol/mg proteins, and in *P. aeruginosa* PA01 ΔwspF1 there is an undetectable amount of this compound [5].

Determination of planktonic and attached cells were carried out after 30 and 60 minutes of incubation (fig. 1). Optical density of inoculums were 0,047 for *P. aeruginosa* PA01 and 0,051 for *P. aeruginosa* PA01 ΔwspF1.

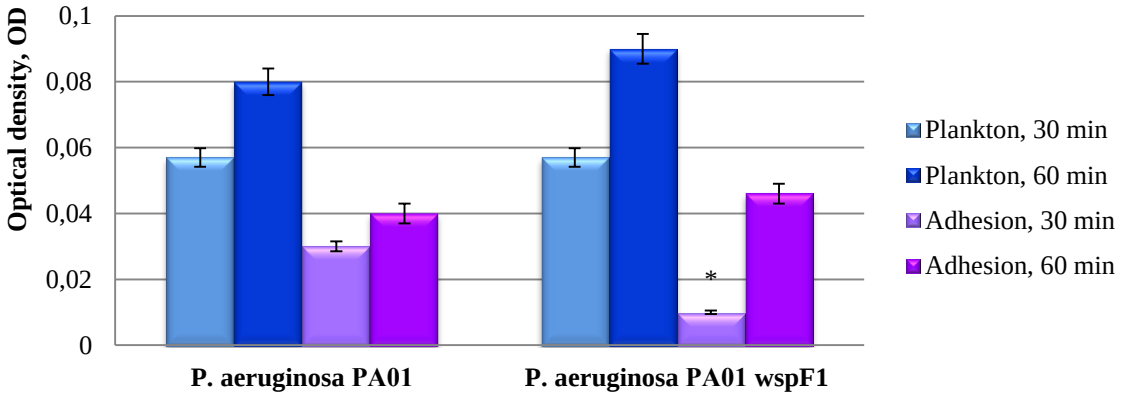

Fig. 1. Attached and free cells amount of *P. aeruginosa* test strains

Note: * - significant difference compared with *P. aeruginosa* PA01

Obtained results show that from the beginning of cultivation *P. aeruginosa* PA01 ΔwspF1 show lower than wild type strain ability of attachment to solid surface. After 30 min of incubation attached cells amount of *P. aeruginosa* PA01 were in 2,5 higher. In the next 30 min of incubation attached cells amount increased in both cases, but it was on 20% higher in case of *P. aeruginosa* PA01 ΔwspF1 compare the

P. aeruginosa PA01. After 60 min of incubation there is a tendency of higher increasing of planktonic cells amount in *P. aeruginosa* PA01.

Examination of daily biofilms show that there were a significant differences in there general form and structure (fig. 2). *P. aeruginosa* PA01 biofilm consists of multicellular 3D-structures. At the same time, *P. aeruginosa* PA01 ΔwspF1 biofilm was flat and "monolayer" (fig. 2A). The difference from two strains also was noticeable on microcolony level (fig. 2B). *P. aeruginosa* PA01 microcolonies were good formed that consists of matrix enclosed cells. In addition, there are secondary microcolonies formation that enhance biofilm mass. In *P. aeruginosa* PA01 ΔwspF1 only the small structural units were detected.

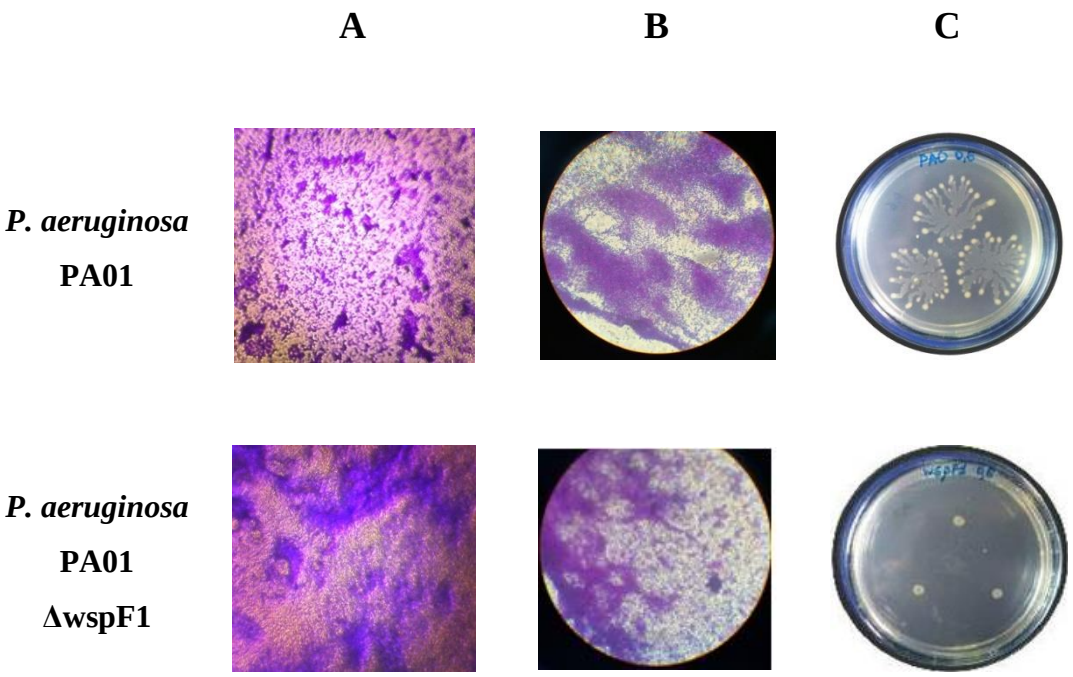

Fig. 2. Images of biofilms (A, B) and swarming motility (C) forming by wild and mutant strains of *P. aeruginosa*
(Magnification: A - × 200, B - × 400, crystal violet staining; C-16 mp)

Swarming experiments showed that *P. aeruginosa* PA01 ΔwspF1 swarming motility zones diameter were 5 ± 0 mm and it were in 0.11 times lower than at *P. aeruginosa* PA01 – 43 ± 3 mm. *P. aeruginosa* PA01 swarming zones have not clearly formed central "core". In the end of each "rays" there is a white thick colony. *P. aeruginosa* PA01 ΔwspF1 not able to motile by swarming (fig. 2C).

Quantity examination of the biofilm formation shows (fig. 3) that *P. aeruginosa* PA01 biofilm have in 1.3 times lower mass than *P. aeruginosa* PA01 ΔwspF1 ($p<0,001$). However, planktonic cells amount were higher in the case of *P. aeruginosa* PA01 ΔwspF1 by 10%. Pel and alginate exopolysacharides amount were similar in all strains biofilm matrix.

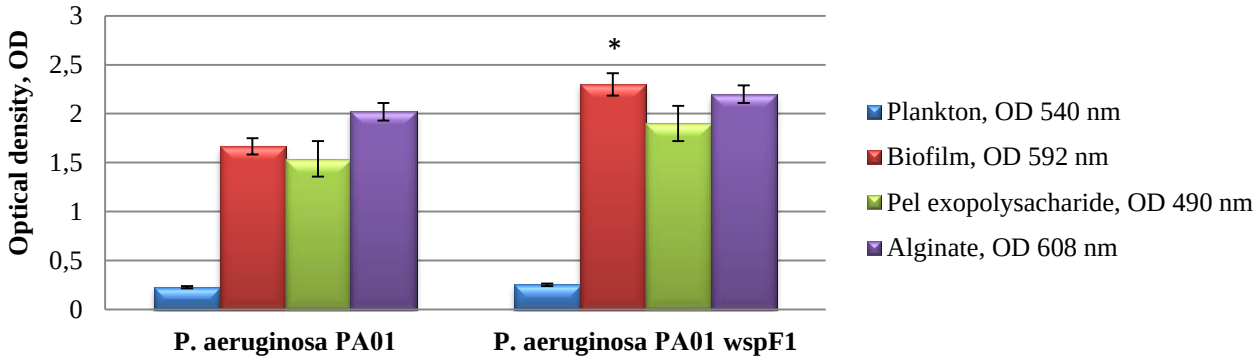

Fig. 3. Biofilm mass and exopolysaccharides ammount.

Note: * - significant difference compared with *P. aeruginosa* PA01

Cell size and physical-chemical properties of the cells surface study show that examined strains have a difference in hydrophobicity. More over, strains hydrophobicity changed during the cultivation (table).

Table

Cell size and physical-chemical properties of the *P. aeruginosa* strains cells surface

Strain	**Cell hydrophobicity, %**		**d, nm**	**z-potential, - mV**
	3 h	24 h		
P. aeruginosa PA01	42,8 ± 2,3	14,2 ± 1,7	666,4 ± 46,4	23,4 ± 1,2
P. aeruginosa PA01 ΔwspF1	41,6 ± 1,9	23,5 ± 1,4*	677,3 ± 46,1	24,7 ± 1,5

Note: * - significant difference compared with *P. aeruginosa* PA01

In logarithmic phase of growth, after 3 h of incubation cell hydrophobicity of *P. aeruginosa* PA01 were higher in 1.03 times then in *P. aeruginosa* PA01 ΔwspF1. Transition to stationary phase accompanied with opposite changes of hydrophobicity

especially for wild strain – 3 fold decreasing and only 1.8 fold decreasing in mutant type strain. Cell diameter in *P. aeruginosa* PA01 overnight culture was 10% less than in *P. aeruginosa* PA01 ΔwspF1. Z-potential of the cells were the same.

More over, decreasing in biofilm formation ability on the background of c-di-GMP decreasing make this system an attractive target for novel antimicrobial drugs.

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UDC 579.6

O. Smazchuk

BIOLOGICAL PROPERTIES OF BACTERIA OF THE GENUS *PSEUDOMONAS* - AROMATIC COMPOUNDS DESTRUCTORS

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Introduction

The danger of the receipt compounds of aromatic nature in the environment is related to their toxicity for biological objects significant resistance to decomposition))) [1]. A microbiological detoxication of phenols is one of the safest and prospective methods of cleaning the environment [1, 2, 3]. Bacteria of the genera *Pseudomonas* are widespread in soils and reservoirs, play an important role in the processes of environmental purification [4].

The aim of the work is to study biological properties of the bacteria *Pseudomonas* - destructions of aromatic connections.

Materials and methods

Experimental part of the work was carried out in the Research biotechnological center of Odesa Mechnykov University. Material for research was 1 collection of strians of bacteria of the biotechnological setting - *P. fluorescens* of ONU328, separated from seawater. These strains are kept in the collection of microbiology, virology and biotechnology department, branches of National collection of microorganisms of Ukraine that has status of National acquisition. Cultivations of bacteria were carried out on a liquid mineral environment M 9. To determine the concentration of phenol we used a photometric method to the uses of 4-aminoantipyrine. The method is based on the formation of colored compounds of phenol, its derivatives and homologs with 4-aminoantipyrine in presence of hexacyanoferate (III). The test was carried out on photocoelectrocolorimeter (PEC) with the wave-length of 540 nm.

The results of research

The results of the study of phenol-oxidative activity of marine strains of microorganisms *P. fluorescens* ONU-328 are presented in Table. 1. The ability of microorganisms to degrade phenol in different temperature regimes was confirmed experimentally. The strain was able to grow at a concentration of phenol in a living medium of 300 mg / l, which is an unwelcome concentration. The strain showed destructive activity at temperature of 4 - 30 ° C during the first 5 days of exposure. Degree of degradation of phenol using *Pseudomonas fluorescens* strain ONU-328 at temperature of 4 ° C during the entire exposure period (5 - 40 days) varied from 18.1 ± 0.9% to 26.7 ± 1.3%. At 18 ° C, the increase in destructive activity to 33.3 ± 1.7% was recorded only for 22 days. After 34 days, this figure reached 89.7 ± 4.5%, and after 40 days - 100%. The highest level of biochemical activity of *Pseudomonas fluorescens* strain ONU-328 was determined at 30 ° C. Already after 5 days of exposure, the degree of degradation of phenol reached 33.6 ± 1.7%, after 18 days, it increased almost 3 times - to 93.5 ± 4.7%, and in 22 days it reached 100%.

Conclusions

1. Complete water dephenolation with the use of strain *P. fluorescens* ONU-328 proceeds under the optimal conditions (30 ° C) for 34 days.

2. Collective cultures of *P. fluorescens* ONU-328 are resistant to the conditions of phenolic loading, which allows them to be considered promising for the development of methods of bioremedation of waters contaminated with compounds of phenolic nature

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UDC 579.64

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EFFECT OF *LACTOBACILLUS PLANTARUM* ON WHEAT GROWTH IN HYDROPONICS

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Treatment of wheat seeds with *L. plantarum* strains increased germination and growth characteristics of seedlings under hydroponics conditions in 10,0 – 41,0 %.

Key words: *Lactobacillus plantarum*, wheat, hydroponics, growth characteristics

Bacteria of the species *Lactobacillus plantarum* can be used in biotechnology to protect and stimulate plant growth [2, 3, 4]. Bacteria in biological preparations can be used both in monoculture and in a form of consortia, and quite often the stimulatory activity of bacteria in consortia becomes higher than in certain strains [3, 4].

The **aim** of this work was to study the effect of *L. plantarum* strains on wheat growth under hydroponics conditions.

Materials and Methods

Hydroponics was modelled by gel Aquasave S. Lactobacilli were cultivated in MRS medium at 37 ºC overnight and diluted till the concentration of the suspension 10^8CFU/ml. This cell suspension was used to serial dilutions as: 1%, 0,1%, 0,01%, 0,001%, 0,0001%, 0,00001% for each of the strains and consortia, and mixtures of the strains. A mixture was prepared by pouring together cultures of different strains of lactobacilli in a ratio 1:1 and after this using them immediately for an experiment.

A consortium was prepared by mixing overnight cultures of lactobacilli in a ratio 1:1 and cultivating them together not less than one week with passages in every two days.

Stimulation activity of bacterial strains from the Collection of cultures of microorganisms of Microbiology, Virology and Biotechnology Department of Odessa National I.I. Mechnikov University was studied.

The next strains were brought into experiments: *L. plantarum* ONU 12, *L. plantarum*ONU 311, *L. plantarum*ONU 355; mixtures *L. plantarum* ONU 12 + *L. plantarum*ONU 311, *L. plantarum* ONU 12 + *L. plantarum*ONU 355 and consortia *L. plantarum*ONU 12 + *L. plantarum* ONU 355, *L. plantarum* ONU 12 + *L. plantarum* ONU 311.

Surfaces of wheat seeds (*Triticum aestivum* L.) of the cultivar Odeska ozyma were sterilized by 25% of hydrogen peroxide and washed three times in sterile distillated water.

Seeds were soaked for one hour in prepared bacterial suspensions. Control seeds were soaked in water. After, seeds were planted in gel under greenhouse conditions at 25°C. After 7 days germination and growth characteristics of the seedlings were evaluated.

Results and Discussion

Soaking seeds in suspensions of lactobacilli resulted in changes of germination depending on bacterial strain and dilution of the suspension (Table 1). Treatment with suspensions of lactobacilli improved germination of test plants in 10,0 – 40,0 %.

The highest increase was found after the treatment of seeds with mixtures and consortia of *L. plantarum* ONU 12+311 and *L. plantarum* ONU 12+355.

Table 1

Germination of wheat after treatment with *L. plantarum*

Strain	1%	0,1%	0,01%	0,001%	0,0001%
ONU 12	60,0±2,5%	80,0±2,1%	70,0±2,4%	60,0±2,5%	60,0±2,5%
ONU 311	80,0±2,1%	90,0±1,5%	90,0±1,5%	90,0±1,5%	60,0±2,5%
ONU 355	90,0±1,5%	60,0±2,5%	70,0±2,4%	60,0±2,5%	80,0±2,1%
12+311 Mixture	70,0±2,4%	100%	90,0±1,5%	80,0±2,1%	70,0±2,4%
12+355 Mixture	90,0±1,5%	70,0±2,4%	80,0±2,1%	80,0±2,1%	90,0±1,5%
12+311 Consortium	80,0±2,1%	80±2,5%	70,0±2,4%	80,0±2,1%	80,0±2,1%
12+355 Consortium	80,0±2,1%	80,0±4,4%	80,0±4,4%	70,0±2,4%	80,0±2,1%

Note: germination in a control was 60±2,5%.

The same combinations of bacterial strains positively effected on growth of wheat seedlings.

Thus, the height of plants increased in 37,0 % after soaking of seeds in suspensions of the consortium *L. plantarum* ONU 12+311 (Fig. 1).

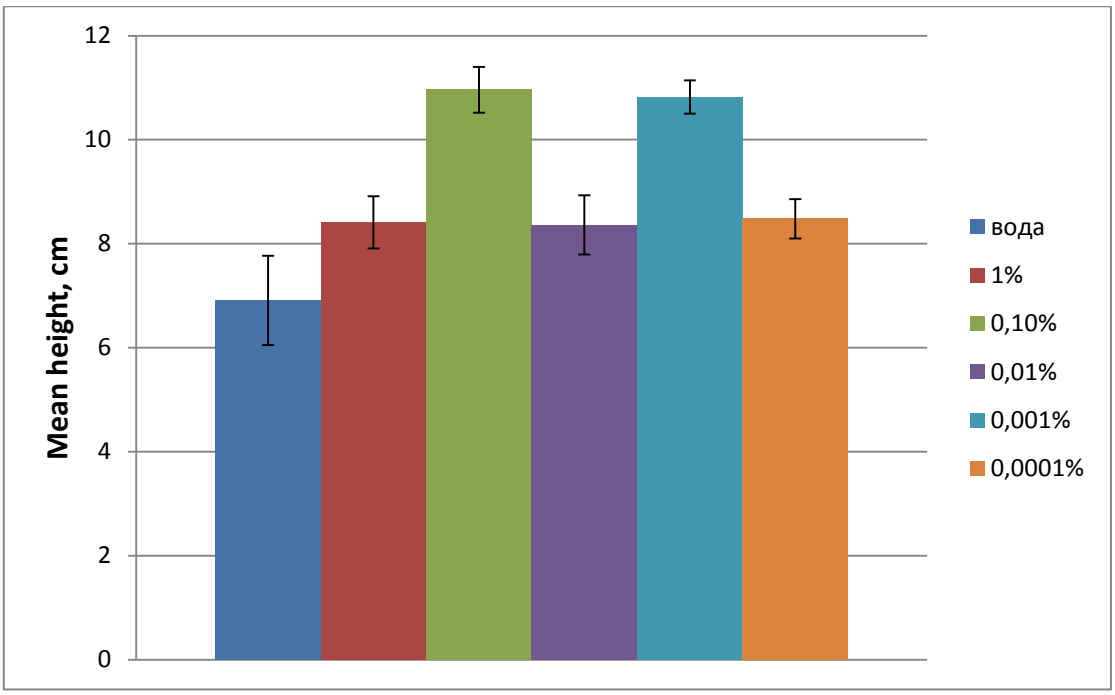

Fig. 1. Mean height of wheat seedlings after the treatment with the consortium *L. plantarum* ONU 12+311.

Treatment with the consortium *L. plantarum* ONU 12+355 increased mean height of the plants in 41,0% (Fig. 2).

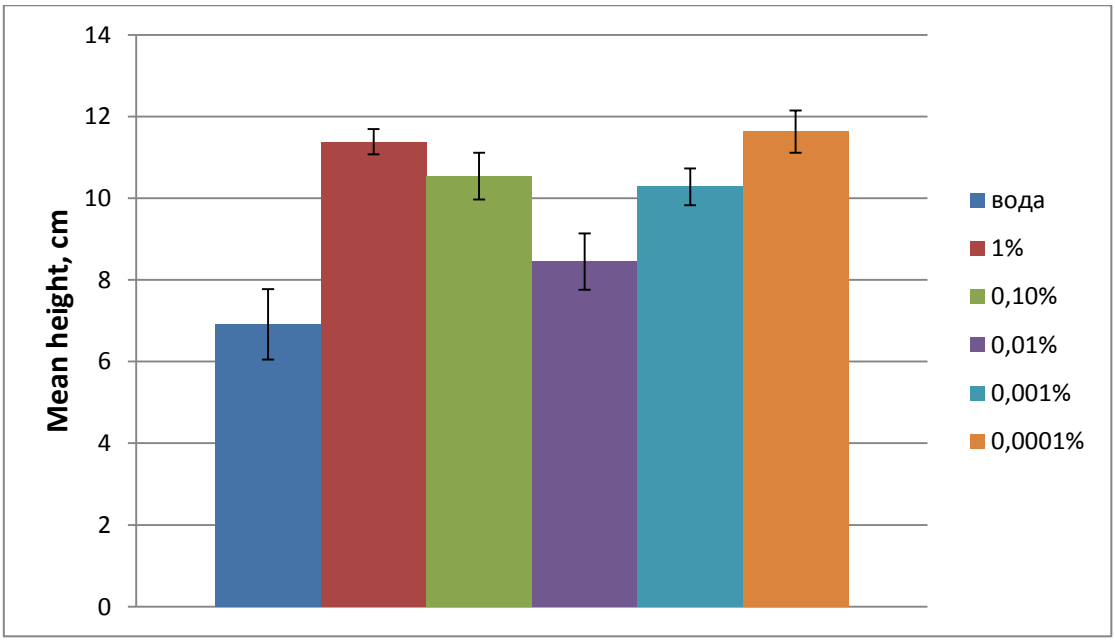

Fig. 2. Mean height of wheat seedlings after the treatment with the consortium *L. plantarum* ONU 12+355.

Stimulating effect of lactobacilli can be explained by the synthesis of auxin hormones [1]. The data of our study proof the perspective of using lactobacilli for stimulation of plant growth.

Conclusions

Consortia of *L. plantarum* strains ONU 12+311 and ONU 12+355 are the perspective agents for biotechnological stimulation of plant growth.

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UDC 579.64

N. Sokolova

EFFECT OF *LACTOBACILLUS PLANTARUM* ON SURVIVABILITY OF WHEAT UNDER OSMOTIC STRESS CONDITIONS

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Lactobacillus plantarum ONU 12+311 positively affected survivability and osmotolerance of wheat seedlings under drought conditions. Pre-treatment of wheat seeds with the bacterial suspension led to an increase in plant survivability by 27.6% under sudden osmotic stress conditions.

Key words: lactobacilli, osmotic stress, stimulation activity

Osmotic stress of valuable crops during droughts is a major problem for modern agriculture. It is known that some endophytic bacteria and fungi are capable of alleviating the effects of osmotic stress in plants [Das et al., 2016]. Enhanced protection against phytopathogens, which are more likely to harm stressed plants, may account for the beneficial effect of endophytic microorganisms [Zhu et al., 1997].

On the other hand, lactic acid bacteria *Lactobacillus plantarum* are known to produce plant growth promoting and antagonistic compounds like phytohormones and bacteriocins [Goffin et al., 2010].

The aim of the work was to study the effect of bacterial suspensions of a mixture of *Lactobacillus plantarum* strains ONU 12 and ONU 311 on osmotolerance and survivability of wheat cv Kuyalnyk.

Materials and Methods

Overnight cultures of lactobacilli with concentration 10^8 cells/ml were used for preparation of 0,01% suspensions. Seeds were treated with the suspensions for 1 hour. *Lactobacillus plantarum* ONU 12 and ONU 313 strains used in this experiment were initially isolated from grape must.

Control seeds were soaked in sterile distilled water instead of bacterial suspensions. Germination was carried out under greenhouse conditions in soil.

Osmotic stress was modeled by adding 20% PEG-6000 (polyethylene glycol) to the soil. In total, 200 seeds were processed: 100 were soaked in distilled water, and 100 – in the biopreparation.

During grounding the seeds were divided into four groups: control (water); control (water + PEG); lactobacilli pre-treatment + PEG; lactobacilli pre-treatment + PEG added on the 3rd day of the experiment (in order to model sudden stress conditions).

The results were recorded after 2 weeks. Seed germination, turgor intensity and survivability of plants was taken into consideration.

Results and Discussion

It was revealed that the initial seed germination was approximately the same in all four variants, which may be explained by the addition of water during seed grounding.

By the 14th day of the experiment, reduced turgor was observed in most of the plants (Table 1).

Survivability of plants was the worst in the case of using PEG without lactobacilli treatment: 2,9 ± 2,3% (instead, in the absence of PEG, 16,5 ± 5,2% of the seedlings survived).

Pre-treatment of seeds with lactobacilli yielded some interesting results. In the case of the addition of PEG immediately to the soil, the results did not differ significantly from the control ones.

Table 1

Seed germination and survivability of wheat seedlings under drought conditions following pre-treatment with lactobacilli

Group	Seed germination, %	Plants with decreased turgor, %	Dead plants, %	Normal plants, %
Control (water)	72,0±6,3%	55,5±7,0%	28,0±6,3%	16,5±5,2%
Control (water + PEG)	68,0±6,6%	59,0±6,9%	38,1±6,8%	2,9±2,3%
Lactobacilli pre-treatment + PEG (added immediately)	72,0±6,3%	39,0±6,8%	47,2±7,0%	13,8±4,9%
Lactobacilli pre-treatment + PEG (added on the 3rd day)	72,0±6,3%	58,3±7,0%	11,2±4,5%	30,5±6,5%

However, when PEG was introduced into the soil on the 3rd day of the experiment (in order to model sudden stress conditions), treatment with lactobacilli contributed to the survival of 30,5 ± 6,5% of plants (against the control 2,9 ± 2,3%). In addition, only 11.2 ± 4.5% of the plants died (against the control 38.1 ± 6.8%).

Conclusion

Pre-treatment of wheat seeds with *Lactobacillus plantarum* ONU 12+311 contributes to an increase in plant survivability under osmotic stress conditions by 27.6%.

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UDC 60:- 634.8: -579.64

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IMPROVING OF ADAPTATION OF THE THORNLESS BLACKBERRY MICROCLONES WITH USING OF BACILLUS MEGATERIUM AND ENTEROCOCCUS ITALICUS STRAINS

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Summary

The effect of bacterial cultures on the processes of adaptation to the in vivo conditions of the thornless blackberry microclones, which were cultivated in vitro on the Murashige and Skoog medium, was studied. Evaluating the growth and development rates of experimental plants over time, it was possible to establish optimal dilution of pure culture of Bacillus megaterium, which contributed to the increase of survivability of the laboratory plants in the soil and positively influenced on the external characteristics of the adapted blackberry plants.

***Key words**: in vitro culture, in vivo adaptation, microclones, Bacillus megaterium, Enterococcus italicus.*

Microorganisms and products of their metabolism find their application in solving of a wide range of different tasks. It is very promising to use microbial preparations as auxiliary agents in plant biotechnology. The convenient and rapid method of microclonal propagation, which allows to get hundreds of genetically homogeneous healthy plants for the short time, is facing with a number of methodological problems today. Different species and plant varieties need correction in the classic propagation technique. The peculiarities arise from the stage of introduction into the culture and cultivation itself [3, 5]. Nevertheless, if these stages are successfully overcome, the last and most difficult period comes for plants then - it is adaptation from in vitro to in vivo conditions.

When growing in a sterile box, plants develop in perfectly comfortable conditions, which can not be achieved in the open field. These are illumination according to the schedule, constant temperature, humidity, nutrients and all necessary compounds from the nutrient medium. Also the intensity of photosynthesis is reduced in plants under such conditions. But, what is the most important, plants are completely deprived of contact with any infections. Thus, the process of adaptation, which means getting used to normal soil conditions with every day changes, is a huge stress for the plants and a possible risk for the researcher to lose a large amount of propagated material [1]. It is expedient in this stage to use bacterial cultures to increase efficiency of adaptation and viability of the planting material. Due to their antagonistic properties to plant pathogens, some microorganisms demonstrate great potential for using them as preparations to improve the resilience of plants on the field. Literature sources [2, 4, 5] indicate a probable positive effect from some strains of the *Bacillus* and *Enterococcus* genus.

The **aim** of our study was to use the strains of *Bacillus megaterium* and *Enterococcus italicus* on thornless blackberry culture *in vitro* to improve the adaptation process to the *in vivo* conditions.

Materials and methods

Our experiment included using of *Bacillus megaterium* ONU500 and *Enterococcus italicus* ONU547 strains that were obtained at the laboratory of the Odessa National University.

Bacillus megaterium are a rod-like gram-positive spore-forming bacteria that are often associated in pairs and chains. Bacteria got their name due to large sizes that far exceed others, especially in comparison with *E. coli*. *B. megaterium* can be found in a large range of environments, but it is traditionally considered to be a soil microorganism. It is also widely used in biotechnology as a producer of recombinant proteins. Some strains have shown themselves to be powerful antagonists to phytopathogens, and some are able to accumulate phosphorus and make it an affordable component of mineral nutrition of plants [2].

For the experiment on improving the efficiency of adaptation, a two-day culture of *B. megaterium* was grown on a liquid nutrient medium LB in a thermostat at a constant temperature of 28°C [4]. Two concentrations of the microbial culture were used. There were 50 percent and 25 percent dilutions with the number of viable cells $4.70*10^7$ in ml and $2.35*10^7$ in ml, respectively. Distilled water was added to the culture just before the experiment to obtain the necessary concentration.

Enterococcus italicus - gram-positive cocci which often can be observed united in pairs and short chains. They are optional anaerobes and typical habitants of intestines of some mammals. These microorganisms can be also isolated from several kinds of cheese - they are involved in maturation processes of this product. *E. italicus* is commensal and does not have a pathogenic effect on humans or animals [3].

In previous studies, the antagonistic activity of the strain *E. italicus* ONU547 against phytopathogens in vitro was detected, but efficiency in application on live plants was not yet verified [4].

A daily culture of *E. italicus*, grown on a liquid MRS medium in a thermostat at a temperature of 36°C, was used for the experiment [3]. Two concentrations of the microbial culture were used as well. There were 50 percent and 25 percent dilutions with the number of cells, on average, $2.94*10^8$ in ml and $1.47*10^8$ in ml, respectively.

The microclones of thornless blackberry *Rubus caesius* (the Thornfree variety) were used as a subject for adaptation. Microclones for the experiment had a height above 3.5-4 cm, 4-6 leaves, and a well-developed root system. They also did not form calluses. The material for adaptation was obtained by cultivating plants in vitro in a nutrient medium of Murashige and Skoog (MS) with addition of 20 g/l of sucrose, 9 g/l of agar, and 1 mg/l of 6-benzylaminopurine (6-BAP).

Adaptation was carried out in two stages. The first one was the habituation of plants to non-sterile conditions. To this end, plants were gradually allowed access to normal air for seven days, removing the covers from sterile containers with microclones [1]. Then the plants were planted into the prepared soil. At this stage, two potentially useful bacterial cultures were tested.

After the first stage of adaptation microclones were divided into three groups. The roots of the first group of plants were kept in a 50 percent solution of bacteria for 30 minutes before planting, the second group was kept in 25 percent solution and the third was a control with sterile distilled water.

After that, the plants were planted in individual containers with prepared soil. Processes of growth and development in experimental plants were observed during 100 days after planting. The experiment was conducted separately for two potentially effective bacterial cultures.

Results

Three-time frequency of the experiments with *E. italicus* did not show a positive effect on the survivability or any other parameters of the researched plants. Both the control and the experimental plants grew almost identically, hence no significant difference was observed. However, considering the positive results of in vitro studies, it can be assumed that *E. italicus* may exhibit its potential at other stages of plant cultivation and adaptation, which requires further research.

The results of experiments with *B. megateruim* revealed significant differences.

On average, 63% of control samples were lost and only 37% survived on the 100th day of the experiment, while viability of experimental plants was at the level

up to 73%. The 25% concentration of *B. megateruim* has shown the best results - when used, the most effective adaptation of plants to the soil was observed (Table 1).

Table 1

Average growth and survivability performance of thornless blackberry microclones during the adaptation process using *B. megaterium* strain ONU 500 (average data according to three replications of the experiment)

Time passed from the planting, days	Culture dilution of *B.megaterium*, %	Average survivability of the microclones, %	Average shoot height, cm	Average number of nodes, pieces	Average number of additional shoots,
14th	50	73,33	3,73	3,76	0
	25	**83,33**	**4,20**	**4,50**	0
	Control (H_2O)	63,33	3,36	3,30	0
30th	50	66,66	4,43	4,90	0,66
	25	**80,00**	**5,56**	**6,33**	0,66
	Control (H_2O)	46,66	3,76	3,63	0,33
100th	50	50,00	7,50	8,30	0,66
	25	**73,33**	**9,73**	**10,36**	1,66
	Control (H_2O)	36,66	6,30	7,20	0,66

Adaptation experiments with *B. megaterium* allowed to establish the following:

- the 25% dilution of the *B. megaterium* culture was the most effective for optimization of the process of adaptation for the studied species.
- the 50% dilution of the *B. megaterium* culture revealed intermediate results between the control and the 25% dilution.

- exposure of roots to the 25% solution of the *B. megaterium* culture before planting allowed to increase the viability of the thornless blackberry plants 2 times compared to the control on 100th day of cultivation.
- experimental plants, which were exposed to the 25% solution, on average, grew 1.3 times more rapidly and formed 1.2 times more nodes than the control did.

Also, the plants, which were exposed to the 25% solution of *B. megaterium*, had the biggest leaves with the most saturated shade of green among all other plants in the experiment (Fig. 1). This fact confirms more intensive photosynthesis in plant tissues and, as a result, the most effective adaptation within our research.

Fig. 1. Appearance of the blackberry plants after 4 weeks of adaptation (on the left - experimental plants, roots of which were kept in the 25% solution of the *B. megaterium* culture, on the right - control).

Thus, the first results of studies on the the thornless blackberry microclones allowed to confirm experimentally that *B. megateruim* strain ONU 500 positively influences on adaptation of laboratory plants to the soil conditions.

Conclusions

In our experiments, a positive effect of the 25% solution of the two-day *B. megaterium* culture on the processes of microclones adaptation of a valuable thornless blackberry variety from in vitro to in vivo conditions was established. The

obtained data testify to the real possibility of using the ONU 500 strain to increase the survivability of the micropropagated plants in the open field conditions and to improve the growth rate and quality of the seedlings.

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UDC 579.6

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THE PRODUCTION PHENAZINE COMPOUNDS BY SOME STEMS OF *PSEUDOMONAS AERUGINOSA*

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The work was carried out in the Biotechnology Research and Training Center I. I. Mechnikov National University. The various *Pseudomonas* phenazine synthesis intensity and the compound production dependence on the forms of microbial existence were compared.

Key words: *Pseudomonas genus, phenazine-1-carboxylic acid, oxychlororaphine, pyocianine, planktonic culture, biofilm*

One of the most important tasks of modern biotechnology is the creation of highly productive strains of microorganisms capable of super-synthesis biologically active substances [1]. Especially promising for solving the corresponding problems turned out to be bacteria of the genus *Pseudomonas*, which has a natural ability to synthesize more than 300 different antimicrobial substances. The most diverse group of antimicrobial compounds produced by bacteria of the genus *Pseudomonas* constitutes phenazine pigments. Different strains of the bacteria *Pseudomonas* produce oxychlororaphine, blue pigment – pyocyanin and phenasine-1-carboxylic acid [3, 9].

The research was carried out in the Biotechnology Research and Training Center I. I. Mechnikov National University. The work was used *Pseudomonas* strains: *Pseudomonas chlororaphis* ONU 305, *P. fluorescens* (ONU 303, 13225 ATCC) and *P. aeruginosa* (ATCC 9027, ATCC 15692). Cultivation of bacteria was performed in liquid culture medium King B a duration of 8 days at 37 °C in the first

variant, experiments were carried out on the swing to obtain planktonic cultures, and the second in the wells of polystyrene tablet for the formation of their biofilm [5, 6].

Researches have shown that phenazine-1-carboxylic acid, which is the precursor of all other derivatives of the phenazine pigments, is synthesized by all used *Pseudomonas* strains [8]. The cells of the studied microorganisms during the transition from the exponential to the stationary phase of development produced the highest number of phenazine compounds.

As for oxychlororaphine, it was found that it is synthesized in large quantities in the absence of oxygen. The highest rates were found in strains *P. chlororaphis* ONU 305 and *P. aeruginosa* ATCC 15692 in biofilm. The highest rates of the amount of compounds formed by the strains *P. fluorescens* ONU 303, ATCC 13225 and *P. chlororaphis* ONU 305 corresponded to the second (for pyocyanine) and the third (for phenazine-1-carboxylic acid and oxychlororaphine) added.

The bacteria, which present in the biofilm, produced more intensively the corresponding metabolite in comparison with the planktonic way of existence. During the formation of biofilm, the studied strains formed, on average, 1.5 to 15 times more than phenazine-1-carboxylic acid and oxychlororaphine, than planktonic cultures. In the case of the synthesis pyocyanine was recorded the opposite trend: the planktonic form of existence of microorganisms more active, 1.3 – 2.7 times more intensively produced is derived [7]. In general, the formation of this metabolite by strains of the *Pseudomonas* that do not belong to the species *P. aeruginosa* is uncommon. Therefore, it was suggested that the investigated strains of *P. fluorescens* ONU 303 and *P. chlororaphis* ONU 305 received a gene responsible for the synthesis pyocyanine, when interacting with other microorganisms; it was isolated from the rhizosphere [4].

Therefore, the study of environmental factors and mechanisms of intercellular interaction, as defined by numerous studies, significantly affects the biosynthesis of phenazine compounds is a very important direction of modern biotechnology [2].

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UDC 579.6

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STRATIFICATION OF ENDOSPOREFORMING BACTERIA IN BOTTOM SEDIMENTS OF BLACK SEA

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Aerobic Endospore-forming Bacteria (AEB) represent an interesting group of mesophilic bacteria for geological and environmental studies. The optimal temperature for their growth are in the range of 25-30 ° C. The main places of their habitation are soil, active sludge and bottom sediments of fresh water basins. In the marine environment they come from land with annual and rainy runoff and through the air. From the upper aerobic zone of the sea, along with organic residues, these planktonic bacteria sediment to the bottom of the sea. It can be argued that in conditions of low temperatures, complete absence of molecular oxygen, high concentrations of hydrogen sulfide and methane in the deep sea of the Black Sea, these bacteria are unable to carry out their life processes and multiply. But their endospores are resistant to the negative effects of environmental conditions, which makes them able to maintain viability in sedimentary matter for hundreds of years [1, 2]. Thus, the amount and taxonomic composition of the endosporeforming aerobic bacteria found on certain horizons in the bottom sediments can serve as a characteristic of environmental conditions (including hydrological) of a certain historical period. It is accepted that one centimeter bottom sediments accumulate over 20 years [3].

Samples of the Black Sea sediments were collected in 2011 during the international expedition on the German vessel Meteor at five points (with numbers 242, 233 on the Turkish shelf, and 258 and 269, on the shelve of the Crimean peninsula, respectively), at depths from 880 to 2000 m and were gifted to us by Professor Yu.P. Zaytsev from the Institute of Marine Research (Odessa). Kerns were

cut into sectors of 5 cm (0 corresponds to the surface of the bottom). The work was carried out using classical microbiological and statistical methods. Pasteurized and non-pasteurized sediment suspensions were inoculated on meat-peptone agar prepared from 1.8% sea salt solution and cultivated at 25 ° C for counting bacteria (as colony forming units - COO) of mesophilic lifestile and 5 ° C for psychophilic, respectively. The research was carried out in five repeats.

The number of psychophilic AEBs, depending on the depth of the siege horizon, took values in a wide range (from 0 to 10^6 CFU / g), which may be explained by the assumption of the existence of local ecological gradients in the process of their formation. The number of mesophilic AEB co-livated in the same range (from 0 to more than 300×10^6 CFU / g), but more evenly compared to psychophytes. In general, the results of changes in the number of AEB in bottom sediments can be a reflection of climatic changes in the Black Sea region during the last 1000 years. According to the analysis of fatty acid composition and PCR analysis, the bacteria studied from the corresponding horizons are identified as: *B. cereus, B. pumilis, B. megaterium, B. licheniformis, B. subtilis, B. atrophaeus, B. mycoides, B. viscosus, B. luciferensis, B. oleronius, B. halmapalus, B. thuringiensis, B. thuringiensis israelensis, Paenibacillus macerans, Paenibacillus polymixa, Paenibacillus alvei, Paenibacillus larvae pulvifaciens, Brevibacillus choshinensis, Brevibacillus parabrevis, Brevibacillus reuszeri, Lysinibacillus sphaericus, Virgibacillus pantothenticus.*

The proposed microbiological approach for detection of climatic changes by the content of AEB can be applied to paleo-climatic researches and also the study of the effects of long-term anthropogenic climate change [3], but the authors consider it necessary to compare the results of microbiological studies with the results of paleogeological, physical and chemical studies.

Keywords: endospore, deepwater sediments, *Bacillus*

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UDC:602.6:582926:577.218

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OBTAINING OF TRANSIENT EXPRESSION OF GUS AND GFP GENES IN PHYSALIS PERUVIANA PLANTS

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Abstract. *Physalis peruviana* plants were infiltrated with lines of *Agrobacterium rhizogenes* (strain A4) and *A. tumefaciens* (strain GV 3101). Lines of *A. rhizogenes* carried the following constructions: pICH 5290 (*gfp* gene), pICBv19 (*gus* gene) *A. tumefaciens* carried the construction pCB 131 (*fbp*B (ΔTMD)::*gfp* gene, *fbp*B gene codes the tuberculosis antigen Ag85B). For several variants of experiment was additionally used suspension of *A. tumefaciens* (GV 3101) which carried construction pICH 6692 (with suppressor of gene silencing). Positive results for transient expression were obtained, using the constructions (pICH 5290 and piCBv19) with reporter genes in *Physalis* plants. Also, optimal conditions for transient expression of the transferred genes into *Physalis peruviana* plants were identified. Maximum of *gfp* expression was observed between 5^{th} – 12^{th} days after infiltration. According to the results of *gus*- and *gfp*- activity, the most suitable for infiltration were young leaves with intensive growth (2-nd, 3-rd and 4-th from the

plant apex). Constructions pICH 5290 and piCBv19 are suitable for further use in transformation experiments with *Physalis peruviana* plants.

Keywords: *Physalis*, *Agrobacterium*, transient expression, *gus*, *gfp*.

Introduction. *Physalis* is a genus of *Solanaceae* family. Due to rich biochemical composition (the main components are 15-desacetylphysabubenolide and betuline), *Physalis* species have antitumor effect and used to treat abscesses, coughs, fevers, and sore throat. Also, biochemical composition of *Physalis* plants can be improved by biotechnological methods in order to obtain pharmaceutical proteins.

Material and Methods. The aim of research was to detect *Agrobacterium*-mediated transient expression to identify the optimal conditions for transient expression of the transferred genes into *Physalis peruviana* plants.

The object of the research was plants of *Physalis peruviana*. The seeds were germinated in the glass flasks on MS medium [1]. The 2-month age plants were transferred into the pots with soil and were grown in the greenhouse (22-26^{0}C, 14-hour light period, illumination - 3000 - 4500 lx).

After 2 weeks of growing in greenhouse, plants were infiltrated with lines of *Agrobacterium rhizogenes* (strain A4) and *A. tumefaciens* (strain GV 3101). Lines of *A. rhizogenes* carried the following constructions: pICH 5290 (*gfp* gene), pICBv19 (*gus* gene) [2]. *A. tumefaciens* carried the construction pCB 131 (*fbp*B (ΔTMD)::*gfp* gene, *fbp*B gene codes the tuberculosis antigen Ag85B).

Infiltration of *Agrobacterium* suspensions was conducted for both pure lines and lines mixed with *A. tumefaciens* (GV 3101) which carried construction pICH 6692. Construction pICH 6692 contained gene of p19 protein – suppressor of gene silencing [3]. Infiltration of whole plants was carried out in a vacuum chamber, under pressure 0,1 mPa.

Transient expression of *gfp* gene began in 3 day after infiltration. Detection was carried out every day during eighteen days. Fluorescence of *gfp* was observed under UV light (400 nm) in dark room [4, 5].

Detection of *gus* genes was carried out by histochemical assay on 4^{th}, 5^{th}, 6^{th} days (GUS activity) by Jefferson [6].

Results and discussion. *Gfp*- and *gus*-positive results were obtained for all infiltrated plants. Separate areas with green fluorescent were detected. Maximum of *gfp* expression was observed between 5^{th} – 12^{th} days after infiltration (fig.1). According to the results of *gus*- and *gfp*- activity, the most suitable for infiltration were young leaves with intensive growth (2-nd, 3-rd and 4-th from the plant apex).

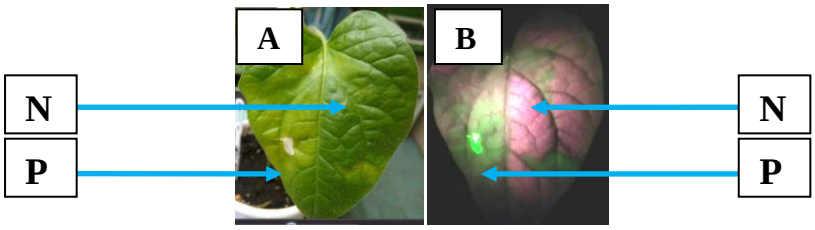

Fig.1.Transient expression of *gfp* gene (construction pICH 5290) on the 12^{th} day after infiltration. A – 2^{nd} leaf of *Physalis* under daylight, B - the same leaf under the UV light, N – zone without *gfp* expression, P – zone with *gfp* expression

Fluorescence was weak when pCB 131 was used. In this case, zones of necrosis on the 5^{th} day appeared. It might be caused with some toxic effects of tuberculosis proteins.

There was no significant difference in expression levels of genes neither with the suppressor of gene silencing nor without it.

It was shown the possibility of transient expression using the constructions (pICH 5290 and piCBv19) with reporter genes in *Physalis* plants.

In future we plan to continue use the transformation protocol for production pharmaceutical proteins.

Conclusions. *Agrobacterium*-mediated transient expression of *gus* and *gfp* genes was detected in *Physalis*. Possitive results for transient expression were obtained, using the constructions (pICH 5290 and piCBv19) with reporter genes in *Physalis* plants.

Also, optimal conditions for transient expression of the transferred genes into *Physalis peruviana* plants were identified.

Maximum of *gfp* expression was observed between 5^{th} – 12^{th} days after infiltration.

According to the results of *gus*- and *gfp*- activity, the most suitable for infiltration were young leaves with intensive growth (2-nd, 3-rd and 4-th from the plant apex). Constructions pICH 5290 and piCBv19 are suitable for further use in transformation experiments with *Physalis peruviana* plants.

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UDC 579.64

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EFFECT OF LACTOBACILLUS PLANTARUM ON GROWTH OF SOME ORNAMENTAL PLANTS

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Summary. Mixture of strains *L. plantarum* ONU 12 and *L. plantarum* ONU 311 could improve growth of lawn grasses, clove and matthiola increasing root length and height of seedlings.

Key words: lactobacilli, growth characteristics, stimulation of plant growth

Introduction. *Lactobacillus plantarum* can be used to protect and stimulate plant growth [2, 3, 4]. These microorganisms have stimulating effect on growth of some plants, namely, wheat, tomatoes, lettuce, lettuce and radish [2]. That is why our goal was to check the activity of the mixture of these strains in a wider spectrum of plants, namely, on some monocotyledonous and dicotyledonous ornamental plants.

The **aim** of this work was to study the effect of a mixture of strains *L. plantarum* ONU 12 and *L. plantarum* ONU 311 on growth of some ornamental plants.

Materials and Methods. The materials of the study were two strains of bacteria of the species *Lactobacillus plantarum*: *Lactobacillus plantarum* ONU 12 and *Lactobacillus plantarum* ONU 311, isolated from grape must (Ukraine). Lactobacilli were cultured overnight in MRS liquid nutrient medium [1] at 37 ºC until a concentration of 2 - 3 x 10^9 CFU/ml was reached. Then from the obtained cultures a mixture was prepared in a ratio of 1:1, and it was further diluted in sterile distilled water in following concentrations: 1%, 0,1%, 0,01%, 0,001%, 0,0001%. As test plants, the following decorative plants were selected:

- Lawn grass mix "Sportivnaya" 50% - perennial ryegrass *Lolium perenne* L. Gator, 20% - red fennel *Festuca rubra* L. Coral, 30% - thin meadow *Poa pratensis* L. Evora;

- Lawn grass mix «Universal» (20% - perennial perennial ryegrass *Lolium perenne* L. Newman, 30% - red fescue red *Festuca rubra* L. Gerald, 25% - fine meadow *Poa pratensis* L. Evora, 15% - fennel red *Festuca rubra* L. Kazan, 10% - fennel red *Festuca rubra* L. Samantha);

- lawn grass mix "Golf" (45% - reed cane *Festuca arundinacea* Schreb. Mustang, 40% fennel reed *Festuca arundinacea* Schreb 1771 Frrsc1, 15% - meadow *Poa pratensis* subsp. *pratensis* Tzvel (Balin);

- clove pink double blend *Diánthus plumárius* L.

- Matthiola bicolor *Matthiola bicornis* W.T. Aiton

It was these test plants that were chosen because they grow better in conditions of acidic pH of the soil (5.5 - 6.5), namely, such, with an excess of peat, traditionally used in greenhouse complexes.

Seeds of ornamental plants were soaked in suspensions of lactic acid bacteria for one hour and then transferred to the soil. In control, the seeds were soaked for an hour in water. 1 g of seeds of each of the lawn variants; 0,4 g of mathiols; 0,1 g of cloves were sown. The treated seeds after the exposure were sown in 2,5 liters of pots with a substrate, which were subsequently placed in greenhouse (Fig. 1).

Fig. 1. Test plants in the greenhouse.

The room temperature was 25 ° C, the light was within 12 hours. Commercial peat substrate "Polissky" was used. After 7 days of germination, the length of the

roots and shoots of plants were measured. Mean values and confidence intervals were evaluated.

Results and Discussion. Treatments of seeds with a mixture of strains *L. plantarum* ONU 12 and *L. plantarum* ONU 311 resulted in improvement of morphological parameters of seedlings, but the positive effect varied depending on the concentrations of the mixture and the type of test plants (Fig. 2).

Fig. 2. Improvement of the morphological indexes of the seedlings of lawn "Universal" after the treatment of seeds by lactobacilli (left) compared with the control (right).

The difference was noted even for various lawn grass mixtures. Thus, the slightest influence was found for the lawn "Golf". The average root length after treatment with lactobacilli remained unchanged, and the height of seedlings increased by 15,7 – 19,6% when treated with *L. plantarum* ONU 12 and *L. plantarum* ONU 311 mixture at concentrations 0,1% and 0,001%.

In case of "Sportivny" lawn, an improvement in root lengths and heights of seedlings was observed for the majority of cases, with the exception of treatment with a concentration of lactobacilli 0,0001%, which had no effect on seedling growth. Treatment of seeds of this lawn mixture with concentrations of lactobacilli from 1,0% to 0,001% caused an increase in average height 3,8 – 8,0%, and in root length – 13,7 – 18,1%.

For the grass mixture of the lawn "Universal" a positive effect was found for almost all tested concentrations. The average length of the above-ground part increased in 5,2 – 11,8%, and the root - in 5,6 – 12,8%.

Lactobacilli influenced even more positive on dicotyledonous plants – cloves and matthiola: mean height of plants increased in 28,3 – 68,7 % and mean root length – in 14,9 - 68,7%.

Conclusion. Mixture *L. plantarum* ONU 12 and *L. plantarum* ONU 311 positively influenced growth of lawn grasses, clove and matthiola. The best dilutions were 0,1 - 0,001 % from overnight culture that was equal to concentration of bacterial cells 10^5 - 10^7 CFU/ml.

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UDC 579.695

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BIOACCUMULATION OF COPPER BY THIOBACTERIA ISOLATED FROM THE BLACK SEA

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Heavy metals are a group of metals with density greater than 5 g/cm^3. They persist in nature and consequently tend to accumulate in food chains. Although relatively high levels of these elements are found in natural environment, their presence as a contaminant in ecosystems results mainly from anthropogenic activities. Some heavy metals such as nickel, iron, copper and zinc are essential to metabolic reactions and are required as trace elements by the organisms. Others like mercury, silver and cadmium have no biological role and are harmful to the organisms, even at very low concentrations [3].

The traditional approaches for removing or recovering metals, such as precipitation, oxidation reduction, ion exchange, filtration, electrochemical processes, membrane separations, and evaporation, all exhibit several disadvantages, such as high cost, incomplete removal, low selectivity, high energy consumption, and formation of toxic slurries that are difficult to be eliminated [3].

Today problem solving process in the field is often associated with the necessity of finding microorganisms with extraordinary abilities to accumulation of heavy metals. Checking the efficiency of laboratory strains in metal accumulation as well as the isolation of microorganisms from natural or secondary habitats often lay among the basic tasks to be performed in such investigations.

The aim of the research was to investigate the biosorption of Cu(II) by the neutrophilic marine strains of genus *Thiobacillus.*

Materials and methods. The variety of different strains of *Thiobacillus sp.* was obtained by growing the strains on the Beijerinck medium (g/l): $Na_2S_2O_3$ – 5,0 г;

$NaHCO_3$ – 1,0; Na_2HPO_4 – 0,2; $MgCl_2$ – 0,1; NH_4Cl_2 – 0,1; H_2O – 1000 ml, with the addition of 0.02% yeast extract (to create mycotrophic conditions) and pH value 7,0 [2].

The initial solution of Cu(II) was prepared at concentrations of 0.1 μM.

A bottle of 250 ml was used for each individual strain, where 100 ml of the initial solution and 1 ml of the bacterial suspension were added.

Liquid Beijerinck medium with addition of 0.02% yeast extract and 1 μM CuSO4 × 7H2O was used as a control. However, it did not contain any microorganisms.

The evaluation of the metal-accumulative activity of thiobacteria was carried out by estimating the degree of purification of water from copper ions:

$$\alpha = [(C0-C) / C0] \times 100\%,$$

where C_0 and C are the concentrations of a specific pollutant before and after treatment [1].

Analysis of solutions for the content of residual copper was carried out using atomic absorption spectroscopy at the AAC-1 (Germany) and C-115PK Selmi (Ukraine) devices.

The reliability of the obtained results was evaluated according to the Student's criterion with probability of $p < 0,05$.

Investigation of the intensity of the biosorption of Cu(II) by the neutrophilic marine strains of *Thiobacillus* was carried out at a temperature of 30,0 ± 2,0 °C during 7 days under standard conditions.

Results. It was shown that on the first day of the study, the metal-accumulation activity of strains of thiobacteria isolated two checkpoints, deep seawater and tide wave line, was almost not observed (table 1). Some strains were able to accumulate from a solution of 10.56 to 17.95 %% copper concentration (table 1). For other strains, the metal-accumulation activity for the first day was nearly at the zero level (table 1). The greatest ability to copper removal was shown by strains *Thiobacillus sp*. BSS_4 and *Thiobacillus sp*. BSS_5 that were isolated from seawater near the Biological Station of Odesa I.I. Mechnikov National University (table 1). The percentage of copper extracted from the aqueous solution had reached 33.97 and 44.71 %%.

Table 1

The degree of biological purification of aqueous solution from $CuSO_4 \times 7H_2O$ at initial concentration of 1.0 µM on the first day of the study

Isolation place	Strain name	MCI of copper	Copper accumulation	
			Absolute value	% of extraction
Tide wave line, Biological Station, ONU	*Thiobacillus sp.* BSZ_1	0,01 M	51,75 ± 1,20	17,95
	Thiobacillus sp. BSZ_3	0,02 M	54,26 ± 1,25	13,97
	Thiobacillus sp. BSZ_5	0,02 M	56,41 ± 3,51	10,56
Tide wave line, Kovalevskiy Dacha	*Thiobacillus sp.* DKZ_2	0,02 M	56,52 ± 1,31	10,39
	Thiobacillus sp. DKZ_3	0,02 M	53,70 ± 1,24	14,87
	Thiobacillus sp. DKZ_4	0,02 M	62,97 ± 1,46	0,16
Seawater, Biological Station, ONU	*Thiobacillus sp.* BSS_4	0,01 M	41,65 ± 0,96	**33,97**
	Thiobacillus sp. BSS_5	0,02 M	34,87 ± 0,81	**44,71**
	Thiobacillus sp. BSS_6	0,02 M	56,92 ± 1,32	9,75
	Thiobacillus sp. BSS_8	0,01 M	54,01 ± 1,25	14,36
Seawater, Kovalevskiy Dacha	*Thiobacillus sp.* DKS_2	0,01 M	58,34 ± 1,35	7,50
	Thiobacillus sp. DKS_3	0,02 M	57,89 ± 1,34	8,22
	Thiobacillus sp. DKS_4	0,01 M	51,75 ± 1,33	8,69

However, on the tenth day of observations, almost all experiments showed significant decrease in the residual content of copper ions in the solution. The maximum level of copper extraction reached 89.24%, in case of *Thiobacillus sp.* DKZ_4. Strains *Thiobacillus sp.* BSS_4, *Thiobacillus sp*. BSS_5 and *Thiobacillus sp.* BSS_6 also demonstrated comparatively high clearance rate of copper ions (85.6 – 88.42 %%) (table 2).

A high degree of copper extraction from the solution was reported for strains *Thiobacillus sp*. DKS_2 and *Thiobacillus sp*. DKS_3 which were isolated from seawater near Kovalevskiy Dacha (87.27 - 88.97 %%) (table 2).

Table 2

The degree of biological purification of aqueous solution from $CuSO_4 \times 7H_2O$ at initial concentration of 1.0 μM on the tenth day of the study

Isolation place	Strain name	MCI of copper	Copper accumulation	
			Absolute value	% of extraction
Tide wave line, Biological Station, ONU	*Thiobacillus sp.* BSZ_1	0,01 M	22,29 ± 0,53	6,17
	Thiobacillus sp. BSZ_3	0,02 M	24,01 ± 0,57	61,41
	Thiobacillus sp. BSZ_5	0,02 M	9,61 ± 0,21	**84,55**
Tide wave line, Kovalevskiy Dacha	*Thiobacillus sp.* DKZ_2	0,02 M	11,35 ± 0,26	82,00
	Thiobacillus sp. DKZ_3	0,02 M	11,59 ± 0,27	81,62
	Thiobacillus sp. DKZ_4	0,02 M	6,78 ± 0,16	**89,24**
Seawater, Biological Station, ONU	*Thiobacillus sp.* BSS_4	0,01 M	8,95 ± 0,21	**85,60**
	Thiobacillus sp. BSS_5	0,02 M	8,02 ± 0,19	**87,12**
	Thiobacillus sp. BSS_6	0,02 M	7,17 ± 0,17	**88,42**
	Thiobacillus sp. BSS_8	0,01 M	6,89 ± 0,16	**89,07**
Seawater, Kovalevskiy Dacha	*Thiobacillus sp.* DKS_2	0,01 M	46,43 ± 1,07	26,37
	Thiobacillus sp. DKS_3	0,02 M	6,95 ± 0,16	**88,97**
	Thiobacillus sp. DKS_4	0,01 M	8,03 ± 0,19	**87,27**

To conclude, according to the results obtained, it can be said that the thiobacteria of the Black Sea can be used as an object for the design and development of a new biotechnological approaches to the purification of contaminated water from heavy metals.

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Наукове видання

СУЧАСНІ ПРОБЛЕМИ МІКРОБІОЛОГІЇ ТА БІОТЕХНОЛОГІЇ

Міжнародна конференція молодих вчених

Тези доповідей

20-23 червня 2018, Одеса

За редакцією авторів

Підп. до друку 26.06.2018. Формат 60х84/16.
Ум.-друк. арк. 7,56. Тираж 50. Зам. № 1777.

Видавець і виготовлювач
Одеський національний університет імені І. І. Мечникова
Свідоцтво суб'єкта видавничої справи ДК № 4215 від 22.11.2011 р.
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