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Borodin S.V., Huet A.S., Dvorshchenko K.O.

LIPID PEROXIDATION AND EXPRESSION OF *NOS2* GENE IN BLOOD OF PATIENTS WITH OSTEOARTHRITIS

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Abstract. *Osteoarthritis is the most common joint disease and a major cause of functional limitation, pain in adults and development of complications. This disease is a common cause of disability worldwide. The pathogenesis of this degenerative process is not completely understood. But it is known that the development of osteoarthritis is associated with a low-grade inflammation. In this study, we determined the content of lipid peroxidation products and Nos2 gene expression in blood of patients with osteoarthritis of the knee II-III degree. The increase in the content of diene conjugates, TBA-active products and Schiff bases in the blood plasma in the group of osteoarthritis patients has been established. Also, Nos2 gene expression increased compared with the group of apparently healthy people. The obtained data indicate the activation of inflammatory and oxidative stress processes in osteoarthritis patients.*

Key words: *osteoarthritis, lipid peroxidation products, Nos2 gene expression.*

Introduction

Osteoarthritis is a chronic degenerative joint disease characterized by the loss of cartilage in the joints and is commonly associated with pain, stiffness, swelling, limited mobility, and general deterioration of the body. Degenerative changes in the cartilage result from metabolic disorders in the joints, due to traumas, inborn errors of development, genetically predetermined mutations, adiposity, and the activation of free-radical processes [1]. These factors may lead to an excessive synthesis of proinflammatory cytokines, which, in turn, stimulate the expression of proinflammatory genes, such as *NOS2* (encoding the inducible nitric oxide synthase (iNOS), an important mediator of inflammatory reaction). For example, due to the induction, iNOS produces a large amount of nitric oxide (NO) with a nonspecific microbicidal effect destroying the surrounding cells [1, 2].

Considering the above, the goal of the study was to analyze the intensity of free radical processes and the expression of *Nos2* gene in the blood of patients with osteoarthritis.

Methods

The study involved 21 people aged 39 to 70 years. Volunteers were divided into 2 groups: the first group (n=10) - relatively healthy people, the second group

(n=11) - patients with osteoarthritis of the knee II-III degree. The content of diene conjugates was determined by spectrophotometric method, and that of Schiff bases, by fluorometric method. The content of TBA-active products was determined by reaction with thiobarbituric acid. The total protein content was determined by the Lowry method [3].

RNA was obtained by Chomczynski method (guanidinium thiocyanate-phenol-chloroform extraction). Using a «Thermo Scientific Verso SYBR Green 1-Step qRT-PCR ROX Mix» commercial kit we carried out the cDNA synthesis and qPCR. After each amplification cycle, the SYBR Green I fluorescence was read, and the melting curve was built upon completing the reaction to control the formation of the primer dimers and the reaction specificity. The initial quantification of mRNA was calculated using the $\Delta\Delta C_T$ Method, a comparative C_T method, and the efficiency of PCRs was equal ($Ex = (10^{-1/slope})-1$, slope < 0. The relative quantification of mRNA of the indicated gene was normalized to mRNA of *Actb* gene.

The study results were statistically treated using the «GraphPad Prism 8.4.3» software parcel. They were tested for normal distribution using the Shapiro–Wilk test. The subsequent calculation was performed using Student's t-test. The obtained results were reduced to the mean arithmetic±standard deviation (SD). The results were considered significant at $p \leq 0.05$.

Results

It was found that in the group of patients with osteoarthritis of the knee joints, the content of diene conjugates, TBA-active products and Schiff bases in the blood plasma increases by 2,1; 1,9 and 2,4 times ($p \leq 0,01$) respectively compared with the group of apparently healthy people.

In our further studies, we have shown that in the blood the level of *Nos2* gene expression in the group of patients with osteoarthritis of the knee joints was higher by 5,7 times ($p \leq 0,001$) if compared to the group of apparently healthy people (Fig. 1).

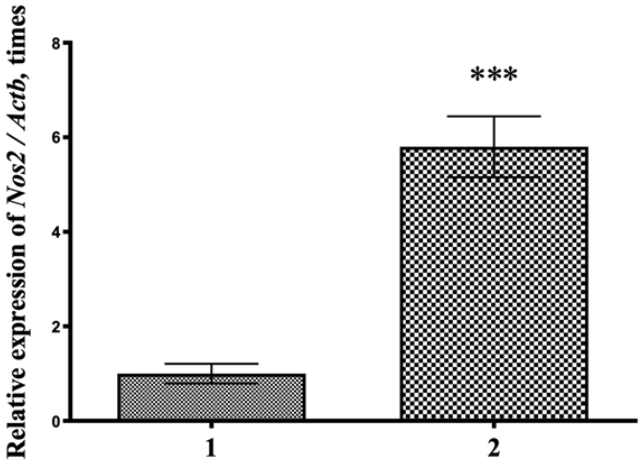

Figure 1. *Nos2* gene expression in the group of patients with osteoarthritis of the knee joints. 1 - the group of apparently healthy people; 2 - patients with osteoarthritis of the knee II-III degree; ***$p \leq 0.001$ relative to the group of apparently healthy people

Discussion

At the onset of the disease, free radical processes are activated in chondrocytes and synoviocytes, the formation of reactive oxygen species (ROS) increases, oxidation processes are significantly enhanced, which leads to the development of oxidative stress - OS (in particular, lipid peroxidation - LPO), which disrupt cell metabolism, damage enzymes and structural proteins of cartilage tissue, which leads to cell death [1, 4].Therefore, an increase in the level of ROS can activate catabolic signaling, cause OS, inhibit anabolic signaling pathways, and increase the expression of a number of genes involved in the development of degeneration and inflammation [5].

Conclusions

It has been established that in patients with osteoarthritis of the knee joints the content of both diene conjugates and Schiff bases, as well as the content of TBA-active products and *Nos2* gene expression increased compared with the group of apparently healthy people. The findings suggest the activation of inflammatory and destructive processes in the tissue.

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PEST CONTROL STRATEGIES AND GMOS

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Abstract. *An overview of the main possibilities of plant protection against insect pests due to genetic modifications is presented. The main strategies to prevent the formation of resistance to Bt toxin have been identified. The problems of optimization of Ukrainian legislation in the field of GMO products regulation are highlighted.*

Key words: *GMO, plant protection, product regulation.*

Results and Discussion

Before genetically modified plants appeared, chemical pesticides, which are relatively expensive and potentially dangerous, were used to control insects harming plant crops. In 2007, 15-20 billion dollars were spent on chemical pesticides. Biological insecticides are usually highly specific to certain insect species and safe for humans and other higher mammals.

As for genetically modified plants, there are two main groups of strategies for increasing their resistance to insects: plant production of the *Bacillus thuringiensis* insecticidal protoxin and strengthening of the plant's own protection mechanisms against insects (plant proteins, for example, α-amylase inhibitor, protease inhibitor, lectin).

During the evolutionary processes plants have acquired their own mechanisms of insect protection, but they are not so strong and effective in a natural environment, so several strategies, which are based on the principles of natural plant resistance to insects, have been developed. During the process the plant resistance gene is isolated, a strong promoter is added, and a transgenic plant culture, which produces high levels of defensive proteins, is created. Protease inhibitors based on *Vigna unguiculata* genetic modification are used. Beans containing about 2 mg of inhibitor per mg of plant protein showed high toxicity to insects but were safe for humans and mammals. To increase safety, the gene can be cloned into the tissues of plants that are not consumable by humans.

Vigna unguiculata has an α-amylase inhibitor in its beans, which has a protective effect against four-spotted and Chinese grainworm. The α-amylase inhibitor gene was isolated and placed under the control of a strong bean-specific phytohemagglutinin gene promoter.

Then, the field pea (*Pisum sativum*) was transformed. The level of resistance to grain eaters was found to be proportional to the α-amylase inhibitor amount.

Cholesterol oxidase is a bacterial enzyme catalyzing the oxidation reaction of 3-hydroxysteroids to ketosteroids and H_2O_2. Small amounts of this enzyme have high insecticidal activity against ball weevil larvae. Cholesterol oxidase destroys

the epithelial membrane of the insect midgut, thus killing them. The cholesterol oxidase gene was isolated from a *Streptomyces* strain and cloned into a vector. The gene was under the control of the *Scrophularia nodosa* virus promoter and the terminator of the nopaline synthase gene.

Currently, about 60 subspecies of *B. thuringiensis* are known, all of which produce toxins (Cry-toxins – Bt proteins, cry proteins, δ-endotoxins, crystal proteins (proteinaceous inclusions)), which have a highly specific entomopathogenic effect on certain groups of insects. Studies have shown that the N-end of the molecule is highly conserved (~98%), and the C-end is more variable (~45% of conserved sequences). It was determined that the entomopathogenic property of the protoxin depends on the first 646 amino acids at the N-end [4].

Genetically modified cotton, in which the Cry1Ac gene is expressed, is now actively used in Pakistan. However, there are concerns that insects (cotton bollworm) may develop toxin resistance [3]. Therefore, a vector-based on two Cry1Ac+Cry2A genes was constructed. Cry1Ac gene under the control of two 35S promoters of cauliflower mosaic virus and 35S terminator; Cry2A under the control of common figwort mosaic virus promoter and nopaline synthase terminator [3]. As a result, several lines of vectors were obtained, one of which showed very good results when transformed into a plant (insect mortality was 93%). High expression of target genes in the samples was observed (from 3,28 to 7,72 μg/g) [3]. Currently, a combined strategy of controlling insect pests prevails in the world: genetically modified crops, resistant to both herbicides and insects, are bred.

What are the main strategies for the insect resistance to Bt toxin development preventing? First, growing the non-transgenic crops on 20% of the field area. Transgenic crops are grown on 80% of the field, where 99.9% of insects do not survive, and on the other 20% – many more insects with the lack of resistance genes survive. Therefore, the principle is that any resistance genes will be lost during multiple crossings of insects in the same field [4].

Second, two or more Bt toxins usage. Resistance appearance to two toxins at the same time is less probability.

Third, a transformation of plants simultaneously in two ways: Bt toxin and another biological insecticide (for example, α-amylase inhibitor).

Fourth, combining chemical and biological methods. Creating transgenic plants with the Bt toxin gene, but additionally spraying small amounts of chemical insecticides.

Fifth, expression of the toxin in transgenic plants for a short period (specific inducible promoters) should reduce the selection pressure for resistant insects.

In 2015, genetically modified plants were grown in 28 countries. In another 34 countries, the import of genetically modified products was allowed. Another 11 countries allowed field research on genetically modified plants. Top 5 countries with the largest cultivation of genetically modified plants: USA (over 70 million hectares), Brazil (>40 million hectares), Argentina (>24 million hectares), India (11 million hectares), Canada (~ 11 million hectares) [2].

The legal status of genetically modified plants differs significantly in different countries. In 2015, the cultivation and sale of genetically modified plants were prohibited in 38 countries (19 of them are in Europe, including Ukraine) [1, 2].

Biotechnological use of genetically modified plants is allowed in 28 countries. In total, over 1000 legislative acts have been adopted in all countries regarding the circulation and use of genetically modified plants. In 2020, genetically modified plants were grown in 42 countries [1].

In 2009, Ukraine introduced a requirement for mandatory labeling of all food products with the inscription "without GMOs" or "with GMOs". In 2012, an attempt was made to optimize the legislation on product labeling in accordance with global practice. The bill was not adopted [1].

As a result, no GMO has been registered in Ukraine yet. It is worth noting that the import and circulation of GMOs and GMO products in Ukraine are actually prohibited. The creation, testing, transportation, and use of genetically modified organisms in Ukraine are allowed only for organisms entered into the state register (2017). Ukraine has a situation where the register of allowed-for-use genomes exists, but it is still not filled. And that is why it looks like GMOs are allowed in our country, but de facto they are prohibited.

Conclusion

There is a big variety of modern pest control methods with the help of GMOs, which may result in products cheapening. As for Ukraine no GMO has been registered yet. And since this is so, no one conducts proper checks on the content of GMO products. Ukrainians consume products and do not even know whether they actually contain GMOs. On the basis of such a situation, a huge shadow market of GMO products, which are cheaper than ordinary ones, has formed in Ukraine.

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Finohenova M.O., Semenets A.S., Galkin M.B.,
Galkin B.M., Filipova T.O.

INFLUENCE OF THE CELL-FREE MARINE BACTERIUM SUPERNATANTS AFTER MONO- AND CO-CULTIVATION ON *PSEUDOMONAS AERUGINOSA* ATCC 27853 STRAIN

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Abstract. *In the last 20-25 years, the number of studies in the field of marine biology has been rapidly growing in the world. Marine microorganisms are a source of natural compounds with new structural characteristics and unique biological properties. They synthesize secondary metabolites different in structure, specificity, and biological activity: antibiotics, antitumor compounds, biosurfactants, etc. [4,11]. Most biosurfactants also have antibiotic and antitumor activity. An important step in the study of marine microbiota was the study of the taxonomic composition of marine biofilms and their role in the fouling of biotic and abiotic structures [2]. In separate publications, some issues of regulation of interspecies relationships within biofilms [9] and their significance for marine biocenoses [10] are considered. Black Sea bacteria remain practically unstudied and were found mainly by metagenomic methods in samples of Black Sea origin samples [8]. The* ***purpose*** *of this work was to study the co-cultivation of Black Sea bacteria and to determine the antimicrobial activity of secondary metabolites that they synthesize. Based on the topic, the* ***task*** *was: assess the antimicrobial and antibiofilm properties of secondary metabolites of marine microorganisms.*

Key words: *secondary metabolites, marine, antimicrobial activity, mono- and co-cultivation.*

Introduction

Co-cultivation systems have long been used to study interactions between populations of cells and are fundamental to studies of interactions between cells of any kind. Recently, such systems have become especially interesting for the study and design of complex multicellular synthetic systems. At a basic level, co-culture is a cell culture setup in which two or more different populations of cells are grown with a certain degree of contact between them. The motivation for using such a scheme includes: studying natural interactions between populations, improving the success of culturing certain populations, or establishing interactions between populations. Examples of the study of natural interactions between populations include the study of infections, the study of other natural interactions, and the creation of experimental models and biomimetic environments of natural systems such as artificial tissues [7].

Co-cultures are extremely relevant for drug research because they provide a more representative human tissue model similar to in vivo than animal models, as well as allow for high productivity testing and in-depth monitoring of drug effects on cell-cell interactions. Improving cultivation success is necessary for certain pop-

ulations. Some cells cannot be easily monocultured in vitro because they do not exhibit the desired in vivo physiological features, but having a different cell population may improve culture success or cell behavior [5].

Materials and methods

All studies were carried out at the Biotechnological Scientific and Educational Center of Mechnikov ONU in 2020-2021.

Strains isolated from the surface of mussels in the Odessa bay of the Black Sea were used in the work: 3 strains of *Pseudomonas aeruginosa*, designated as M1, M3, M4; 2 strains of bacillus - *Bacillus subtilis* (MC3) and *Bacillus atrophaeus* (MC4), as well as 1 strain of *Alcaligenes faecalis*. To determine antimicrobial activity, the collection strains *P. aeruginosa* ATCC 27853 were used as test strains.

Cultivation of marine bacteria was carried out in the LB medium with the composition g/l: peptone - 15; yeast extract - 10; NaCl - 5; sea water - 1 l.

Growing in liquid cultures was carried out in 300 ml flasks containing 120 ml of nutrient medium. The inoculum concentration of bacteria was 6 ml of a daily culture with an optical density of 0.2 at 540 nm. Cultivation was carried out at 30 C for 5 days under stirring conditions at 150 rpm. After 5 days, the number of cells in each variant was spectrophotometrically determined and the culture was centrifuged 20 for min at 12,000 rpm to obtain cell-free supernatants.

Growth in biofilm cultures was carried out in 6-well flat-bottom Nuclon plates for 5 days at 30°C. 5 ml of liquid medium was added to each well. Bacteria were added to five wells, and one was left for sterility control. At the end of the culture, the liquid layer of the five wells was pooled and the content of planktonic cells was determined and centrifuged as described for liquid cultures. Biofilms formed at the bottom of the wells were washed with saline and fixed with 96% ethanol for 10 min, dried and stained with 1% crystal violet solution. After 15 min, the dye was removed, the wells were washed, and after drying, 1.5 ml of a lysing buffer containing 0.1 M NaOH and 1% sodium dodecyl sulfate was added. Optical density was measured at a wavelength of 592 nm [3]. The mass of biofilms was expressed in units of optical density, OD595.

Determination of antimicrobial activity was carried out according to the method of serial dilutions using flat-bottomed 96-well plates, which makes it possible to evaluate the effect of the studied substances not only on the content of planktonic cells, but also on the formation of biofilms by them [1]. From 1 to the 12 column were obtained dilution of the supernatants from 1:2 to 1:4096. Then in each wells of one plate were added 100 µl of a daily culture of the test microorganism: *P. aeruginosa* ATCC 27853. Cultivation was carried out at 37°C for 24 hours. A day later, plankton cells were transferred to clean plates and the amount was determined spectrophotometrically. Biofilms at the bottom of the wells were washed from non-adherent cells and their mass was determined.

All experiments were carried out in 3 independent experiments with 3 repetitions in each. Statistical processing of the research results was carried out using the generally accepted methods of variational analysis. Mean values of indicators (X) and their standard error (SX) were calculated. The significance of differences between the means was determined by Student's t-test, evaluating the probability of

the results obtained at a significance level of at least 95% ($p \leq 0.05$). Mathematical calculations were performed using the Excel computer program [6].

Results and discussion

The study was made of the effect of cell-free supernatants of mono- and co-cultures of marine microorganisms that were obtained during cultivation in suspension and biofilm cultures on their growth and biofilm formation. According to the degree of inhibition of the mass of biofilms, the samples were divided into moderately active (20-35% inhibition) and highly active (36-55% inhibition or even more).

In the tables 1-2 shown the results of determining the effect on the formation of biofilms by test strains. The results are shown in the table 1 showed that *P. aeruginosa* ATCC 27853 biofilm formation was more effectively inhibited by samples of co-cultures grown in suspension.

So, in the case of monocultures, an inhibitory effect was found in 36% of the samples, of which only one third showed a high level of inhibition - 36-50%. The range of active dilutions of cell-free supernatants was from 1:4 to 1:64. When using supernatants of co-cultures, antibiofilm activity was detected in 63 samples out of 96, which is 64%. Of these 63 samples, 26 showed high levels of activity and reduced biofilm weight by 50% or more. Also in the case of co-cultures, the range of active dilutions was wider - from 1:2 to 1:1024.

Samples obtained from biofilm cultures showed a higher ability to inhibit biofilm formation by *P. aeruginosa* strain ATCC 27853 (Table 2). 43% of monoculture samples showed an average level of activity. It should also be noted the effective inhibition of biofilm mass by culture supernatants diluted 2048 and 4096 times. 98% of the supernatant samples of all variants of co-cultures suppressed film formation of the studied test strain. Moreover, 69% of the samples revealed a high level of activity even in dilutions of 1:2048 and 1:4096.

It should also be noted that the secondary metabolites of *P. aeruginosa* marine strains effectively suppressed biofilm formation by the reference strain of the same species.

In mature biofilm cultures, a dynamic balance is formed between planktonic, free-floating cells and those contained in biofilms. In fact, cultures are the only plankton-biofilm system. Therefore, in the course of the study, we studied the effect of supernatants of marine mono- and co-cultures on the content of planktonic cells of test strains.

The results are shown in tables 3-4 showed differences in the influence of the studied supernatants on the components of the supernatant-biofilm system. Thus, the supernatants of suspension cultures had a similar effect on the content of planktonic cells on the *P. aeruginosa* ATCC 27853 biofilm. That is, their number was reduced, and the products of monocultures turned out to be more active (Table 3).

In general, the inhibitory effect was recorded in 44 out of 84 variants. At the same time, the supernatants of biofilm cultures (Table 4) had almost no effect on the content of planktonic cells, although they effectively reduced the mass of biofilms.

It should also be noted that the supernatants of biofilm co-cultures had a greater inhibitory effect of all the studied combinations.

Table 1

The secondary metabolites influence obtained by mono- and co-cultivation of marine bacteria in suspension cultures on the biofilm formation of *P. aeruginosa* ATCC 27853

	Dilutions of supernatant											
	2	4	8	16	32	64	128	256	512	1024	2048	4096
Control	1,160											
	Mono-culture											
A.faecalis	1,496	0,949	0,856	1,068	0,820	0,969	0,944	0,963	0,994	1,140	1,350	1,595
M1	1,215	0,793	0,822	1,003	0,728	0,998	1,100	1,087	1,228	1,202	1,214	1,501
M3	1,476	0,762	0,706	0,940	0,636	1,066	1,099	1,013	1,082	0,737	1,368	1,150
M4	1,614	0,860	0,755	0,769	0,891	0,812	1,063	0,983	0,942	1,184	1,334	1,324
MC3	1,575	0,896	0,764	0,667	0,590	0,647	0,909	1,053	0,999	0,905	0,908	1,498
MH4	1,308	0,966	0,592	0,549	0,764	0,828	0,992	1,094	0,999	0,948	1,136	1,024
PA01	1,221	0,832	0,670	0,697	1,057	0,668	0,945	1,115	1,307	1,306	1,413	1,094
	Co-culture											
MC3+A.f	0,580	0,537	0,485	0,565	0,823	0,632	0,770	0,921	0,795	0,938	1,564	1,812
MC3+M1	0,984	0,831	0,462	0,538	1,457	0,521	0,677	0,733	0,855	1,600	0,936	1,213
MC3+M3	1,562	0,721	0,614	0,776	0,889	0,761	1,423	0,743	1,017	0,839	0,698	0,966
MC3+M4	1,276	0,547	0,583	0,803	0,843	0,517	0,872	0,640	0,832	1,144	1,080	0,980
MH4+A.f	0,842	0,633	0,713	0,871	1,165	0,769	0,826	1,050	0,965	0,791	1,468	1,294
MH4+M1	1,445	0,876	0,511	0,864	0,857	0,681	0,729	0,564	1,248	1,051	0,634	0,842
MH4+M3	1,129	0,591	1,004	0,589	0,969	0,837	0,819	0,763	0,825	0,880	0,971	1,069
MH4+M4	1,050	1,410	0,620	0,887	0,862	0,728	0,909	0,636	0,929	0,871	0,805	1,271

Note: data shown against a light green background reflects a 20-35% inhibitory effect; data shown on a green background - 36-55% inhibitory effect; data shown on a red background indicate an inhibitory effect of more than 60%

Table 2

The secondary metabolites influence obtained by mono- and co-cultivation of marine bacteria in biofilm cultures on the biofilm formation of *P. aeruginosa* ATCC 27853

	Dilutions of supernatant											
	2	4	8	16	32	64	128	256	512	1024	2048	4096
Control	1,777											
	Mono-culture											
A.faecalis	2,324	2,211	1,988	1,700	1,531	1,376	1,696	1,350	1,314	1,412	1,674	1,770
M1	1,801	1,312	1,236	1,417	1,480	1,246	1,506	1,134	1,648	1,783	1,080	1,371
M3	1,944	1,530	1,486	1,817	1,575	1,790	1,227	1,138	1,389	1,385	1,834	1,856
M4	2,435	2,013	1,512	1,480	1,442	1,680	1,419	1,155	1,565	2,050	1,038	1,292
MC3	2,096	1,852	1,688	1,577	1,226	1,247	1,247	1,085	1,216	1,522	1,190	0,892
MH4	2,200	1,429	1,355	1,487	1,343	1,254	1,803	1,307	1,384	1,190	1,133	1,141
PA01	1,613	2,691	2,427	1,832	1,772	1,590	1,934	1,197	1,952	1,512	1,322	1,410
	Co-culture											
MC3+A.f	0,971	0,790	1,145	1,277	1,215	1,135	1,125	1,065	1,262	1,059	1,519	1,282
MC3+M1	0,872	0,714	0,956	1,126	0,993	0,981	1,318	1,122	1,306	1,232	1,228	1,106
MC3+M3	0,918	0,742	0,507	0,912	0,968	1,117	1,088	1,157	1,209	0,817	1,468	1,037
MC3+M4	0,575	0,724	0,980	1,398	0,985	1,074	1,174	1,180	1,015	0,835	1,406	1,205
MH4+A.f	0,626	0,740	0,993	0,892	1,062	1,120	0,944	1,087	1,027	1,015	1,145	0,768
MH4+M1	0,623	0,629	1,013	0,774	0,837	0,897	0,832	0,928	1,164	1,010	0,711	0,615
MH4+M3	0,840	0,785	0,983	0,827	1,084	0,890	1,174	1,079	0,886	0,756	0,988	0,709
MH4+M4	1,209	1,023	1,064	0,773	0,992	0,949	0,992	1,092	1,344	0,730	1,197	1,209

Table 3

The secondary metabolites influence obtained by mono- and co-cultivation of marine bacteria in suspension cultures, on the content of planktonic cells of *P. aeruginosa* ATCC 27853

	Dilutions of supernatant											
	2	4	8	16	32	64	128	256	512	1024	2048	4096
Control	0,987											
	Co-culture											
A.faecalis	0,817	0,796	0,904	0,842	0,957	0,858	0,927	1,189	0,934	0,941	0,978	1,089
M1	0,735	0,854	0,909	0,866	0,924	0,846	0,897	0,975	0,880	0,854	0,886	0,786
M3	0,770	0,940	0,891	0,845	0,949	0,966	0,981	0,875	0,907	0,997	0,963	0,802
M4	0,763	0,973	0,830	0,861	0,817	0,886	0,890	0,888	0,956	1,026	0,830	0,792
MC3	0,741	0,932	0,895	0,906	0,869	0,921	0,879	0,955	0,967	0,904	1,124	0,868
MH4	0,737	0,864	0,966	0,933	0,936	0,972	0,906	0,952	0,970	0,940	0,995	0,795
PA01	0,765	0,867	0,935	0,879	1,033	0,897	0,979	0,886	1,050	0,944	0,973	0,857
	Co-culture											
MC3+A.f	0,907	0,913	1,071	0,986	0,928	1,055	1,037	1,053	0,981	0,960	0,844	0,962
MC3+M1	0,869	0,904	1,063	0,992	0,914	1,005	1,009	0,984	1,039	1,017	0,983	1,067
MC3+M3	0,706	0,857	0,999	1,022	0,815	1,007	1,041	0,883	0,864	0,950	1,014	1,061
MC3+M4	0,813	0,932	1,092	1,020	0,888	1,056	1,058	0,945	1,075	0,986	1,036	0,993
MH4+A.f	0,822	0,979	0,860	0,896	0,887	0,913	0,951	0,991	0,903	0,812	0,839	0,975
MH4+M1	0,731	0,926	1,039	0,977	0,893	0,993	1,064	1,046	1,134	0,985	1,031	0,832
MH4+M3	0,792	0,982	0,992	0,913	1,083	0,932	0,906	0,922	0,979	0,923	0,850	0,984
MH4+M4	0,840	0,848	0,963	0,893	1,112	0,992	0,754	0,970	0,934	0,962	0,974	0,902

Note: data shown against a light green background reflects a 20-35% inhibitory effect; data shown on a green background - 36-55% inhibitory effect; data shown on a red background indicate an inhibitory effect of more than 60%

Table 4

The secondary metabolites influence obtained by mono- and co-cultivation of marine bacteria in biofilm cultures, on the content of planktonic cells of *P. aeruginosa* ATCC 27853

	Dilutions of supernatant											
	2	**4**	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**2048**	**4096**
Control	1,001											
	Mono-culture											
A.faecalis	0,872	0,928	0,885	1,004	0,833	0,864	0,969	0,995	0,833	0,887	0,924	0,810
M1	1,166	0,974	1,114	1,138	0,873	1,276	0,975	0,962	0,963	1,078	0,978	1,050
M3	1,139	0,989	1,101	1,071	1,202	0,931	1,129	0,784	0,842	0,931	0,900	0,908
M4	0,723	0,751	0,860	0,948	1,050	1,036	0,966	1,012	0,950	1,162	1,100	0,966
MC3	0,767	0,687	0,817	0,900	0,963	1,066	1,211	1,006	0,837	0,890	1,005	0,881
MH4	1,003	1,377	1,029	1,439	1,069	1,173	1,040	0,952	0,888	0,888	1,043	0,975
PA01	0,827	0,899	0,992	0,887	1,001	1,055	0,915	0,990	0,863	0,846	1,144	0,977
	Co-culture											
MC3+A.f	0,896	1,044	0,888	0,900	0,974	0,926	0,957	1,086	0,968	1,042	0,996	0,882
MC3+M1	0,864	1,015	0,921	1,055	0,934	0,961	0,908	1,017	0,918	0,907	0,956	0,940
MC3+M3	0,789	0,976	0,960	0,946	0,913	0,937	0,878	0,983	0,922	1,039	0,950	0,879
MC3+M4	0,894	0,933	0,962	0,917	0,997	0,971	0,874	0,958	1,104	0,976	0,988	0,922
MH4+A.f	0,889	0,903	0,859	0,886	0,978	0,984	0,832	0,942	0,894	0,932	1,063	0,757
MH4+M1	0,908	1,016	0,847	0,840	0,987	0,916	0,827	0,873	1,020	1,009	0,931	0,653
MH4+M3	0,852	0,879	0,847	0,836	0,955	0,838	0,858	0,939	0,985	0,832	0,973	0,581
MH4+M4	0,904	0,846	0,863	0,891	0,917	0,897	0,908	0,875	0,914	0,968	1,010	0,991

Note: data shown against a light green background reflects a 20-35% inhibitory effect; data shown on a green background - 36-55% inhibitory effect; data shown on a red background indicate an inhibitory effect of more than 60%

Thus, in the course of studying the antimicrobial activity of mixtures of secondary metabolites of marine bacteria obtained in this work, several curious facts were discovered. First, it was found that the metabolites of monocultures of *P. aeruginosa* strain can have an inhibitory effect on reference strain of the same species of microorganisms. Secondly, supernatants of co-cultures shown a higher level of activity compared to monocultures.

Conclusions

Determination of the antimicrobial and antibiofilm activity of the supernatants of the studied cultures in relation to the reference strain of *P. aeruginosa* ATCC 27853 showed that the planktonic culturing and biofilm formation of this strain react differently to the action of metabolites of marine bacteria. *P. aeruginosa* ATCC 27853, a significant inhibition of the process of biofilm formation was noted, which was especially pronounced in the supernatants of biofilm cultures. The number of *P. aeruginosa* ATCC 27853 cells not only did not decrease under the influence of the studied supernatants, but also slightly increased compared to the control. This can be explained by the fact that against the background of the inhibitory effect on biofilm formation, some cells of the test strain do not attach to the bottom of the plate wells and remain in a free state.

A high level of synthesis by co-cultures of marine bacteria of antimicrobial agents inhibiting the formation of P. aeruginosa ATCC 27853 biofilms by 30-60% was established.

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CYTOTOXIC ACTIVITY OF MARINE BACTERIA - DESTRUCTORS OF ORGANIC COMPOUNDS IN HUMAN CULTURE *IN VITRO*

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Abstract. *The cytotoxic properties of the Black Sea strains of bacteria - destructors of organic compounds were studied on the model of human cell culture Hep-2. Pseudomonas fluorescens 55, Pseudomonas fluorescens ONU328, Bacillus megaterium 64, Microbacterium barkeri 5 and Microbacterium barkeri 13, Pseudomonas maltophilia ONU32 strains of marine bacteria-decomposers of organic compounds can be recommended for use in biotechnologies for cleaning the marine environment from organic pollutants.*

Key words: *marine bacteria - decomposers of organic compounds, Black Sea, cytotoxic properties, human cell cultures*

Introduction

The problem of pollution of the marine environment with organic waste of man-made origin is becoming more acute, as it affects people's health. Anthropogenic activity intensively affects the state of aquatic resources, especially in the coastal densely populated areas of the Black Sea [1, 5].

In this regard, research on the development of methods for the detoxification of organic compounds is of particular importance. A promising direction is the use of the biochemical activity of microorganisms, which makes it possible to develop effective methods for purifying sea water [8].

However, the use of strains of bacteria-destructors is possible only if they do not have pathogenic properties [7]. Therefore, the study of the cytotoxic activity of bacteria-destructors of organic compounds in model systems in vitro is of practical interest.

There are data in the literature on the use of cell cultures to determine the cytotoxic properties of bacteria, including potential biodestructors [3, 4, 6, 9, 10].

The aim of the work was to evaluate the cytotoxic properties of the Black Sea strains of bacteria - decomposers of organic compounds on the human cell culture model Hep-2.

Materials and Methods

The object of research was strains of marine bacteria-destructors of organic compounds (oil hydrocarbons, phenol) selected for use in biotechnologies for

wastewater, sea water and soil treatment from the collection of microorganism cultures of the Odessa I.I. Mechnikov.

The tested strains were isolated from samples of sea and pore water of the interstitial cavities of the psamocontour zone (splash zone), taken in the recreational zones of the Black Sea coast with different levels of anthropogenic pollution:

1. Odessa coast: - in the area of the oil harbor; - in the area of wastewater discharge into the sea at Kovalevsky's Dacha; - in a conditionally clean area of the coast - at the hydrobiological station of the ONU;

2. Snake Island: - in the area of discharge of domestic and fecal waste into the sea on the beach "Devichiy". In total, 8 strains of marine destructor bacteria were studied:

- *Pseudomonas fluorescens* ONU328 and *Pseudomonas maltophilia* ONU329, isolated from oil-contaminated sea water of the Odessa coast in the oil harbor area;

- *Pseudomonas fluorescens* 55 isolated from sea water and *Bacillus* sp. 66, isolated from pore water taken in the area of the hydrobiological station of the Odessa National University named after I.I. Mechnikov;

– *Pseudomonas sp.* 47 and *Bacillus megaterium* 64 isolated from pore water taken from the area of wastewater discharge into the sea at Kovalevsky's Dacha;

- *Microbacterium barkeri* 5 and *Microbacterium barkeri* 13 isolated from oil-contaminated coastal soil of Zmeiny Island. The pathogenic strain *Pseudomonas aeruginosa* 2-9, a decomposer of organic dyes, was used as a control.

To study the cytotoxic and invasive properties of destructor bacteria *in vitro*, we used transplanted cell cultures of human laryngeal carcinoma Hep-2.

When cultivating cells, medium 199 containing 10% bovine serum was used. The inoculum dose was 3-5x10^4 cells per 1 ml of growth medium. The cells were cultured at 37°C for 72 h until a cell monolayer was formed [2].

The cell culture was inoculated with a suspension of an 18-hour bacterial culture, which was grown on meat-peptone agar at a temperature of 30°C. The inoculum dosage was 1x105 bacterial cells in 1 ml of growth medium 199.

After inoculation of cell cultures with bacteria at certain time intervals (24, 48, 72, 96 h), cytological studies of native unstained and permanent fixed with Carnoy's fixative and stained with azure-eosin (Romanovsky Giemsa stain) were carried out.

The cytotoxic effect of marine bacteria after 24–96 h was determined visually by microscopic examination of permanent preparations by morphological changes in individual cells and the degree of monolayer degeneration, taken into account by the number of non-viable cells.

After 96 hours, cells were removed from the glass surface, stained with trepan blue (at a concentration of 0.01%), while non-viable cells were stained diffusely in blue, their number was expressed as a percentage (%). The criterion for cytotoxicity was an increase in the number of non-viable cells in the monolayer up to 10% or more compared to the control [2].

Results

The results of the study of the cytotoxic properties of marine strains of bacteria-destructors of organic compounds *in vitro* are shown in the table.

Table

Cytotoxic properties of marine bacteria degrading organic compounds *in vitro*

Station	Source of isolation of bacteria	Strain	Number of non-viable cells in the monolayer, %*
Hydrobiological station	Sea water	*Pseudomonas fluorescens* 55	0
	Pore water	*Bacillus sp.* 66	16±3
Oil harbour	Oil-contaminated sea water	*Pseudomonas fluorescens* ONU328	0
		Pseudomonas maltophilia ONU329	5±1
Dacha Kovalevsky	Pore water	*Pseudomonas sp.* 47	25±5
		Bacillus megaterium 64	0
Zmiiny Island	Oil-contaminated coastal soil	*Microbacterium barkeri* 5	0
		Microbacterium barkeri 13	0
P. aeruginosa 2-9			97±12

Note:* 96 hours

Looking at the data in the tables, it can be seen that for a period of 96 hours, the guard strains *Pseudomonas fluorescens* 55, *Pseudomonas fluorescens* ONU328, *Bacillus megaterium* 64, *Microbacterium barkeri* 5 and *Microbacterium barkeri* 13 did not cause morphological changes and destruction of the monosphere of human Hep-2 cultures. Under the age of the *Pseudomonas maltophilia* ONU329 strain, weakly pronounced morphological changes in a few cells were recorded in monoballs - the number of non-viable cells did not exceed 5 ± 1% after 96 hours of exposure. On the basis of these data, it is possible to collect conclusion about the presence of cytotoxic activity in strains isolated from sea water from various regions of the Odessa coast and the naphtho-encrusted coastal soil of the Zmiïny island.

In contrast, strains of *Bacillus* sp. 66 and *Pseudomonas* sp. 47, isolated from pore water at the Hydrobiological Station (conditionally clean coastal area) and in the area of wastewater discharge into the sea at Kovalevsky's Dacha, had weakly pronounced cytotoxic activity - the number of non-viable cells after 96 hours of exposure reached, respectively, 16±3 % and 25±5 %.

The *P. aeruginosa* 2–9 strain already on the first day of observation caused a pronounced cytopathic effect in the monolayer of transplanted human cell cultures Hep-2, which manifested itself in cell rounding, vacuolization and granulation of the cytoplasm, deformation, wrinkling and pyknosis of the nuclei. Complete degeneration of the cells of the monolayer of transplanted Hep-2 cell cultures was recorded, which indicated a pronounced cytotoxic activity of the *P. aeruginosa*

2-9 strain. After 96 hours, the monolayer was completely destroyed - the number of non-viable cells reached 97 ± 12 %, which indicated the presence of cytotoxic properties in *P. aeruginosa* 2-9 bacteria.

Thus, the results of our experimental studies indicate that marine strains of bacteria-destructors of organic compounds *Pseudomonas fluorescens* 55, *Pseudomonas fluorescens* ONU328, *Bacillus megaterium* 64, *Microbacterium barkeri* 5 and *Microbacterium barkeri* 13 Hep-2.

Conclusion

1. Strains of bacteria-destructors isolated from pore water *Bacillus* sp. 66 (in the conditionally clean area of the hydrobiological station) and *Pseudomonas* sp. 47 (in the area of wastewater discharge into the sea at Kovalevsky's Dacha), had weakly expressed cytotoxic activity.

2. Strains of marine bacteria-destructors of organic compounds *Pseudomonas fluorescens* 55, *Pseudomonas fluorescens* ONU328, *Bacillus megaterium* 64, *Microbacterium barkeri* 5 and *Microbacterium barkeri* 13, *Pseudomonas maltophilia* ONU isolated from the sea water of different regions of the Odessa coast and the oil-contaminated coastal soil of Zmeiny Island did not have cytotoxic properties and can be recommended for use in biotechnologies for cleaning the marine environment from organic pollutants. Isolated from the sea water of different regions of the Odessa coast and the oil-contaminated coastal soil of Zmeiny Island did not have cytotoxic properties and can be recommended for use in biotechnologies for cleaning the marine environment from organic pollutants.

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ENCAPSULATION PROTECTS CELLS FROM THE HARMFUL EFFECT OF H_2O_2 AND EXTENDS STORAGE TIME AT POSITIVE TEMPERATURES

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Abstract. *Human mesenchymal stromal cells (MSCs) are promising cell type for biomedical research and clinical medicine. Cell storage at ambient temperatures may simplify transportation and escape disadvantages of cryopreservation. Here we study the viability, metabolic activity of MSCs during storage at ambient temperature in the form of the monolayer, suspension, and encapsulated in alginate capsules (AMS). Viability and metabolic activity of MSCs in monolayer and suspension decreased by more than 2 times at day 7 of storage. Encapsulated cells were more resistant and had significantly higher values of viability and metabolic activity. It was found that 64% of cells released from AMS showed ability to adhere to plastic and following proliferation.*

The study of MSCs response on H_2O_2 exposure showed that the metabolic activity of cells in the monolayer was less that 30% at concentration of 400 μM H_2O_2. Cells in AMS were more resistant to the injured effect of reactive oxygen species (ROS) induction and kept metabolic activity higher 80% up to 500 μM of H_2O_2. The basal and H_2O_2-induced level of ROS was significantly lower in encapsulated MSCs compared to monolayer. Thus, the results demonstrate that encapsulation of MSCs in AMS supports resistance to storage conditions and ROS toxicity.

Key words: *mesenchymal stromal cells, hypothermic storage, oxidative stress, alginate capsules.*

1. Introduction

Human mesenchymal stromal cells (MSCs) - are increasingly used in various fields of biology, are promising cell type for tissue engineering and regenerative medicine. This is due to the high proliferative potential, the ability to multilinear differentiation [1, 2, 3, 4], low immunogenicity and immunomodulatory properties of MSCs [5, 6, 7].

To realize the potential of MSCs in regenerative medicine and research, it is necessary to create effective ways to store cells. There are currently two approaches to cell storage: cryopreservation and hypothermic storage. Hypothermic storage - storage of biological objects at temperatures lower physiological. Hypothermal storage can solve the following problems associated with cryopreservation: complexity, the need to attract qualified personnel, the use of special cost equipment, and at the same time greatly simplify transportation between research and clinical centers [8, 9]. This method, in contrast to cryopreservation, allows the preservation

of biological material without the use of toxic cryoprotectants.

There are studies that demonstrate that the enclosure of cells in alginate capsules allows their effective storage at ambient temperatures [10]. In our laboratory [11] it has been shown that encapsulation in AMS supports the viability and metabolic activity of MSCs under conditions of short-term storage in culture medium at temperatures of 22 ° C for up to 3 days at the level of 80-90%. But the possibility of using this approach for longer storage of MSCs has not been investigated. In addition, the protective mechanisms of encapsulation in alginate capsules on cell viability during hypothermic storage remain unclear.

One of the most important factors of damage under hypothermia is oxidative stress, which causes the activation of free radical reactions in biological systems. Thus, high levels of reactive oxygen species (ROS) cause damage to cell membranes, genetic material and cell dysfunction [12]. At the same time, it is believed that low basal levels of ROS play an important role in the regulation of processes such as proliferation, differentiation and survival of MSCs. However, the resistance of cells encapsulated in alginate capsules to oxidative stress remains unclear.

2. Materials and Methods

The experiments were performed on human dermal MSCs derived after adult donors informed consent. MSCs were cultured in alfa-MEM supplemented with 10% of fetal bovine serum under standard culture conditions. For alginate microspheres (AMS) capsules formation MSCs suspension in 1% sodium alginate was extruded through a needle connected to the high-voltage generator into 100 mM $CaCl_2$ solution for solidification. Then AMS were washed out with Hank's solution. MSCs in form of monolayer, suspension and in alginate capsules were stored in sealed containers at 22 °C in culture medium.

Viability, metabolic activity, ability for adhesion and growth in culture were estimated at 0, 3, 7, 10, and 14 day. Viability was determined by fluorescein diacetate/ethidium bromide (FDA/EB) dual staining. Metabolic activity was measured by Alamar Blue test in the relative fluorescence units (RFU). ROS level was assessed with Abcam Cellular ROS Assay Kit (Deep Red). Cell cycle analysis were transduced with the Premo™ FUCCI Cell Cycle Sensor with confocal laser scanning microscope Olympus FV10i-LIV and Olympus cellSense Software.

3. Results and Discussion

3.1. Viability and Metabolic activity of MSCs during storage at ambient temperature

Cell viability measured by FDA-EB staining was sharply decreased in suspension at 3d day of storage at ambient temperature. During storage, spheroid formation was revealed at times. Viability in monolayer decreased from the 7th day of storage. At the same time encapsulation in AMS kept significantly higher viability during storage period compared to monolayer and suspension groups. Thus, cell viability in AMS was 80% on the 7th day, 60% on the 10th day, and 40% on the 14th day.

There was a sharp drop in metabolic activity in suspension and monolayer during the subsequent storage. At the same time encapsulation in alginate made it

possible to maintain the metabolic activity during storage more effectively compared to other groups. Encapsulation in alginate microspheres decreased the basal level of metabolic activity and maintained this parameter during storage at 22°C. Thus, cell metabolic activity in AMS was 2563 RFU in the basal level, 1700 RFU on the 3rd day, 1403 RFU on the 7th day, 870 RFU on the 10th day, 220 RFU on the 14th day.

3.2. Return to the culture conditions after storage MSCs from AMS and suspension

The ability of MSCs to adhere to plastic and proliferate after 7 days of storage was evaluated. For that alginate gel was dissolved by incubated in 50 mm sodium citrate solution for 5 min. A great difference in the adhesion capacity of cells stored in different conditions was revealed. 64% of cells which were extracted by AMS dissolving showed ability to adhere to plastic whereas only 12% of cells stored in suspension were able to attachment.

3.3. ROS levels and Resistance to H_2O_2

3.2.1.Resistance to different concentrations of H_2O_2 (by Alamar Blue test)

MSCs in monolayer and in AMS were incubated with different concentrations (from 50μM to 500 μM) of hydrogen peroxide during 24 hrs then metabolic activities by Alamar Blue test were evaluated.

MSCs in AMS were more resistant to the action of hydrogen peroxide in all used concentrations, compared to MSCs in monolayer.

Table 1

Metabolic activity MSCs after exposure with different concentration of H_2O_2 for 24 hrs

H_2O_2, μM	monolayer	AMS
0 (Control)	100%	100%
50	78,6±3,75%	88,1 ±7,1%
100	81,1±6,25%	98,8 ±3,55%
200	53,9±2,5%*	99,6 ±1,78%
300	48,9±2,5%*	98,6±3,55%
400	29,5±1,25%*	95,9 ±1,78%
500	28,3±1,25%*	79,7 ±7,1%

Note: * - differences are significant compared to AMS ($p < 0,05$).

As the concentration of hydrogen peroxide increased, the difference in the sensitivity of the samples was higher. After exposure with 200μM of H_2O_2 the metabolic activity of MSCs in monolayer was less twofold compared to the

MSCs in AMS. At 400 μM of H_2O_2, cells in AMS were more stable than cells in a monolayer by more than 3 times. The concentration, when metabolic activity decreased by three times relative to the control, was considered critical. The critical concentration for MSCs in the monolayer was taken as 400 μM with a drop in metabolic activity to 29.5±1.25%.

In the case of MSCs in AMS, we did not reach the critical level of hydrogen peroxide even for the maximum used concentration. We can observe that at H2O2 concentration 500 μM (the maximum used in the experiment) metabolic activity remained about 80%.

The metabolic activity of cells in monolayer decreased in a dose-dependent manner after action of H_2O_2. Described results show that MSCs encapsulated in AMS were more resistant to the injured effect of H_2O_2.

3.3.2. ROS levels

The basal level of ROS and the level after short term (2 hrs) incubation with a high concentration of hydrogen peroxide (3mM) were studied. A great difference in the basal level of ROS in cells in monolayer and AMS was revealed.

Each cell in the monolayer was ROS-positive and has slightly red colored appearance, while in AMS only 10% of calls were ROS-positive.

Table 2

ROS level MSCs after exposure with 3mM of H_2O_2 for 2 hrs

concentrations of H_2O_2	monolayer	AMS
0 mM	32,8 ± 5,2 RFU/sell*	2,32 ±0,25 RFU/sell
3 mM	154,5 ± 7,8 RFU/sell*#	11,78 ± 0,44 RFU/sell

Note: * - differences are significant compared to AMS ($p < 0,05$); # - differences are significant compared to control ($p < 0,05$).

Therefore, the basal level of ROS was significantly lower in encapsulated MSCs compared to cells cultured in monolayer. It was detected that in a monolayer after incubation with H_2O_2 intensiveness of staining increased several times. At the same time, ROS-positive cells comprised 35% in AMS after H2O2 exposure, other cells remain unstained indicating.

Conclusions

The benefits of encapsulation in alginate microspheres for MSCs short-term storage and transportation under ambient temperature were revealed.

The encapsulation in alginate microspheres supports MSCs viability parameters during the short-term storage at ambient temperature.

The decrease in overall metabolic activity and lower basal level of ROS in alginate encapsulated MSCs were revealed compared to monolayer.

The decrease in overall metabolic activity, and lower basal level of ROS in alginate encapsulated MSCs were revealed compared to monolayer. The resistance to hydrogen peroxide action was higher in cells within alginate microspheres.

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Kornienko A., Tesliuk N.

THE INFLUENCE OF VARIOUS ORGANIC COMPONENTS ON THE PRIMARY STAGES OF MICROCLONAL PROPAGATION OF *PHALAENOPSIS* ORCHIDS

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Abstract. ***The aim*** *of this work was to study the influence of various organic components on the initial stages of microclonal propagation of Phalaenopsis orchids.* ***Materials and methods.*** *The influence of organic components (yeast extract, coconut water) at the stages of introduction of Phalaenopsis explants into in vitro culture was studied. Determination of the most productive for microclonal propagation organic components of nutrient medium was performed by the indication of Phalaenopsis explants survival rate.* ***Results.*** *It was found that the rate of engraftment of explants on the medium with yeast extract is better than on the control nutrient medium (by almost 70%) and the MS medium with coconut water (by almost 30%).* ***Conclusion.*** *It is recommended to use yeast extract for initial stages of Phalaenopsis in vitro micropropogation.*

Key words: *Phalaenopsis, in vitro culture, survival rate, yeast extract, coconut water.*

Introduction

Orchids are mainly grown because of their beautiful flowers. For this reason, methods of reproduction of this family of flowers *in vitro* culture are actively developed and improved in order to obtain more plants for further commercial sale.

Although foreign scientists have accumulated a lot of experience in orchid propagation, today they do not have a single opinion on the method of microclonal propagation of orchids, especially monopodial species (including *Phalaenopsis*) due to the difficulty of choosing effective nutrient medium for the initial stages of *in vitro* culture.

The task of the study was to determine the influence of organic components of nutrient media for the primary stages of microclonal propagation of *Phalaenopsis* orchids on the survival processes of *Phalaenopsis* explants in in vitro culture.

Materials and Methods

Experimental studies were conducted on the basis of the Biotechnological Research and Training Center of Odesa National I.I. Mechnykov University in 2020 – 2021.

As experimental plants young orchids of the *Phalaenopsis amabilis* species in the stage of intensive growth with healthy, strong flower stalks, free from fungal,

bacterial and viral infections were used. Flower stalk sections with dormant buds were used as initial explants for the experiment.

For sterilization explants were wiped with 95% ethanol, and immersed for about 15 min in a 10% solution of sodium hypochlorite. Then explants were re-immersed in a 5% sodium hypochlorite solution with three drops of Tween 20 and shaken occasionally for 10 min [1]. After this period the cylinder were filled again with autoclaved distilled water for rinsing. Explants were then placed on 30 ml of solid medium.

Three basal media were used: a modified [5] nutrient medium Murashige-Skuga (MS) [4] as a control medium; modified MS medium with 200 ml of coconut water; modified MS medium with 2 g 1^{-1} yeast extract. These media were supplemented with 2 mg 1^{-1} IAA, 10 mg 1^{-1} BAP and 20 g 1^{-1} sucrose and solidified with 8 g 1^{-1} agar. Explants were cultivated at 24 °C under 16 h photoperiods [2]. Survival percentage of explants, the beginning of proliferation and starting of callus formation were recorded for 14 days.

Results and Discussion

Flower-stalk cutting explants were used to determine the effects of different modifications of MS media on survival rate (Table 1).

Table 1

Survival rate of flower-stalk cutting explants on different nutrient media

Nutrient medium	Number of explants	Survival rate (days)					
		3	5	7	9	11	14
Modified MS	15	15	15	15	2	2	2 (12%)
Modified MS + yeast extract	15	12	12	12	12	12	12 (80%)
Modified MS + coconut water	15	9	9	9	9	6	6 (40%)

N = 15

Most of explants on control modified MS medium showed signs of fungal infection after 9 days of culturing. Also the color of the stem darkened faster than on media with organic additives. The modified MS medium with coconut water showed worse results, but explant tissues devoid of bacterial and fungal contamination are also capable of callus formation because they had shown the signs of viability, such as swelling of the explant.

It was found that the survival of explants on the modified MS medium with yeast extract is better than on the control nutrient modified MS medium and modified MS medium with coconut water. After 14 days of culture, survival rates were more than 80% on modified MS with yeast extract but 40% on modified MS with coconut water. Also explants on yeast extract MS medium remained mostly green without necrosis and on day 5, swelling of the axillary bud was observed in 1 explant. On day 14, the beginning of axillary bud proliferation in explant on the MS medium with yeast extract was noticeable (Fig. 1).

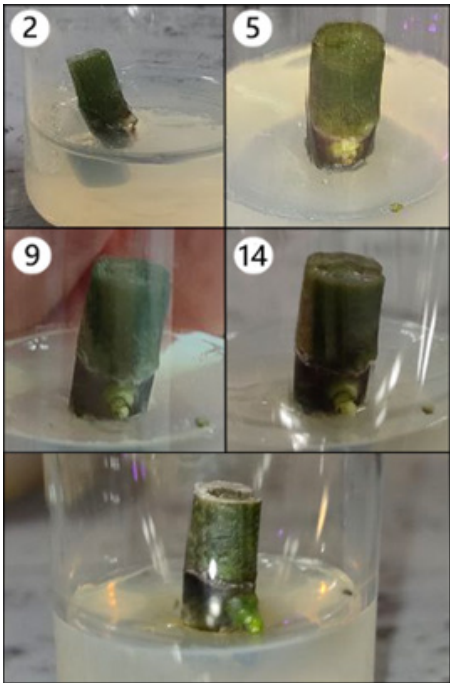

Fig. 1. Proliferation of flower-stalk cutting explant on modified MS medium with yeast extract on 2, 5, 9 and 14 days

Darkening was observed on all nutrient media due to the presence of phenolic compounds in the tissues of the explants. To prevent the death of the explant, it should be transplanted to a new medium or compounds that adsorb phenols should be added to the nutrient medium [3].

Conclusion

It was found that the survival rate of explants on the medium with yeast extract is better than on the control nutrient medium MS modified (almost 70%) and MS medium with coconut water (almost 30%). We recommend further studies with yeast extract.

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Kurjaninov E., Posheluzna J., Kovalenko E., Rusakova M.

ADAPTATION OF MICROORGANISMS TO THE LIMITATION OF FERRIC ION IN THE ENVIRONMENT

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Abstract. *Iron is an essential trace element for most organisms. A common way for bacteria to acquire this nutrient is through the secretion of siderophores, which are secondary metabolites that scavenge iron from environmental stocks and deliver it to cells via specific receptors.*

Solving the issue of production efficiency, there was decided to study some Pseudomonas species as producers of siderophores and influence of different cultivation conditions on their productivity.

Key words: *siderophores, Pseudomonas strains, iron ion, growth conditions.*

Introduction. Siderophores (Greek for "iron carrier") are low-molecular-weight, high-affinity iron-chelating compounds that are produced by organisms to solubilize Fe^{3+} for uptake [2, 6]. They can be used in green biotechnology, for example as plant protection agents against fungi.

While there has been tremendous interest in understanding the molecular basis of siderophore synthesis, uptake and regulation, questions about the ecological and evolutionary consequences of siderophore secretion have only recently received increasing attention [3]. Siderophores hindered the presence of plant pathogens in biocontrol strategies [7]. Bioremediation studies on siderophores discuss mostly the mobilization of heavy metals and radionuclides; the emulsifying effects of siderophore-producing microorganisms in oil-contaminated environments are also presented [3, 8]. The different applications found in literature based in medicine and pharmacological approaches range from iron overload to drug delivery systems and, more recently, vaccines [5].

The **aim** of the work was to determine the influence of different cultivation conditions, in particular the composition of nutrient media, on the spectrum and level of synthesis of siderophores by some *Pseudomonas* species.

Materials and methods

Pseudomonas strains were used in the work: Pseudomonas *chlororaphis* ONU 306, *Pseudomonas fluorescens* ATCC 13325 and *Pseudomonas aeruginosa* ATCC 10145, which were obtained from the collection of cultures of microorganisms of the Department of Microbiology, Virology and Biotechnology.

Maintenance of microorganisms was carried out on nutrient King B and MM9 media. The pH of the media was 7.4 ± 0.2. When using a dense variant of nutrient media, 1.5% agar-agar was added to them, after which it was autoclaved at 1.0 atm (King B medium) or 0.5 atm (MM9 medium) [4]. To prevent iron contamination, all glassware was soaked in 10% nitric acid solution and then washed with distilled water [9].

Previously studied strains of microorganisms were cultured on minimal medium at 22° C for 24 hours. After that, using a sterile saline solution prepared cell suspensions, the optical density of which at 600 nm was equal to 2.0. 2.0 ml were taken from the appropriate samples and added to 100 ml of the liquid version of the minimum medium. Cultivation was carried out under similar conditions, with constant shaking 150 rpm. Cells in the exponential growth phase were collected by centrifugation (11,000 g, 10 min), washed with sterile saline and re-introduced into fresh minimal medium containing 29 mg/l of ferric chloride ($FeCl_3$). The final culture step was performed for three days, during which 5 ml of cell suspension was taken every 24 hours and siderophores were determined. In this case, the growth of crops occurred under the above conditions.

The CAS method with chrome azurol S (CAS) in the modification of Alexander and Zuberer was used to determine the ability of the studied strains of bacilli to produce siderophores [4]. The basis of the CAS-method is the interaction of the formed bacterial siderophores with the CAS-reagent. A mixture prepared with this reagent and distilled water was also used for comparison. Samples of culture fluid containing siderophores were able to change the color of CAS - reagent from the original (dark blue) to orange [4].

Two methods were used to study the type of microbial siderophores: in the case of siderophores of the catechol type, the Arnow method [1], and the hydroxamate type method - the Atkin method [9].

Statistical processing of research results. All experiments were performed twice, the number of repetitions in each was 5. The data presented are given as the arithmetic mean ± standard deviation.

Results and discussion

The first stage of the study was to determine the growth dynamics of strains of *P. chlororaphis* ONU 306, *P. fluorescens* ATCC 13325 and *P. aeruginosa* ATCC 10145 in liquid media, MM9 and King B. The obtained data show that microorganisms quickly adapted to the composition of nutrient media. In both cases, 24 hours after the start of cultivation, the development of the stationary phase of development of the studied pseudomonads was observed.

The largest increase in biomass was recorded for *P. aeruginosa* ATCC 10145: the optical density of the respective suspensions was almost 4 times higher than the corresponding non-aeriginosa strains. It was also determined that when culturing microorganisms in King B medium, the duration of the stationary phase of development was only, on average, 12 hours.

Thus, starting from 36 hours in the suspension was not only the cessation of bacterial growth, but, conversely, there was cell death. This is evidenced by the obtained decrease in the optical density of the suspension of pseudomonad cells.

With regard to the nutrient medium MM9, the term of the stationary phase of growth of the studied strains was longer. Thus, starting from 24 hours of cultivation and during the next two days, the number of cells in the suspensions of *Pseudomonas* sp. did not change.

It is known from the literature that microorganisms most actively begin to produce secondary metabolites, in particular siderophores, during the stationary phase of development [6, 8].

Thus, at the next stage of research, the type of siderophores and the level of their production were determined on the first day of cultivation of pseudomonads, which corresponded to the beginning of the stationary phase of growth of these microorganisms. Based on the obtained data, we can assume that the most intensive production of siderophores occurred during the cultivation of the studied microorganisms in King B. The concentration of iron chelators in this case exceeded the corresponding values for the medium MM9 from 23% (in *P. chlororaphis* ONU 306) to 66% (in *P. aeruginosa* ATCC 10145).

According to the intensity of the synthesis of siderophores at the beginning of the stationary phase, the studied producer strains can be arranged as follows:

P. fluorescens ATCC 13325> *P. chlororaphis* ONU 306> *P. aeruginosa* ATCC 10145.

However, when King B medium was used for cultivation of pseudomonads, instead of MM9, the last two strains changed places, *P. aeruginosa* ATCC 10145 became a more efficient producer of siderophores.

Determining the type of siderophores synthesized by pseudomonads revealed the presence of representatives of two classes of biological chelators − siderophores of hydroxamate and catechol type.

However, if the cultivation of microorganisms in MM9 medium caused mainly the appearance of siderophores of the hydroxamate type, then when using King B, two types of chelators were observed. In the latter case, there was a more intensive synthesis of compounds.

Conclusions

Thus, using different types of nutrient media, in particular MM9 or King B, in the cultivation of pseudomonads, you can significantly affect the production of siderophores. In this case, there are not only changes in the intensity of education, but also their spectrum.

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Kostandinovska S., Atanasova-Pancevska N., Kungulovski D.

ISOLATION AND CHARACTERIZATION OF POTENTIAL ANTIBIOTIC-PRODUCING *BACILLUS* AND ACTINOMYCETES FROM UNDISTURBED AND CONTAMINATED SOILS FROM NORTH MACEDONIA

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Abstract. *The Actinomycetes and Bacillus are diversely distributed microorganisms in nature. The geographical diversity of North Macedonia is suitable for adaptation of various species of these bacteria. Also, the distribution of the actinomycetes and Bacillus is dependent upon the altitude, nature and contamination of the soil. The goal of this study was to determine how common culturable antibiotic-producing Actinomycetes and Bacillus bacteria are in undisturbed and contaminated sites.*

A total of 5 soil samples were collected from different regions of North Macedonia and were processed. Different isolates were characterized by observing colony characteristics and microscopic characteristics. Screening of the antimicrobial property was based upon perpendicular line streaking method.

From the identification tool used, 120 were found to be Bacillus spp., 112 were Actinomycetes. Out of total samples, 28 isolates of Actinomycetes and 26 isolates of Bacillus spp. were tested effective against the tested bacteria by primary screening using the cross streak method. Among the primarily screened, the Actinomycetes and Bacillus spp. from undisturbed sites showed better antimicrobial properties towards the tested bacteria that the ones from the contaminated sites.

In summary, antibiotic-producing microorganisms were found in most soils even by using a relatively limited screening assay.

Key words: *Actinomycetes, Bacillus, antimicrobial property, screening.*

1. Introduction

One of the most used metabolites that soil *Bacillus* and *Actinomycetes* make is antibiotics.A chemical produced by one bacteria that prevents the growth of another microorganism was the original definition of an antibiotic. The term "synthetic" today denotes a material produced by a single microorganism or a compound that is similar to it but is created entirely or in part chemically, and which, in small concentrations, inhibits the growth of other microorganisms [1].Gram-positive, endospore-forming, chemoheterotrophic, rod-shaped *Bacillus* species are anaerobic or facultatively anaerobic bacteria that are typically motile and have peritrichous flagella. They are also catalase positive [2]. Members of the *Bacillus* genus are typically found in soil and exhibit a wide range of physiological capabilities, enabling the organism to grow in any environment and compete advantageously with other organisms there thanks to its ability to produce metabolites that are antago-

nistic to those of other microorganisms and form extremely resistant spores [3]. Numerous *Bacillus* species are extremely significant because they produce antibiotics [2]. When used as a biological control agent, the ability of *Bacillus* species to synthesize a wide range of metabolites with antimicrobial activity has been widely utilized in medicine and the pharmaceutical industry. One of its capabilities is to control different diseases in animals, humans, and plants [4-5].

The most common class of microorganisms in nature, *Actinomycetes* are present in all types of environments, including soil and water sources. They have been extremely helpful in spontaneously producing antibiotics. *Actinomycetes* can be used to make a variety of antibiotics, including tetracycline, macrolide, chloramphenicol, nucleosides, and polyenes. Many antibiotics, including streptomycin, erythromycin, gentamycin, and rifamycin, are made from soil actinomycetes [6]. Depending on the soil's texture and level of cultivation, several antibiotic-producing *Actinomycetes* were available [7].*Actinomycetes* have undergone extensive research in the area of biotechnological application and have proven to be the most beneficial bacteria. One of the main sources of antibiotics is *Actinomycetes* [8].

This study aimed to assess the prevalence of culturable antibiotic-producing *Actinomycetes* and *Bacillus* bacteria in uncontaminated and contaminated environments.Numerous types of these bacteria can adapt to the diverse geography of North Macedonia.

2. Materials and methods

2.1. Sampling and Sample Site

Five soil samples were collected in sterile bags asepttically from several areas with distinct geographic and altitude distributions. The collected samples were taken to the laboratory of the Deparment of Microbiology and Microbial Biotechnology, Institute of Biology, Faculty of Natural Sciences and Mathematics, Skopje, North Macedonia. The pH measurement and moisture content of the samples were carried out immediately after the samples reached the laboratory.

2.2. Isolation of *Bacillus* spp.

For the isolation of *Bacillus* spp., serial dillution techinique was used considering different dilutions (10^{-1} – 10^{-5}) using 0.9% saline solution. 10 g of the soil samples were suspended in 90 ml 0.9% saline solution and shaken vigorously for 2 minutes. The samples were heated at 70°C for 30 minutes in a water bath. Then, the liquid was serially diluted in 0.9% saline solution, and the dilution from 10^{-1} – $10^{-5}$was plantes on nutrient agar medium using the spread method. Plates were incubated at 37°C for 48 hours. After incubation, the streak method was used to obtain pure culture.

2.3. Isolation of *Actinomycetes*

For the soil samples, 10 g were weighed and mixed well in a sterile bottle containing 90 ml 0.9% saline solution, for each sample. It was then serially diluted up to 10^{-5}dilution. From the serial dilution of 10^{-1} – 10^{-5}, 0.1 ml were spread planted on the Starch Nitrate Agar (SNA) plate. The plates were incubated at 28°C for 7-14 days. To obtain pure culture, streaking was used repeatedly during subculture on

the SCA [9]. Based on the macroscopic observation, different colored isolated colonies with a hard texture, powdery form, and musty smell were distinguished from another bacterial colony [10].

2.4. Characterization of *Bacillus* spp. and *Actinomycetes*

The potential antibiotic-producing *Bacillus* and *Actinomycetes* were characterized based on microscopic observation and colony morphology.

The microscopic observation was performed using Gram staining for observation of of the thin thread-like mycelial and hyphal form for *Actinomycetes* and rod-shaped *Bacillus* spp. [11].

2.5. Test microorganisms

Different Gram-negative and Gram-positive test bacteria such as *Salmonella enterica* ATCC 10708, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 13393, *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 used in this study were obtained from the Culture Collection of the Microbiology Laboratory at the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Sciences and Mathematics in Skopje, North Macedonia. The bacteria were incubated at 37°C and all were activated by incubation for a period of 24 hours in a nutrient broth.

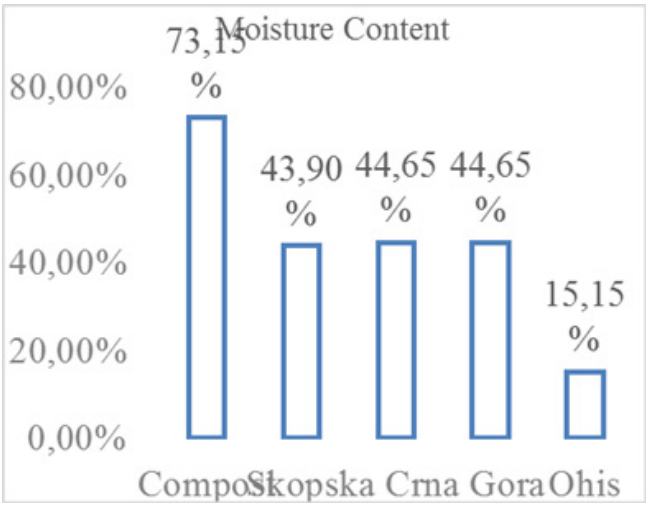
2.6. Primary screening for antagonistic effect

The isolated *Bacillus* and *Actinomycetes* were tested using the single line streak method for and antagonistic impact against test bacteria. A single streak of each test microorganism was inserted perpendicular to the *Bacillus* and *Actinomycetes* streak using this method after and isolated strain was inoculated in a single streak through the middle of a plate of screening media (Mueller Hinton agar) and incubated [12]. The *Bacillus* strains were incubated at 37°C for 48 hours, while the *Actinomycetes* strains were incubated at 28°C for 7-14 days. A zone of inhibition was looked for after the plates had been incubated.

3. Results and discussion

Five soil samples from different regions of North Macedonia were used for screening purpose. Furthermore, the collection of the samples was based on different sites, such as undisturbed and contaminated, one of the sample that was used was compost and the last sample was collected from a mountain forest, under a tree.

The moisture content of the samples from undisturbed soils was between 44 – 73%, while the moisture content from the contaminated soils was between 15-44%. The pH of the most samples was between 7-8 (Figure 1).



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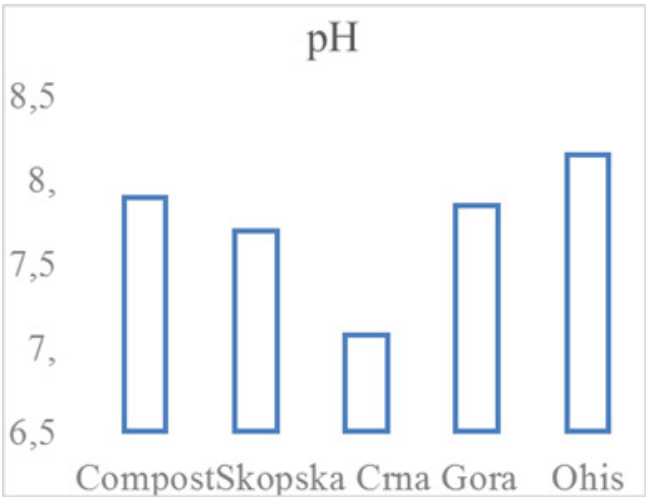

B

Figure 1. A. Graphic with % of the moisture content for the five samples; B. Graphic with pH values for the five soil samples

120 were discovered to be *Bacillus* spp., and 112 were *Actinomycetes*, according to the identification tool utilized. The zone of inhibition against the perpendicularly streaked bacterial lawn was also seen in the perpendicular line streaking on the Mueller Hinton Agar (MHA).By adopting the cross streak method for primary screening, 28 actinomycetes isolates and 26 isolates of *Bacillus* spp. were found to be efficient against the tested bacteria.Furthermore, in order to observe the zone of inhibition and determine the antibiotic property, only those with macroscopic identification were employed.*Bacillus* spp. and *Actinomycetes* were utilized to observe the antibiotic effect on the ATCC cultures *of S. enterica, E. coli, P. aeruginosa, L. monocytogenes, B. subtillis*and *S. aureus*, but the antibiotic effect was mostly seen in *B. subtillis*, followed by a few instances in *E.coli* and *S. aureus* (Figure 2).

Figure 2. Zone of inhibition of the isolates using the line streaking method for primary screening

However, we found out that most of the isolates were effective against Gram-positive, rather than Gram-negative bacteria. *Actinomycetes* and *Bacillus* spp. from undisturbed locations outperformed those from contaminated sites in terms of having antibacterial activities against the tested bacteria among those that were principally screened.Chemical medicines and pesticides have been used to stop the spread of disease for a very long time, yet it is still unclear how they affect the environment and ecology. In their study, Al-Ajlani et al. [13] found that B*acillus* sp. produces antimicrobial compounds. The findings of our work were verified by Prescott et al. [14] who found that the bacitracin generated by *Bacillus* sp. inhibits *S. aureus* and *E. coli*.Additionally, the likelihood of developing antibiotic-resistant strains rises due to the widespread use of chemical pesticides and antibiotics in fields and culture ponds [15], [16]. There have been numerous research on the utilization of the genera *Bacillus* and *Actinomyces* to reduce plant and human infections [17], [18].

5. Conclusion

The goal of the current study was to assess how well a recently discovered *Bacillus* species and *Actinomycetes*from soil produced antibiotics. The data obtained indicated that 26 isolates of *Bacillus*sp. and 28 *Actinomycetes*isolates are capable of generating antibacterial compounds.Among the primarily screened, the actinomycetes and *Bacillus* spp. from undisturbed sites showed better antimicrobial properties towards the tested bacteria that the ones from the contaminated sites.The soil samples of North Macedonia provide suitable environments for *Bacillus* and **Actinomycetes** towards obtaining novel antimicrobial agents. Thus, exploitation ofvarious regions of North Macedonia for the discovery of an effective antimicrobial agent is helpful in providing a solution to the cost-effectivetherapy and action against antibiotic resistance.

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THE *PSEUDOMONAS* GENUS AS A SOURCE OF PHENAZINE COMPOUNDS

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Abstracts. *The phenazines include upward of 50 pigmented, heterocyclic nitrogen-containing secondary metabolites synthesized by some strains of Pseudomonas spp. and a few other bacterial genera. Pseudomonas strains are increasingly attracting considerable attention as a valuable bacterial host both for basic and applied research. It has been considered as a promising producer of bioactive secondary metabolites, particularly phenazines. Apart from the biotechnological perspective, these aromatic compounds have the notable potential to inhibit plant-pathogenic fungi and thus are useful in controlling plant diseases. Nevertheless, phenazines production is quite low by the wild-type strains that necessitated its yield improvement for large-scale agricultural applications. The aim of the work was to the culture media optimization for some Pseudomonas strain cultivation to improve their phenazine compound production.*

Key words: *phenozine, metabolite, Pseudomonas strain cultivation, nutrient medium composition, microbial cell biomass.*

Introduction

Phenazines comprise a large group of nitrogen-containing heterocyclic compounds that differ in their chemical and physical properties based on the type and position of functional groups present. More than 100 different phenazine structural derivatives have been identified in nature, and over 6,000 compounds that contain phenazine as a central moiety have been synthesized [11].

Phenazines are also known as a large class of redox-active secondary metabolites produced by many Gram-positive (e.g. *Streptomyces*) and Gram-negative bacteria (e.g. *Pseudomonas*). The core structure of phenazines is a pyrazine ring (1,4-diazabenzene) exhibiting two annulated benzenes. Their early discovery in the mid-19th century has been facilitated by the fact that phenazines are intensively colored compounds [1]. In 1859 Fordos described the extraction of a blue pigment, which is responsible for the coloration of the "blue pus", observed in patients suffering from severe purulent wounds, and named the blue pigment "pyocyanine" (nowadays more common as pyocyanin) from the Greek words for "pus" and "blue" [3, 4]. A few years later, in 1882, Gessard realized that pyocyanin was produced by an aerobic motile bacterium [5], which he subsequently named "*Bacillus pyocyaneus*". Gessard was, however, not the first to discover this organism: production of a blue-green pigment by bacteria, albeit without identification of the responsible compound, had already been described by Schroeter who called the responsible organism "*Bacteridium aeruginosum*" after "aerugo", the Latin word for

verdigris, the blue-green coating that develops on the surface of copper exposed to air [2]. In 1900, Migula finally replaced these long forgotten names of the pyocyanin-producing species by "*Pseudomonas aeruginosa*", which is the name still in use today. Since then more than 100 different phenazine compounds of microbial origin have been reported in the literature [7, 11, 12].

Phenazines were long considered to be pigments of uncertain importance – a stark contrast to current recognition that they are versatile metabolites involved in numerous aspects of bacterial physiology. Early studies revealed the broad cross-phylum inhibitory properties of these compounds, leading many phenazines to be classified as antibiotics. Antibiotic-producing species are common in microbial communities throughout nature, and natural antibiotics are traditionally associated with roles in microbial defense, fitness, interference competition, and the protection of plants and insects against pathogens [2, 4, 6, 9, 13]. More recently, research on natural antibiotics has focused on their role in microbial physiology, communication and gene regulation [3, 4, 10]. However, few of these functions have been studied in the field and little is known about the frequency, amounts and physiology of antibiotic production in nature. In the past decade, phenazine-producing *Pseudomonas* spp. have emerged as a model system for addressing these fundamental questions.

Material and methods

At work there were used the following strains of *Pseudomonas aureofaciens* (ONU 304, ONU 305, ONU 306), *P. fluorescens* ONU 303, *P. aeruginosa* (ATCC 15692, ATCC 27853, ATCC 10145). Bacterial pre-cultivation was carried out on the MPA for 24 hours and then bacterial biomass was transferred to a following nutrient medium: standard liquid medium, MPB enriched by 5 % glucose and King B Agar without agar-agar supplementation. Initial cell concentration was $5 \cdot 10^7$ CFU/ml. The studied microorganisms were grown during 7 days at 25 °C and 37 °C based on strain physiological characteristics. Every 24 hours the cell suspension density was measured by the spectrophotometer at 540 nm. The phenazine antibiotic extraction of the *Pseudomonas* studied strains was carried out according to the Levitch [8]. Each experiment was conducted twice; the number of repeats in each of them was 4.

Results and discussion

The study of the microorganism growth dynamics showed their gradual biomass increasing over time. The maximum growth of the strains was reported on the fifth day. The determination of culture medium composition influence on the intensity of biomass accumulation detected that the highest cell number of all tested cultures was in liquid variant of King Agar. In this case the *P. aeruginosa* cell density is higher than 5 or 10 times for *P. aureofaciens* or *P. fluorescens* strains respectively.

The *Pseudomonas* cultivation in the King liquid medium caused significant changes of its colour that was the evidence of the metabolite formation and accumulation by the studied microorganisms.

The phenazine concentration determination revealed that *P. aeruginosa* ATCC 15692 and *P. fluorescens* ONU 303 were their most active producers. Ac-

cording to the studied metabolite concentration microbial cultures were assigned as follows: *P. aeruginosa* ATCC 15692 > *P. fluorescens* > *P. aureofaciens* ONU 305 > *P. aureofaciens* ONU 304 > *P. aeruginosa* ATCC 10145 > *P. aeruginosa* ATCC 27853 > *P. aureofaciens* ONU 306.

Conclusions

Thus, the phenazine accumulation intensity depends on the strain specificity and nutrient medium composition. The inorganic metal ion presence in King Agar B liquid variant significantly increased the phenazine concentrations that are released by the cultures. However these characteristics do not corresponded to the studied *Pseudomonas* cell biomass accumulation rates.

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UDC 579.2

Okhotnikova O., Rusakova M.

BIOTECHNOLOGY FOR THE DEVELOPMENT OF ANTIBIOTICS FOR THE VICARIOUS GENUS *BACILLUS*

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Abstract. *The antibiotic activity of exometabolites produced by strains of Bacillus subtilis ONU 481, Bacillus megaterium ONU 484, Bacillus atrophaeus MH4, B. subtilis MB1 against the test microorganisms Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 was studied. The results obtained during the research showed that all the studied microorganisms are characterized by the ability to synthesize antimicrobial exometabolites. All of the samples showed activity against against the gram-positive bacterium S. aureus ATCC25923 and one producers against both test microorganisms. This indicates that these strains are perspective producers of antibiotics, which makes it possible to use them for biotechnological purposes.*

Key words: *Bacillus, antibiotics, resistance, biotechnology.*

Introduction

Antimicrobial resistance is becoming more widespread and is one of the biggest threats to the prevention and treatment of an increasing number of infections. Therefore, the search for new antibiotics and their producers to fight such diseases is becoming increasingly urgent [1].

Bacteria of the genus *Bacillus* are one of the largest sources of bioactive antimicrobial natural products (Fig. 1), which are characterized by a wide spectrum of antibiotic activity and are synthesized as low molecular weight polypeptides by ribosomal (bacteriocins) or non-ribosomal mechanisms. Such antimicrobial substances are characterized by two main features: they have high biological activity against organisms sensitive to them and selectivity of action, since each antibiotic shows its biological effect only in relation to individual organisms or their groups, while not having a noticeable effect on other forms of living beings [2].

The number of antibiotics produced by bacilli is close to 167, 66 of them are synthesized by *Bacillus subtilis*, 23 by *Bacillus brevis* and other peptide antibiotics are produced by other species of the genus *Bacillus* [3]. Most peptide antibiotics synthesized by *Bacillus* are active against gram-positive bacteria; however, compounds such as polymyxin, colistin are active exclusively against gram-negative bacteria, while bacillomycin, mycobacillin, and fungistatin are effective as antifungals [3].

The biotechnology of antibiotics production is a multi-stage system consisting of the stages of auxiliary work, obtaining the producer strain, production fer-

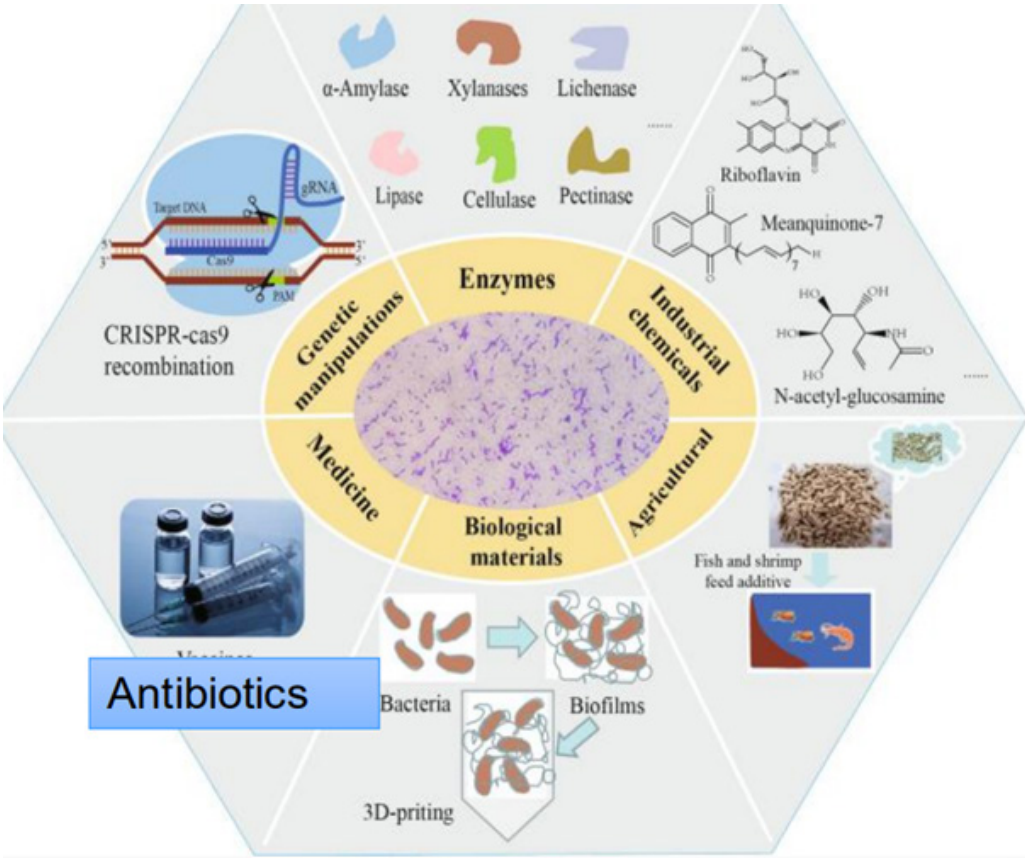

Fig. 1. Representatives of the genus *Bacillus* as producers of a wide range of biologically active compounds [5]

mentation (synthesis of the antibiotic), isolation and purification of the antibiotic, as well as obtaining the target product (packaging, labeling and shipping) [4].

The use of *Bacillus* bacteria as producers in the production of biologically active products has a number of advantages:

1) high rate of culture growth and reproduction, and therefore biosynthesis reactions;

2) a relatively simple and well-known cultivation technology on an industrial scale, which ensures maximum yield of the target antibiotic product and is independent of environmental conditions [5];

3) the possibility of directed influence using methods of genetic engineering on the synthesis of biologically active substances to improve the biological value of the target product;

4) representatives of the genus *Bacillus* show high antagonistic activity;

5) manufacturability in production;

6) stability during storage [6].

The purpose of this work was to determine the antibiotic activity of metabolites synthesized by members of the genus *Bacillus*, carried out at the Biotechnological Research and Training Center of Odesa National University named after I. I. Mechnikov.

Materials and Methods

In the experiment, strains from the microbiology culture collection of the Department of Microbiology, Virology and Biotechnology were used: *Bacillus subtilis* ONU 481, *Bacillus megaterium* ONU 484, *Bacillus atrophaeus* MH4, *B. subtilis* MB1.

To restore strains and maintain them, nutrient medium MPA and MPB were used for deep cultivation with constant stirring. After cultivation, suspensions of cells of the respective strains were prepared on the basis of a physiological solution and their optical density was measured using the McFarland standard [7]. 2.0 ml of suspension was taken from each sample and added to 100 ml of MPB. The main parameters of cultivation were constant shaking on a swing at 150 rpm at a temperature of 25°C.

During 5 days, samples of 1.5 ml of suspensions of each strain were taken and the cultural properties of the producers, as well as the number of cells, were determined. For this, the samples were centrifuged, after which the precipitated cell mass was resuspended in physiological solution and the optical density was determined. The results obtained using the calibration curve were converted into indicators of the accumulated biomass - the amount of CFU in 1 ml of nutrient medium.

Antibiotic activity was checked by the method of wells in agar. Petri dishes were kept in a thermostat for 7 days at 37°C, measuring the growth inhibition zone around the well on the second and seventh days. The absence of growth of microorganisms, that is, the diameter of the zone of growth inhibition (in mm) was calculated as the arithmetic mean of three dimensions of randomly selected projections [8]. For each variant, the number of repetitions was 5.

Results and Discussion

All producer strains were cultivated for 4 days, which corresponded to the course of the idiophase - the period of maximum synthesis of secondary metabolites, in particular antibiotics (Fig. 2). A more intensive development was observed

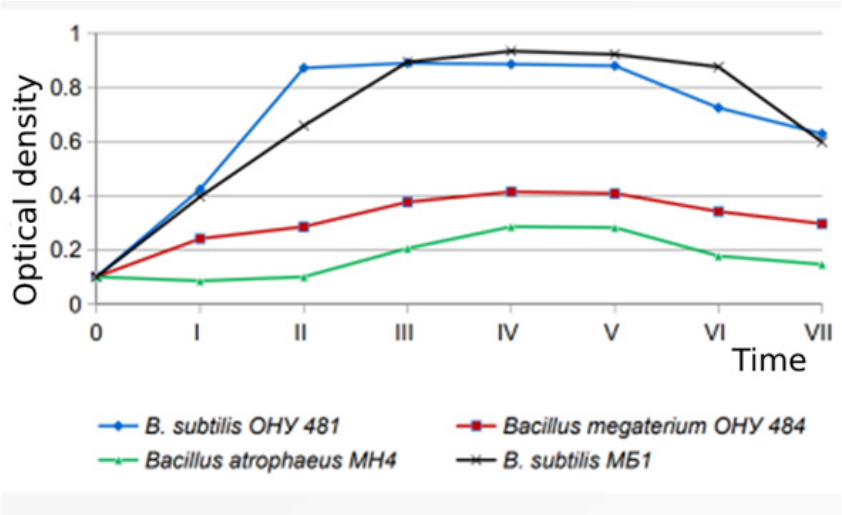

Fig. 2. Biomass accumulation by bacilli of the studied strains

for *B. subtilis* ONU 481: already on the 2nd day of cultivation, there was a noticeable visual turbidity, evenly distributed throughout the entire thickness of the nutrient medium.

The work determined the antibiotic activity of the liquid culture, where the producer strains were developed, in relation to the test microorganisms *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (Fig. 3), All studied strains were characterized by the production of antimicrobial compounds to which different test microorganisms were sensitive. The widest spectrum of activity was recorded for the strain *B. subtilis* ONU481.

Strains	The diameter of the growth inhibition zone of the test-microorganism, mm	
	E. coli ATCC 25922	*S. aureus ATCC 25923*
B. subtilis ОНУ 481	6±1	16±1
B. megaterium ОНУ 484	0	19±1
B. atrophaeus МН4	0	14±1
B. subtilis МБ1	0	16±1

Fig. 3. Zone of test cultures growth inhibition

All studied test microorganisms were sensitive to exometabolites. At the same time, the zones of inhibition of growth of *S. aureus* ATCC25923 were larger than those of *E. coli* ATCC 25922. In some cases, there were no zones of inhibition of growth of *E. coli* at all. The greatest degree of antimicrobial effect, i.e. the most pronounced zone of inhibition of the growth of test cultures, was determined for strain *B. megaterium* ONU 484.

Conclusions

A study of the antibiotic properties of the exometabolites of individual representatives of the genus *Bacillus* was conducted. According to the results, the period of the idiophase - the stationary phase of the growth of microorganisms, characterized by the synthesis of secondary metabolites, in particular antibiotic compounds, began on the fourth day of cultivation. More intensive development and the highest degree of antimicrobial action was demonstrated by the strain *B. subtilis* ONU 481. Among the selected test microorganisms, *S. aureus* ATCC 25923 showed the greatest sensitivity to exometabolites with antimicrobial properties. It can be concluded that the antimicrobial substances synthesized by the studied strains of bacilli are active in the majority of gram-positive bacteria. However, one of the producers, *B. subtilis* ONU 481, showed an inhibitory effect on the gram-negative microorganism *E. coli* ATCC 25922.

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Palamarenko A., Kovalchuk R., Kimurzhyi I., Merlich A.

ANTIBIOFILM ACTIVITY OF THE BACTERIOCIN FROM *ENTEROCOCCUS ITALICUS* ONU547 AGAINST OPPORTUNISTIC PATHOGENS *ENTEROCOCCUS FAECALIS* AND *LISTERIA IVANOVII*

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Abstract. ***Aim.*** *To study an antibiofilm activity of the bacteriocin from Enterococcus italicus ONU547 against opportunistic pathogens Enterococcus faecalis and Listeria ivanovii.* ***Methods.*** *Inhibitory activity of the bacteriocin samples was tested by microtiter plate assay using 1% crystal violet as a dye.* ***Results.*** *The bacteriocin from E. italicus ONU547 inhibited the growth of planktonic cells of L. ivanovii subsp. ivanovii 20750, as well as of E. faecalis ATCC29212. The decrease of the cell number of the plankton was higher in the case of enterococci strain (up to 85.4%), while the observed percentage of listeria plankton inhibition after addition of the bacteriocin was from 36.4 to 76%. The tested bacteriocin inhibited the level of biofilm formation only in L. ivanovii subsp. ivanovii 20750 in 75.8% compared to the control. The inhibitory activity of the bacteriocin against plankton and biofilms was dose-dependent.* ***Conclusions.*** *The bacteriocin produced by the E. italicus ONU547 strain significantly inhibits biofilm formation in L. ivanovii subsp. ivanovii 20750 and effectively suppresses the growth of plankton of E. faecalis ATCC29212. This antimicrobial compound can be perspective candidate for development of new therapeutics.*

Key words: *biofilm, antibiofilm activity, bacteriocin, Enterococcus italicus, opportunistic pathogens*

Introduction

Biofilms are special form of microbial life composed of their cells and produced extracellular substances. Biofilms due to their complex structure are more resistant to antimicrobial compounds compared to the planktonic cells. Thereby, the biofilm formation in pathogenic and opportunistic bacteria is harmful for the medical field [2, 5].

From the scientific sources it is known that bacteriocins from lactic acid bacteria (LAB) can be perspective candidates for development of new therapeutics for use in medical field due to their antibiofilm activity [6]. However, the information on inhibitory activity of bacteriocins from LAB against plankton and biofilm formation is scarce. In our previous work we reported on antibiofilm activity of the bacteriocin from the strain *E. italicus* ONU547 isolated from plant material. However, among the tested indicator strains only one opportunistic pathogen was used [3], and the effect of this antimicrobial substance on biofilms and planktonic

cells of the dangerous opportunistic pathogens *Listeria ivanovii* and *Enterococcus faecalis* is unknown.

The aim of this work was to study an antibiofilm activity of the bacteriocin from *E. italicus* ONU547 against opportunistic pathogens *E. faecalis* and *L. ivanovii*.

Materials and methods

The samples of bacteriocin were prepared in our previous studies. Totally, two samples were took for the present research: CFS1 and CFS2 with bacteriocin activity 160 arbitrary units per milliliter (AU/ml) and 640 AU/ml, respectively, exhibited against *E. faecalis* ATCC 29212 and *L. ivanovii* subsp. *ivanovii* 20750. Briefly, CFS1 was prepared from the culture of *E. italicus* ONU547 grown in autoclave sterilized medium, while CFS2 – from the enterococci culture grown in filtered medium that was not subjected to any heating [4].

The indicator strains *E. faecalis* ATCC 29212 and *L. ivanovii* subsp. *ivanovii* 20750 were kept at – 20 °C with 30% of glycerol and activated before the experiments by inoculation in Nutrient Broth (NB, Himedia, India) and incubation at 37 °C overnight. The cell concentration of the activated cultures was measured by spectrophotometer SmartSpec Plus (Bio-Rad, USA) with the wavelength 600 nm. In the experiments the overnight cultures of *L. ivanovii* subsp. *ivanovii* 20750 and *E. faecalis* ATCC 29212 were used with the cell concentration 5 x 10^8 and 4.77 x 10^8 cell/ml, respectively.

The microtiter plate assay was performed in sterile 24-well plastic plates. The wells were firstly covered by 20 and 100 µl of the both bacteriocin samples and as a control the same volumes of sterile saline were apply. Thereafter, 1 ml of NB and 50 µl of the overnight cultures of the indicator strains were added in each of them. The temperature of incubation was 37 °C [1].

The next day the planktonic cells were carefully removed and their concentration was measured by spectrophotometer using the wavelength 600 nm [3].

The rested biofilms were washed twice in saline and fixed by adding of 1 ml of 96% ethanol for 15 min. After the fixation, the ethanol was removed and the biofilms were stained for 10 min with 1 ml of crystal violet (1%). After that, the staining solution was poured out and the biofilms were washed with tape water. The residual water was removed from the wells. Biofilms were dried and destroyed adding 1 ml of the lysing solution for 2 hrs. The composition of the lysing solution was the next: 0.1 M NaOH, 1% SDS. After the complete lysis of the biofilms the optical density of the cells was measured as described above [1, 3].

All the experiments were performed two times with two - four replications at each. The program Microsoft Office Excel was used for statistical analysis (mean values, standard deviation, confidence).

Results and discussion

As a result of the conducted researches it was revealed that the bactericin from *E. italicus* ONU547 showed the inhibitory activity against plankton as well as biofilm formation in food pathogen *L. ivanovii* subsp. *ivanovii* 20750 (Fig. 1 – 2).

Moreover, this antimicrobial compound also inhibited growth of planktonic cells of the pathogen *E. faecalis* ATCC 29212 (Fig. 3).

Indeed, the studied bacteriocin inhibited the growth of planktonic cells of *L. ivanovii* subsp. *ivanovii* 20750 decreasing the cell number from 36.4% to 76%, depending on the bacteriocin concentration. Thus, adding of 20 and 100 µl of the CFS1 (160 AU/ml) decreased the number of planktonic cells in 36.4% and 57.6%, respectively, while the use of 100 µl of more concentrated CFS2 (640 AU/ml) caused inhibition of the cells growth of this pathogen in 76% compared to the control (Fig. 1).

Besides the planktonic cells the studied antimicrobial peptide also inhibited biofilm formation in *L. ivanovii* subsp. *ivanovii* 20750, but only when it was added in highest concentration. Thus, 100 µl of CFS2 decreased the level of biofilm formation in 75.8% (Fig. 2).

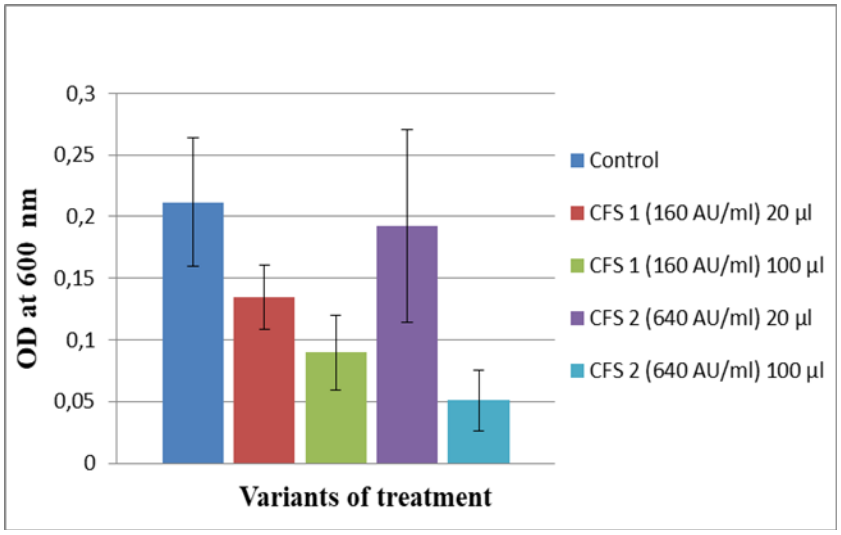

Fig. 1. Effect of the bacteriocin samples from *E. italicus* ONU547 with different activity levels on plankton of *L. ivanovii* subsp. *ivanovii* 20750

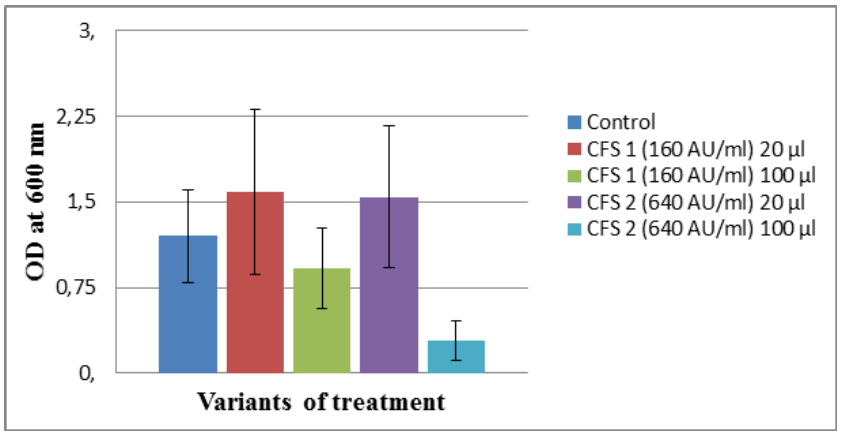

Fig. 2. Effect of the bacteriocin samples from *E. italicus* ONU547 with different activity on biofilm of *L. ivanovii* subsp. *ivanovii* 20750

The other samples containing less of bacteriocin did not show any significant inhibition of formation of biofilms in this listeria strain. The obtained results indicate dose-dependent mechanism of inhibition of biofilm formation in *L. ivanovii* subsp. *ivanovii* 20750 as well as of their planktonic cells growth.

To our knowledge this is the first report on antibiofilm activity of bacteriocin from *E. italicus* species against the opportunistic pathogen *L. ivanovii*.

In our study it was also established that the bacteriocin from *E. italicus* ONU547 inhibited the growth of planktonic cells of *E. faecalis* ATCC 29212, but more intensively when compared to listeria cells. Indeed, the bacteriocin from CFS1 of volume 20 µl decreased the amount of plankton of the enterococci in 68.2% and when added 100 µl – even in 85.4%. The adding of the bacteriocin from CFS2 at the same volumes inhibited plankton growth in 75.7% and 74%, respectively (Fig. 3). Thus, all the tested samples of bacteriocin showed the high inhibitory activity of similar level against plankton of the strain *E. faecalis* ATCC 29212.

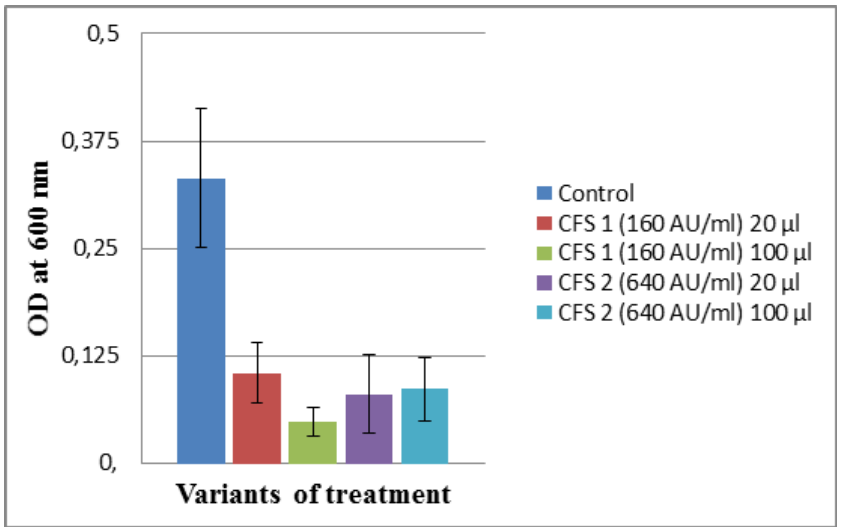

Fig. 3. The effect of the bacteriocin samples from *E. italicus* ONU547 on plankton of *E. faecalis* ATCC 29212

In contrast to planktonic cells, we did not find any inhibitory activity of the tested bacteriocin against biofilm formation in bacteria of *E. faecalis* ATCC29212. This fact indicates the specificity of the antibiofilm activity of the tested antimicrobial compound from *E. italicus* ONU547.

The results on effect of bacteriocins from *E. italicus* species on plankton and biofilm formation in *E. faecalis* and *L. ivanovii* species are completely absent. In our previous work we showed inhibitory activity of this bacteriocin against biofilm formation and plankton of other indicator bacteria: *Pseudomonas aeruginosa* PAO1 and *Lactobacillus sakei* subsp. *sakei* JCM 1157. The percentage of inhibition of biofilm formation in *P. aeruginosa* PAO1 was 48.0% and in *L. sakei* subsp. *sakei* JCM 1157 – 52.5% [3].

The inhibitory activity of the bacteriocins from other lactic acid bacteria such as nisin A and lacticin Q was also tested against biofilms of *S. aureus* MR23 in pub-

lication of other authors. The studied antimicrobial compounds showed inhibitory activity against biofilms as well as planktonic cells. The authors also established that formation of pores in the envelope of sensitive biofilm cells is the main mechanism of the studied inhibitory effect on the biofilms [6].

Conclusions

The bacteriocin from *E. italicus* ONU547 exhibits antibiofilm activity against *L. ivanovii* subsp. *ivanovii* 20750. Because of its ability to significantly decrease the level of biofilm formation in listeria and to inhibit the growth of planktonic cells of *E. faecalis* ATCC29212 in high extent, the bacteriocin from *E. italicus* ONU547 can be perspective for development of new therapeutics.

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UDC 611.93.013-053.13/.31

Popova I.

EMBRYOLOGICAL ESTABLISHMENT OF SOME STRUCTURES IN THE INFRAHYOID REGION OF NECK DURING FETAL PERIOD OF HUMAN DEVELOPMENT

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Abstract. *High incidence of congenital malformations in newborns (including those arising in the neck region) requires profound anatomical and embryological research in this field. Data on normal and also impaired human organogenesis can be practically implemented for a better surgical resolving of congenital pathologies and improve postnatal survivance as well. In our work, by using classical dissection and microscopy methods, as well as three-dimensional remodeling of human prenatal material, we discuss obtained data on normal organogenesis and establishment of neck muscles in the infrahyoid region, their nerve and blood supply, topography of fascial spaces. It is seen that interconnection of components in the carotid triangle, as well as the course of strap muscle have specific peculiarities if compared to adult morphology. Fascial spaces of the neck are established at the beginning of the 4th month of intrauterine development. Components of the carotid triangle tend to vary in the position of magistral vessels at the beginning of the fetal stage (4-5th months) and have defined topography by 8th month of intrauterine development.*

Key words: *human, development, embryology, neck.*

Introduction

Medical embryology is aimed to study normal human morphogenesis, which gives a basis for further investigations of pre- or postnatal possible pathological conditions. Such investigations complement existing data on human prenatal development and moreover, give useful data for medical practitioners such as maxillofacial surgeons, aesthetic specialists and pediatricians. Infrahyoid region includes crucial blood vessels, a system of muscles that is often used in reconstruction surgeries and is a place of regular development of congenital malformations.

Aim

Research is aimed to investigate morphological and topographical peculiarities of some structures in the infrahyoid region of the neck in human fetuses during the prenatal period of human ontogenesis.

Materials and methods

We have examined 12 specimens of human fetuses (4-8th month of prenatal

development (PND); 82,0-311,0 mm of parieto-coccigeal length (PCL)). The material was obtained and studied at Chernivtsi Regional Pathologists Office. In order to visualize necessary structures in the infrahyoid region we have used complex of morphological methods: macroscopy, microscopy, three-dimensional remodeling and statistical analysis. The study was performed in accordance with the provisions of the Declaration of Helsinki on ethical issues of studies conducted with humans (1964-2008), Ukrainian Ministry of Health Orders № 690 (23.09.2009), № 944 (14.12.2009), № 616 (03.08.2012). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave indications of possible malformation. Approval for the study was granted by the Ethics Committee of the HSEE of Ukraine "Bukovinian State Medical University".

Results

We have seen that starting from the early fetal period (middle of the 4th month of PND) infrahyoid structures such as strap muscles and blood vessels are fully distinguished. Infrahyoid group of muscles that include paired omohyoid, thyrohyoid, sternothyroid, sternohyoid already have precise features of attachment places and fasciae coverings. One of their representatives - omohyoid, is seen as a thin muscular plate, arising by a broad inferior belly from the medial part of the superior portion of the scapula at the early stages of fetal period of the PND. In human fetus of the 4th month of PND, the middle ⅓ of the muscle then passes below the sternocleidomastoid. Superior belly arises superiorly over the anterior edge of the sternocleidomastoid (that has already separated from the common precursor mass with the posterior muscles of neck) within the anterior triangle and neck. Omohyoid was attached to the lower edge of the hyoid bone body, that actually merges at this stage the infrahyoid triangles of neck already. It is worth mentioning that at late fetal period (end of the 9th month of PND) a few neck spaces can be already distinguished. For instance, carotid space is composed of two areas that extend from the base of the skull to the aortic arch: supra- and infrahyoid portions of neck and mediastinum. Carotid space contains internal jugular vein, common carotid artery, internal carotid artery, cranial nerves, lymph nodes and vagus nerve. In the infrahyoid region of neck, carotid space is bordered by anterior cervical space in the front, retropharyngeal space medially and by perivertebral cervical space posteriorly. In the late fetal period of human PND (starting from the end of the 8th month of PND) the common carotid artery runs behind the sternocleidomastoid muscle and medially to the internal jugular vein. The internal jugular vein in human fetus runs down along with the lateral wall of the pharynx and posteriorly to the internal carotid artery. Topographical interconnection between the common carotid artery and the vagus nerve is changing in the late fetal period: it changes the direction from a ventral course to an intermediate one between the artery and vein. This is the final dorsal course that is tending to have defined adult features.

Conclusions

Muscles, blood vessels and nerves within the infrahyoid region of the neck tend to have variable course starting from the end of 4th month of intrauterine development and gain determinate topographical interconnections by the 8th month of

prenatal period of human ontogenesis. We find it appropriate to continue research that will consider gender differences of structures course and variations during different stages of intrauterine development.

UDC 579.87

Potapenko K., Chaban M.

STUDY OF THE ACTINOMYCETES MYCELIUM DURING CULTIVATION ON COVER GLASS

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Abstract. *The study of actinomycetes aerial mycelium by dark-field microscopy in combination with molecular methods is an old approach to determining the systematic position of a bacterial strain. Using the method of mycelium immobilization on a coverslip during the cultivation of actinomycetes, we obtained the whole mycelium with its included structures. Thus, the differences in the aerial mycelium formation and the spore's formation of the Streptomyces ambofaciens ONU 561 strain were found and studied when cultivated on Gause 2 and Oat agar nutrient media.*

Key words: *actinomycetes, mycelium, cultivation, microscopy.*

Introduction

During the colony development, actinomycetes form an aerial mycelium followed by the spore's formation. Mycelium formation itself is an important systematic feature in actinomycetes. Thus, the study of the mycelium structure and its formation using the optical microscopy method allows to collect information to establish the isolated actinomycetes strains taxonomic position.

Materials and methods

We conducted a study of some actinomycetes strains aerial mycelium. For this purpose, the method of cultivating bacteria on Gause-2 nutrient medium and oat agar using cover glass was optimized. Indentations, which were made in the prepared medium with a sterile scalpel, were then seeded with actinomycetes strains, and then covered with a sterile cover glass. On the third day, when the growth of colonies appeared on the nutrient medium, the cover glass was removed and transferred with its surface in contact with the medium to a glass slide with a drop of sterile water [4]. Further, microscopic studies were carried out using a Carl Zeiss AXIO microscope in dark-field mode at a magnification of 400 times. While the microscopy, we tested various lighting systems, and settled on the use of dark-field microscopy for the best visual results. The analysis was carried out every week during a month.

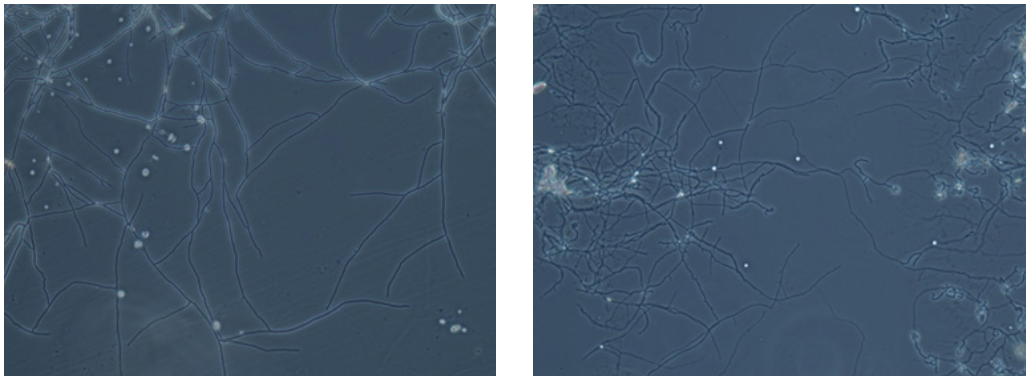
Results

The cultivation method with coverslip use made it possible to preserve and analyze the mycelium structure of the studied bacterial strains. It was possible to detect differences in the mycelium structure of one strain that grew on different media. We also studied the mycelium change during the colony growth on nutrient

media during a month cultivation, where we monitored the mycelium change with the individual spores formation.

The aerial mycelium structure was studied on the example of one of the most promising strains, with pronounced antagonistic activity, isolated from Black Sea mussels - *Streptomyces ambofaciens* ONU 561 (according to the results of 16S RNA sequencing, Illumina HiSeq 4000, 2x150 bp reads).

Preliminary cultivation of the strain on different nutrient media, like Gause No. 2 and Oat agar, made it possible to establish the aerial mycelium structure, the actinospores presence and location in the aerial mycelium hyphae at different time intervals (Fig. 1).



а) **в)**

Fig. 1. The actinobacteria strain *S. ambofaciens* ONU 561 aerial mycelium morphology on media: a) Hause 2, b) Oat agar (photo on the third week of growth)

Within one organism, there is a distinction between substrate (one that grows into the substrate) and aerial mycelium, that is responsible for the synthesis of secondary metabolites, including many antibiotics and antitumor compounds [1].

The aerial mycelium structure of *S. ambofaciens* strain ONU 561 differs depending on the culture medium. The mycelium of the strain on a medium with oat agar has a branched structure, with frequent partitions (septa), spores are conidia, immobile on spore-bearing branches, single or paired, and look like spherical or pear-shaped bundles or tassels [2]. At the same time the formation of individual spores was not observed on Gause 2 medium.

It is well known that many actinobacteria are characterized by colonies pleomorphism on different nutrient media. Thus, actinobacteria, depending on the composition of the medium, form various colonies that differ in structure, edge, height of aerial mycelium, and color of mature spores [3].

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Potapenko K., Korotaieva N., Strashnova I., Vasylieva N.

CHARACTERISTICS OF *STREPTOMYCES AMBOFACIENS* ONU 561 FROM *MYTILUS GALLOPROVINCIALIS* OF ODESA GULF OF THE BLACK SEA

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Abstract. *Marine actinobacteria are one of the most important producers of various groups of secondary metabolites, therefore it is promising to search for new marine actinobacteria, study their main biological properties and determine their biotechnological potential.* ***The aim of the study*** *was to identify and study the main biological properties of a strain of actinobacteria isolated from the Black Sea mussels.* ***Methods****. Isolation was performed on the nutrient media (nutrient agar, Gauze 2, Czapek agar, ISP-4, soil agar) which were prepared in seawater. The cultural, physiological and biochemical properties were studied according to generally accepted methods. Primary identification of strains by fatty acid composition was performed by gas chromatography using an automatic system for the identification of microorganisms MIDI Sherlock. Identification of 16S rRNA sequencing was performed on Illumina HiSeq 4000, 2x150 bp reads. Antagonistic activity was evaluated by agar blok-diffusion method.* ***Results.*** *Strain with pronounced antagonistic activity has been identified as S. ambofaciens ONU 561 his main morphological, physiological and biochemical properties were studied. The antagonistic activity of the strain was investigated against 12 indicator microorganisms represented by gram-positive, gram-negative bacteria and the yeast-like fungus Candida albicans ATCC 18804.*

Key words: *marine actinobacteria, Streptomyces, 16S rRNA sequencing, biological properties, fatty acid, antagonistic activity.*

Introduction

The constantly growing resistance of bacteria to already known antimicrobial substances forces scientists to look for new sources of finding producers of biologically active compounds.

The marine environment contains many microorganisms capable of producing biologically active substances that have the potential to create future drugs against human diseases [1].

Marine actinobacteria are a promising source of new natural compounds with many medical and biotechnological applications; they produce numerous secondary metabolites belonging to different chemical classes [2].

Biological research of the ocean opens up new perspectives for the discovery of new drugs and other useful chemical structures due to the huge unexplored biodiversity of microorganisms found in this environment [3].

Materials and methods

From the Black Sea mussels collected at a depth of 5–6 meters in June 2020 in the area of the Biological Station of the Odessa Mechnikov National University, a strain of actinobacteria was isolated, which received the conditional designation Myt7ch.

Isolation was carried out on the nutrient media (nutrient agar, Gauze 2, Czapek agar, ISP-4, soil agar), prepared in seawater with the addition of antibiotic nalidixic acid at a concentration of 0.01 g/l to prevent the growth of most foreign microorganisms. Sowings on the nutrient media were carried out in three replicate. Incubation had carried out at a temperature of 28 °C during for 21 days [4].

Primary identification was carried out by definition of fatty acid composition using automatic identification system Sherlock Microbial Identification System (MIDI Sherlock version 6.2, MIDI Library ACTIN 3.80) equipped with the flame-ionization detector, with ULTRA 2 column. Fatty acid methyl ester was isolated according to the standard protocol [5, 6].

The species level identification of the isolated strain was carried out by16S rRNA partial gene sequencing and construction of the phylogenetic tree. 16S rRNA sequencing was performed on Illumina HiSeq 4000, 2x150 bp reads. DNA extraction was prepared according to the standard protocol [7].

Morphology properties of the strain were studied on media Oat agar with sea salt, Gauze 1 and Gauze 2 for 14 days at 28 °C [4]. The ability of strain to synthesis melanoid pigment detected on ISP-6 and ISP-7 media [8].

Enzymatic properties (determination of catalase, oxidase, amylase activities; ability to dilute gelatin; H2S formation; reduction of nitrate to nitrite; reduction of nitrate to molecular nitrogen; proteolytic activity) studied according to generally accepted methods [9].

Antagonistic activity was evaluated by agar blok-diffusion method for the twelve strains of indicator microorganisms. Indicator strains represented by gram-positive (*Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* ATCC 4698, *Kucoria rhizophila* DSM 348, *Bacillus subtilis* ATCC 6633), gram-negative (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6896, *Salmonella enterica* NCTC 6017, *Klebsiella pneumoniae* ATCC 131, *Pseudomonas putida* KT 2440) bacteria and the yeast-like fungus (*Candida albicans* ATCC 18804). Pre-cultivation of actinobacteria strain was performed on Gauze 2 medium for 14 days at 28 °C [10]. Ehe experiment was carried out in three replicate. Statistical processing of the results was performed in the Microsoft Excel-2016.

Results and Discussion

Based on study fatty acid composition with dominant fatty acids 16:0 (10.24%) and 16:0 ISO (7.26%) and different indices similarities, strain Myt7ch was identified as genus *Streptomyces*.

Among molecular techniques used, 16S rRNA sequencing is a “gold standard” option due to the possessing the proper gene size, and availability of a large number of sequences in databases for comparison [11].

According to the results of 16S rRNA sequencing, the species name of the strain is *Streptomyces ambofaciens* ONU 561.

Phylogenetic analysis was carried out in the program MEGAX. Phylogeny reconstruction was reconstructed on based on the neighbor-joining (NJ) algorithm. The evolutionary distances were computed using the Tamura 3-parameter method and are in the units of the number of base substitutions per site. The rate variation among sites was modelled with a gamma distribution. The bootstrap consensus trees inferred from 1000 replicates were taken to represent the evolutionary history of the taxa analyzed.

On the basis of the obtained phylogenetic tree, it was shown that our strain was closest in evolutionary terms to the strain *Streptomyces ambofaciens* ATCC 23877 that is a soil bacterium industrially exploited for the production of the macrolide spiramycin which is used in human medicine as an antibacterial and antitoxoplasmosis chemical. This is the type strain isolated from the soil of France: province of Picardie in 1954. Strain identifiers in the database GenBank– CP012382. The similarities between type aried from 89.0 to 93.0% (Fig. 1).

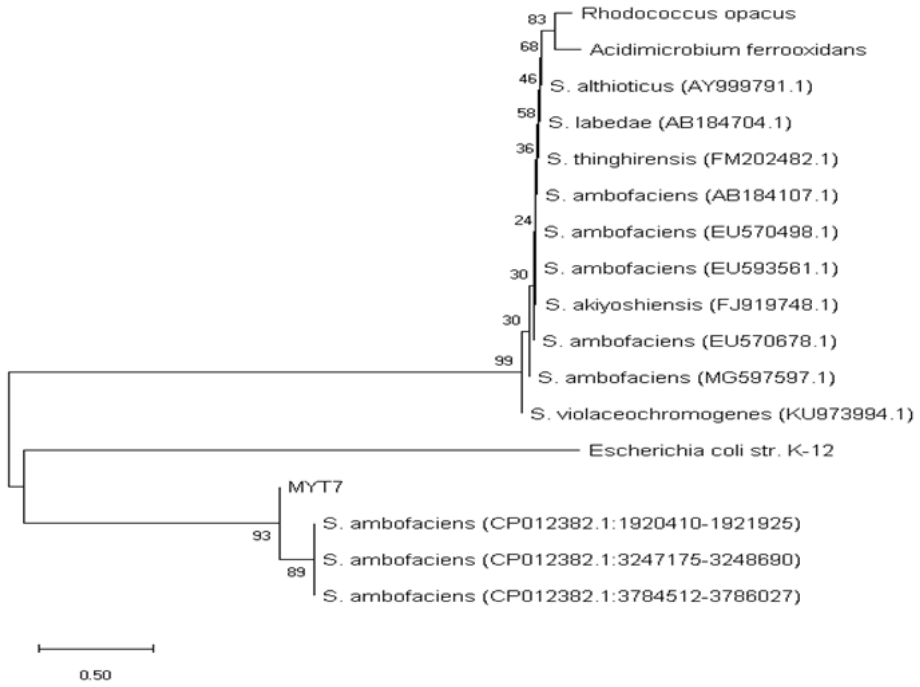


Fig. 1. Phylogenetic relationships among *Streptomyces* strains based on 16S rRNA gene sequences. The tree was constructed using the NJ method. Numbers at nodes represent levels (%) of bootstrap support from 1000 resampled datasets

It should be noted that there were other strains in the basic dataset prepared for phylogenetic analysis and selected after work in BLAST. Most of them were isolated in China and Japan (AB184107, EU570678, EU593561 and EU570498). However, as can be seen from the unrooted tree, they form a separate clade that has a lower evolutionary growth with our strain *Streptomyces ambofaciens* ATCC 23877 (CP012382).

On the unrooted tree, the second clade, which combines most of the strains in the phylogenetic tree, consists of three clusters. We can include strains KU973994.1, FJ919748.1 and AY999791.1 related to representatives of *Streptomyces violaceochromogenes*, *Streptomyces akiyoshiensis* and *Streptomyces althioticus* into first cluster. The second cluster holds strains AB184704.1, FM202482.1 and EU570678.1 related to representatives Streptomyces labedae, *Streptomyces thinghirensis* and *Streptomyces ambofaciens*. The third cluster contains only *Streptomyces ambofaciens* representatives (Fig. 2).

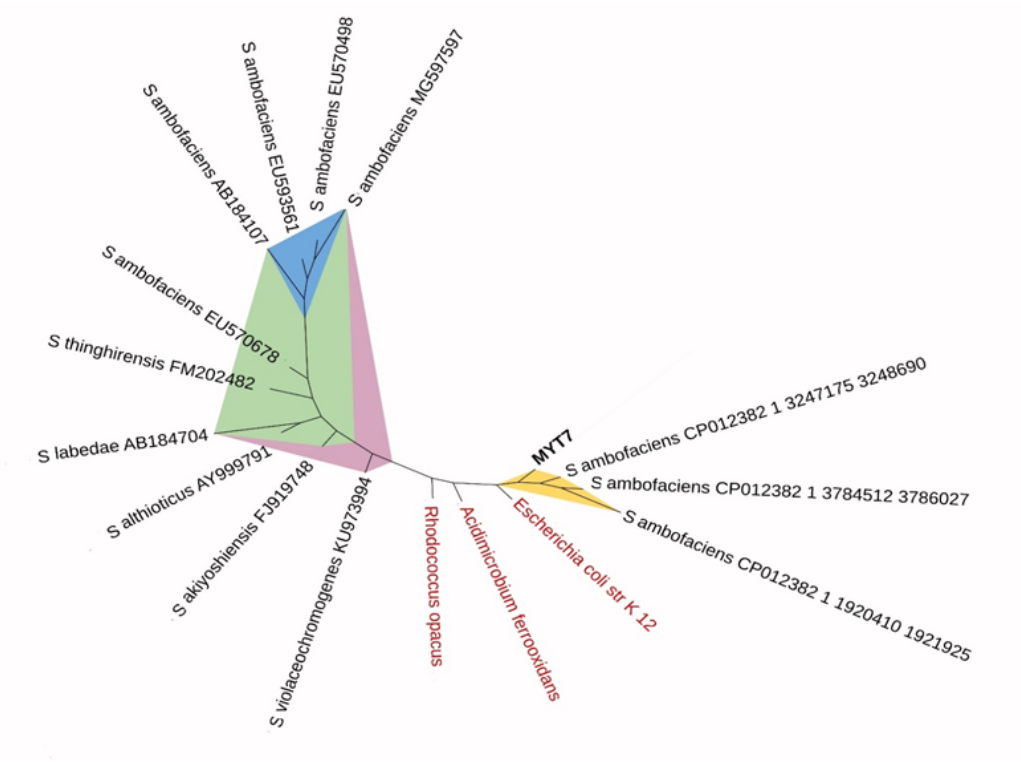


Fig. 2. Unrooted phylogenetic tree among *Streptomyces* strains based on 16S rRNA gene sequences. The tree was drawn using itool (https://itol.embl.de/); an interactive version is available at https://itol.embl.de/shared/izabelcavassim

The isolated strain had different morphological properties (Tab. 1), on media of different composition, which are probably associated with the morphological variability of actinobacteria species, which depends on many factors, including the composition of nutrient media.

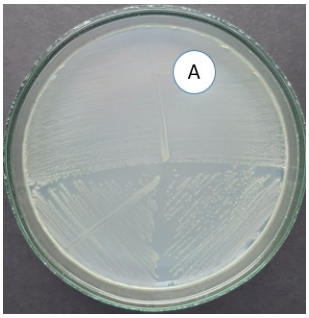
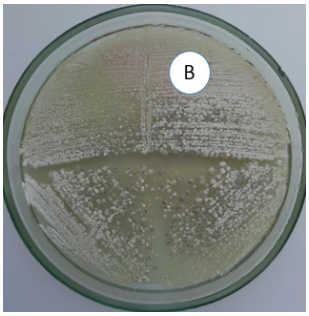
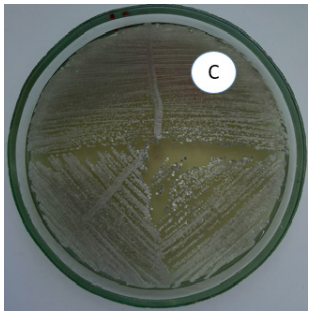
According to the literature date, many actinobacteria were characterized by pleomorphism of colonies during growth on different nutrient media. Actinobacteria grow on media of different composition, forming various colonies that differ in structure, edge and height of aerial mycelium, color of mature spores, the presence of exudate, pigments and the texture of the colonies [12].

Detection of melanoid pigments on two nutrient media ISP 6 and ISP 7 showed that *S. ambofaciens* ONU 561 could synthesize melonoid pigments on the

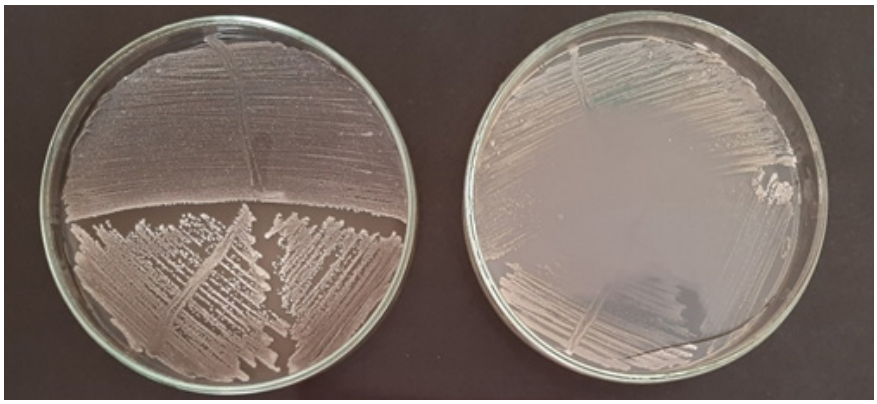
medium ISP-6 (Fig. 3). Melanoid pigments have radioprotective and antioxidant properties that can effectively protect living organisms from ultraviolet radiation and serve as an important taxonomic criterion. Melanins are often used in medicine and pharmacology [8].

Table 1

Morphological properties of *S. ambofaciens* ONU 561 on different nutrient media

Oat agar with sea salt	Gauze 1	Gauze 2
Rounded, colorless colonies, which formed a white mycelium	Rounded, yellow colonies, which formed a white-gray mycelium	Rounded, yellow colonies, which formed a dark gray mycelium
		

Note: *S. ambofaciens* ONU 561 growth on: A) Oat agar with sea salt, B) Gauze 1, C) Gauze 2



ISP 6 *S. ambofaciens* ОНУ 561 ISP 7

Fig. 3. Melanoid pigments of strain *S. ambofaciens* ONU 561 growth on ISP-6 and ISP-7

The strain was characterized by catalase activity, oxidase activity is not clearly expressed, the strain grows on a medium with gelatin, but does not dilute it, coagulates but does not peptonize milk, hydrolyzes starch, forms hydrogen sulfide, does not reduce nitrates.

The most pronounced antagonistic activity of the studied strain was shown against *Micrococcus luteus* ATCC 4698 – 8.75 ± 1, 06 mm, *K. pneumoniae* ATCC 131 – 6.0 ± 0, 7 mm, *K. rhizophila* DSM 348 – 5.25 ± 0, 35 mm and *P. putida* KT 2440 – 5.0 ± 0, 7 mm.

The less active strain was relatively to *E. coli* ATCC 25922 – 4.5± 0, 7 mm *S. aureus* ATCC 25923 – 3.25 ± 0, 35 mm, *B. subtilis* ATCC 6633 – 1.25 ± 0, 35 mm and *C. albicans* ATCC 18804 – 1.25 ± 0, 35 mm.

The studied strain did not show antagonistic activity against *P. vulgaris* ATCC 6896, *S. enterica* NCTC 6017, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853.

According to literature sources, actinobacteria exhibiting high antagonistic activity encode at least one type of polyketide synthase biosynthesis gene (PKS-I, PKS-II), which are responsible for the synthesis of new secondary metabolites and have the potential for the discovery of new drugs [13].

Conclusions

According to the literature data, the *Streptomyce*s genus can produce a wide spectrum of bioactive molecules. Therefore, isolation of actinobacteria from marine environments and study of their metabolite profiles is promising for the subsequent search for new antimicrobial compounds [14].

The search for new bioactive metabolites is of great importance worldwide for pharmaceutical, agricultural and industrial applications, due to the rapid emergence of pathogenic microorganism's resistant to many drugs [15].

The primary identification of actinobacteria strain isolated from the Black Sea mussels, based on the results of a comparative analysis of the spectrum of fatty acids, made it possible to assign it to the genus *Streptomyces* (*Streptomyces sp.* Myt7ch). Based on the analysis of the 16S rRNA sequencing, the isolated strain was identified as a representative of the species *Streptomyces ambofaciens* (*Streptomyces ambofaciens* ONU 561).

For the first time, morphology and enzymatic properties of the strain were studied. It was found that the strain exhibits high antagonistic activity against at least four strains of opportunistic pathogens *Micrococcus luteus* ATCC 4698, *K. pneumoniae* ATCC 131, *K. rhizophila* DSM 348 and *P. putida* KT 2440. Detection of melanoid pigments showed that *S. ambofaciens* ONU 561 could synthesize melonoides on the medium ISP-6.

The obtained results indicate a high potential for further study of the *Streptomyces ambofaciens* ONU 561 strain.

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MORPHOLOGICAL CHARACTERISTICS OF THE BLACK SEA STRAIN OF ACTINOBACTERIA LIM 10

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Abstract. *The morphological properties of the Black Sea strain of actinobacteria Lim 10 from the collection of cultures of microorganisms of Odessa National University I.I Mechnikov. It was found that the strain Lim 10 is characterized by pleomorphism. Based on the study of morphological, tinctorial and phenotypic properties, the belonging of the marine strain Lim 10 to the genus Streptomyces was confirmed.*

Key words: *Actinomycetes, Black Sea, morphological characteristics..*

Introduction

Actinobacteria are a common group of microorganisms in natural ecosystems. They are used as a source to search for beneficial secondary metabolites with antibiotic activity [Ghanem et al., 2000].

The marine environment proved to be a rich source of new actinomycetes - producers of biologically active substances. A very important feature of actinomycetes is their ability to produce antibiotics that can selectively inhibit the life of fungi, helminths, protozoa, rickettsiae. The ability of microorganisms to produce antibiotics is the result of a long evolution in the struggle for existence [Коротаєва та інш., 2021; Страшнова та інш., 2021].

Currently, the level of interest in marine actinobacteria has increased due to the fact that they live in a unique environment that promotes the synthesis of new biologically active metabolites [Ramesh et al., 2009; Ramesh et al., 2019].

The aim of our work was to study the biological properties of the Black Sea strain of actinobacteria Lim 10.

Materials and methods

The object of the study was a strain of marine bacteria Lim 10, which is stored in the collection of microorganisms of the Department of Microbiology, Virology and Biotechnology of Odessa I. I. Mechnikov National University.

Strain Lim 10 was isolated by Strashnova I.V. from the fouling of natural shell rock collected at a depth of 0.2–1.0 m in June 2020 in the Odessa Bay of the Black Sea near the Hydrobiological Station of the Odessa National University named after I.I. Mechnikov (Odesa, Ukraine, 46 ° 27′01′′N 30 ° 46′14′′E).

Nutrient media such as Gauss 2, Chapek, Ashby were used to isolate actinobacteria [Білявська, 2018]. All media were prepared in seawater and the antibiotic

nalidixic acid was added at a concentration of 10 mg / l to prevent the growth of concomitant microbiota. Sowing on the medium was performed in 3 repetitions. Incubation was performed at 28 ° C for 14-1 days.

The presence of actinobacteria was determined visually by colonies with typical mycelium (substrate and air) or colonies with a folded surface. Pigmentation of the nutrient medium and colonies was also taken into account. Colonies characteristic of actinobacteria were removed and subcultured on medium such as Gauze 2, oat agar (OA) and starch-casein agar (SCA), resulting in a pure culture. Incubation was performed at 28 ° C for 7-10 days. Pure isolates were stored at 4 ° C until further use.

Morphology and growth patterns were studied in Gause 2, Chapek, Ashby, TSB and ISP (ISP-1 - ISP-7) culture medium, according to the International Streptomyces Project (ISP) (Shirling and Gottlieb 1966), for 14–21 days at 28 ° C.

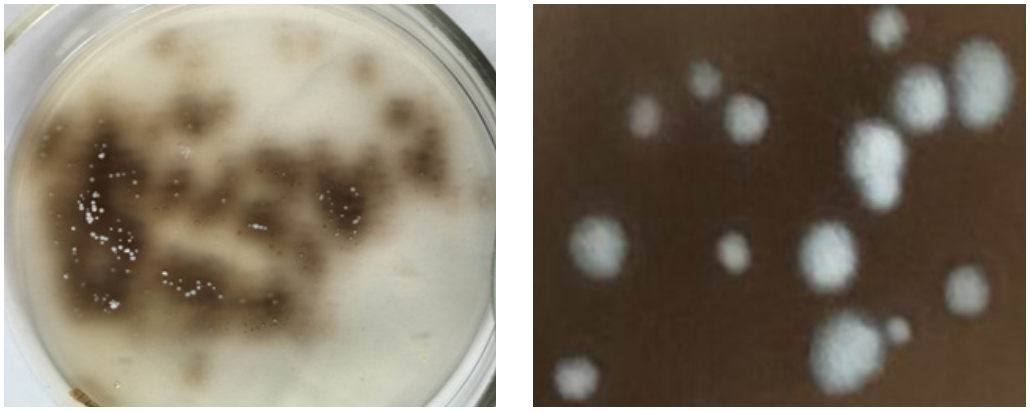
The synthesis of melanoid pigments was studied on ISP-6 media cultured for 14–21 days at 28 ° C.

Cell morphology was examined by light microscopy of fixed preparations stained with an aqueous solution of purple Pfeiffer.

Results

Isolated Lim 10 isolate from mollusk thickets in the Odessa Gulf of the Black Sea on dense media of Chapek, Ashby, starch-ammonia and soil agar was overgrown with thickets in the form of small single colonies (Fig. 1 A).

A study of the nature of Lim 10 culture growth and colony morphology showed that after 5 days of exposure, small colonies of bacteria 0.2–0.5 cm in diameter with a smooth edge grew on Chapek nutrient medium, forming white air mycelium over the entire colony surface. The aerial mycelium was well removed with a loop from the surface of the colony (Fig. 1 B).



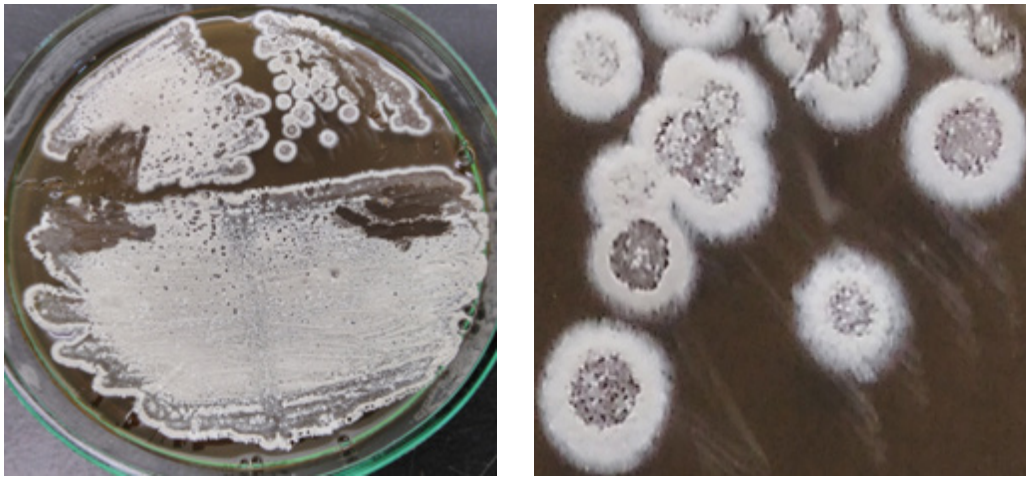
A **B**

Fig. 1. Nature of growth (A) and morphology of colonies (B) of Lim 10 strain on Chapek culture medium. Exposure 5 days

On organic nutrient medium Gauze 2 isolated strain Lim 10 gave abundant growth (Fig. 2 A).

After 7 days of cultivation, round, unpigmented colonies with a smooth edge 0.5–0.7 cm in diameter grew on Petri dishes with dense Gauze 2 culture medium, which formed a white air mycelium along the edge of the colonies. In the center of the colony there was a granular structure of the substrate mycelium, which grew into agar.

Colonies of Lim 10 strain, which formed the substrate mycelium, were poorly removed from the nutrient media because they grew strongly into the agar medium. The strength of mycelial growth depended on the time of cultivation (Fig. 2 B).



A **B**

Fig. 2. Abundant growth of the strain Lim 10 on Gauze medium 2. Exposure 7 days.

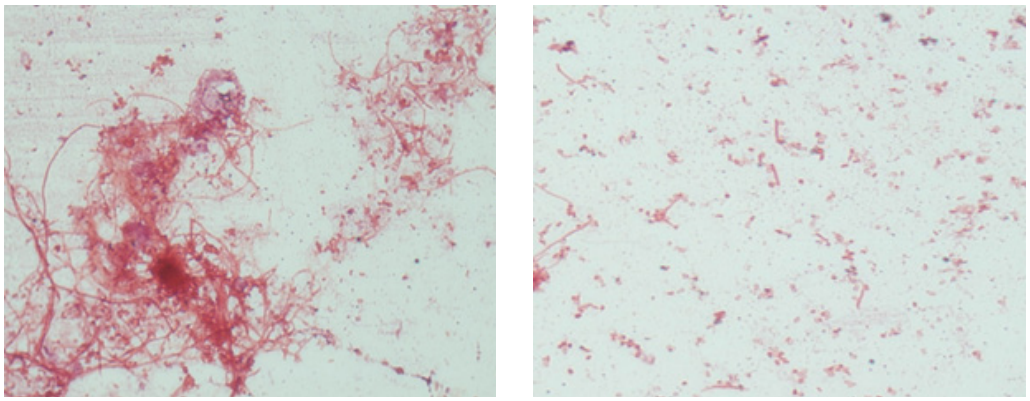
Detection of pigments of the Lim 10 strain showed that on Gauze 2 and Chapek culture medium it forms a dark brown water-soluble pigment that diffuses into a dense medium (Figs. 1–3).

Fig. 3. Accumulation of water-soluble dark brown melanoid pigment during cultivation of Lim 10 strain on liquid nutrient medium TSB. Exposition 10 days

On ISP-6 medium, which is specifically designed to detect melanoid pigments, the Lim 10 strain produced a dark-colored melanoid pigment. During the cultivation of the Lim 10 strain on a liquid culture medium TSB observed the most active production of water-soluble dark brown pigment (Fig. 3).

The ability of microorganisms to form melanoid pigments is often regarded as a protective response to anthropogenic pollution caused by various pollutants. According to the literature, melanoid pigments have radioprotective and antioxidant properties and are an important taxonomic criterion [Страшнова та інш., 2021].

The morphology of the cells of the Lim 10 strain was determined by light microscopy of fixed preparations stained with Pfeiffer's fuchsine (Fig. 4). Different forms of Lim 10 bacterial cells were observed in the preparations: from filamentous to cocci. Cells were mostly represented by short rods of small size, which were arranged singly, in pairs in chains, V-shaped, chaotic. Long filamentous cells were also detected (Fig. 4 A), and some cells had a fragmented cocoid shape (Fig. 4 B).



A **B**

Fig. 4. Photomicrograph of cells of bacteria Lim 10. A - filamentous cells; B - sticks and coca. Light field microscopy, Pfeiffer fuchsine staining (x1500)

This morphological variability of bacterial cells of the Lim 10 strain may be related to a number of factors, including the age of the culture and individual cells, the development of aerial mycelium, substrate mycelium formation, cultivation conditions, and culture medium composition.

Thus, the results of the study of the growth of Lim 10 strain on dense culture medium of different composition for 5 - 7 days showed that the colonies of the isolated strain had different morphological properties, which depended not only on the composition of the medium but also the age of the culture. In fig. 1 - 5 show differences in the morphology of colonies of strain Lim 10 of different ages on different culture medium.

It is known that many representatives of the microworld are characterized by pleomorphism, with the exception of actinobacteria, which in the case of growth on growth medium show a significant variety of structural characteristics of colonies [Коротаєва та інш., 2021].

Actinobacteria of different ages are characterized by pleomorphism of colonies during growth on growth medium. Thus, Yanti, Setyawati and Kurniatuhadi (2019) note that actinomycetes isolated from mangrove sediment grow on medium of different composition, forming different colonies that differ in structure, edge and height of aerial mycelium, color of mature spores, presence of exudate, pigments and colony texture [Yanti et al., 2019; Ghanem et al., 2000].

The isolated strain of Lim 10 in Gause 2, Chapek and Ashby was characterized by different colony morphology, which depended not only on the composition of the media and age of the cultures, but also on pleomorphism (including morphological variability) of actinobacteria species, including heterogeneity in the middle of one species.

Thus, based on mophological and phenotypic properties, the Black Sea strain Lim 10 can be assigned to the genus *Streptomyces*, which confirms the results of preliminary identification of the fatty acid composition of lipids of this strain by other authors [Коротаєва та інш., 2021].

Conclusions

1. Isolated from shellfish Black Sea strain of actinobacteria Lim 10 was characterized by high morphological variability, forming different morphotypes of colonies in the case of growth on growth medium of different composition.
2. The ability to synthesize water-soluble and melanoid pigments in the cultivation of Lim 10 strain on dense and liquid growth medium.
3. The morphology of cells of bacteria of the Lim 10 strain was diverse - along with short rods in the preparations there were filamentous and coccal cells.
4. Based on the study of biological properties of the Black Sea strain of actinobacteria Lim 10 can be attributed to the genus *Streptomyces*.

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Shvets Y. A.

PLANT GROWTH STIMULATION ABILITY OF *BACILLUS MEGATERIUM* ONU 500 ON AN EXAMPLE OF SUNFLOWER SEEDLINGS

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Abstract. *Biological products based on beneficial soil- and plant-associated bacteria can be used as alternative methods to stimulate plant growth, protect them from pathogens and increase chances of survival under stressful conditions. Our aimwasto evaluate the effect of Bacillus megaterium ONU 500 on germination and growth of sunflower seedlings.The treatment with B. megaterium ONU 500 had a positive impact on the development of sunflower seedlings. An increase in length of seedling roots by 21%, leaf area – by 22%, dry weight of seedlings – by 17,8% was observed.*

Key words: *B. megaterium, sunflower, growth characteristics of se edlings.*

Introduction

Bacillus megaterium de Bary – representatives of plant and soil microbiota. *B. megaterium* have antagonistic and phytostimulative properties, which make them useful for study and application in agriculture. Nowadays, protection of the environment against contamination with chemical fertilizers and pesticides becomes increasingly important.

The genus *Bacillus* is one of the largest and most widespread genera in the modern taxonomy of microorganisms, which is characterized by extraordinary phenotypic diversity.In 1884de Barry called the gram-positive bacterium *B. megaterium*as a "large beast" (from the Greek "mega" for large), because it was 100 times larger than the model organism −gram-negative bacteria *Escherichia coli*Migula [Bunk et al., 2010]. *B. megaterium*is ubiquitous and could be found in a variety of habitats, including fresh and salt water, soil, plant rhizosphere, leaves, roots, stems. It also coexists with animals and humans, survives on wooden surfaces and paper coatings, as food contaminants and even in honey [Final…, 2018; Vary, 1994].

B. megaterium are rod-shaped motile bacteria up to 4.0μm in size and up to 1.5 μm in diameter [Final…, 2018]. Bacteria of this species are gram-positive. They are aerobic, but also can grow in anaerobic conditions [Final…, 2018].When nutrients are limited, *B. megaterium*can form endosporesand thus survive in extreme conditions like high temperatures, ultraviolet radiation, disinfectants, drought [Coleman et al., 2010; Final…, 2018].

Strains of *B. megaterium* grow in a wide range of temperatures, with minimum temperatures from 3 °C to 10 °C [Final…, 2018].They are not zoopathogenic or phytopathogenic, but their endospores can beagents of contamination, unwanted decomposition and decay [Final…, 2018].In some situations, *B. megaterium* can be pathogenic for people with compromised immunity.

Plants exist in close connection with complex populations of microorganisms - members of the PGPBgroup (plant-growth-promoting bacteria), which inhabit soil or plant tissues [Santoyo et al., 2016] and play important phytostimulatory and protective functions [Dahmani et. al., 2020]. One of the most important mechanisms for promoting plant growth is the ability to synthesize phytohormones [Ortiz-Castro et al., 2008]. *B. megaterium* are one of the most widespread members of the PGPB group in the soil. Microorganisms of this species play an important role in colonization of rhizosphere and can be used as a sustainable alternative to chemical fertilizers responsible for the worldwidepollution [Santoyo et al., 2016]. Microorganisms *B. megaterium* are able to synthesize phytohormones that promote plant growth and increase yield of horticultural crops. They also cause visible improvement of morphological characteristics of plants and reduce seed germination time. *B. megaterium* strains have the ability to produce indole-3-acetic acid (IOC) [Olanrewaju et al., 2017], which is a key regulator of plant growth and development (cell division and elongation, lateral root formation, flowering),and abscisic acid (ABA) necessaryfor regulation of environmental stress and control of plant growth [Wang et al., 2020].

They also produce cytokinins (CCs), which play an important role in the regulation of cytokinesis [Ortíz-Castro et al., 2008]. Plant growth promotion induced by cytokinin-producing bacteria has been well documented for several species of rhizobacteria, including *B. megaterium* [Wang et al., 2020]. Studies have shown that the newly discovered strain of *B. megaterium* (UMCV1) promotes the growth of *Arabidopsis thaliana* L. and *Phaseolus vulgaris* L. *in vitro* and in soil. Inoculation of *B. megaterium* affected the root system of *A. thaliana* plants and demonstrated phytohormone-mediated effects, including inhibition of primary root growth with subsequent increase of growth of lateral rootand root hair [Ortíz Castro et al., 2008].

Nowadays, protection of the environment to prevent contamination with chemical fertilizers and pesticidesbecomes increasingly important. Modern crop production is based on the ideas of using biological preparations containing microbial phytohormones due to the simplicity and cheapness of their production [Biological…, 2022]. Use of different chemicals leads to depletion of soil fertility and reduced quality of agricultural products. Biological products based on beneficial soil- and plant-associated bacteria can be used as alternative methods to stimulate plant growth, protect them from pathogens and increase chances of survival under stressful conditions [O'Brien et al., 2017]. The growing concern about the impact of pesticides on the environment stimulates the development of new modern methods of control and treatment of plant diseases in orderto obtain a high-quality crop [O'Brien et al., 2017].

Strains of *B. megaterium* effectively produce a wide range of bioactive compounds involved in plant growth and protection. Their ability to form spores resistant to stress conditions is important for development of new products for agriculture and bioremediation especially for the regions with conditions stressful for plants (eg, high salinity) [Nascimento et al., 2019]. Summarizing, *B. megaterium*get into the rhizosphere, quickly colonize the root system and interact with the plant, causing growth stimulating effect and biocontrol of phytopathogens. Growth

stimulation is provided by the ability of bacteria of this genus to produce phytohormones.The direct effect of biological preparations based on *B. megaterium* bacteria is to support plant growth and to develop the root system, which is associated with the transport of more nutrients from the soil, especially nitrogen, phosphorus and iron [Plaza et al., 2021].

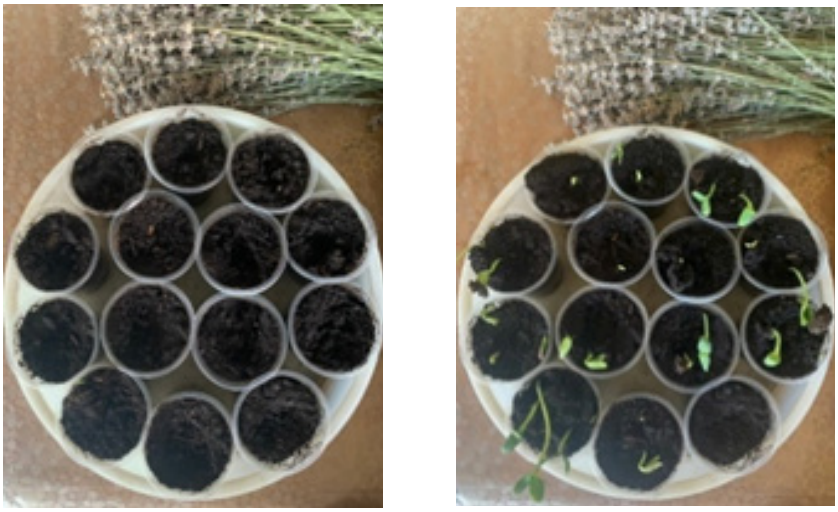
In one of the recent research, the effect of *B. megaterium* bacteria on themorphologyof seedling root was observed [Dahmani et al., 2020]. The results of the analysis showed that the strain *B. megaterium* RmBm31 has many genetic features associated with plant growth stimulation, including the ability to improve the accumulation of plant nutrients and to synthesize phytohormones. The main changes in root morphology caused by RmBm31 were the formation and development of the lateral root and the increase of the length of the root hair [Dahmani et al., 2020]. Consequently, plant-related microorganisms have the ability to modulate hormone levels and metabolism in plant tissues, especially in biochemical processes that can prevent the harmful effects of external stresses such as drought, salinity, nutrient deficiencies or heavy metal contamination [Hashem et al., 2016]. Production of growth-promoting substances is beneficial to microorganisms becauseit increases the release of root plant exudates, which provide nutrition to the microorganisms. They can stimulate plant development, provide resistance to various abiotic and biotic stress factors, improve nutrient uptake and protect plants from various diseases, which can be located in the soil [Egamberdieva, 2009]. Therefore, optimization of beneficial bacteria phytohormone balance in stressed tissues can be an important strategy in development of sustainable approaches to crop production [Egamberdieva, 2009]. Prominentphytostimulating and antagonistic properties can explain the active use of *B. megaterium* and their further study in order to develop and optimize effective biological products.

Due to large number of properties of bacilli useful for agriculture, the **aim** of the work was to study the effect of *B. megaterium* ONU 500 on germination and growth of sunflower seedlings.

Materials and Methods

The culture of *B. megaterium* ONU 500 was cultivated overnight at 28 °C in LB broth [Bertani, 2004]. The concentration of overnight culture was measured using a SmartSpec spectrophotometer (BioRad). The culture was diluted with distilled water to 10^6 CFU/ml according to Tverdokhlib et al. (2018) [Tverdokhlib et al., 2018].To study the phytostimulatory properties of *B. megaterium* ONU 500 in soil conditions, sunflower seeds [Seeds…, 2002] were sown in plastic containers with peat soil (fig. 1).

Three independent experiments of 200 seeds in each variant were conducted. The seeds were watered with a suspension of bacteria in concentration of 10^6 CFU/ml once after sowing – on the first day of the experiment. Control seeds were soaked in water. Measurements were performed on 22nd day of the experiment. Seed germination, average seedling height, seedling root length and seedling leaf area were calculated. Average dry weight of seedlings was measured. Average values and confidence intervals were calculated using the Excel application package.

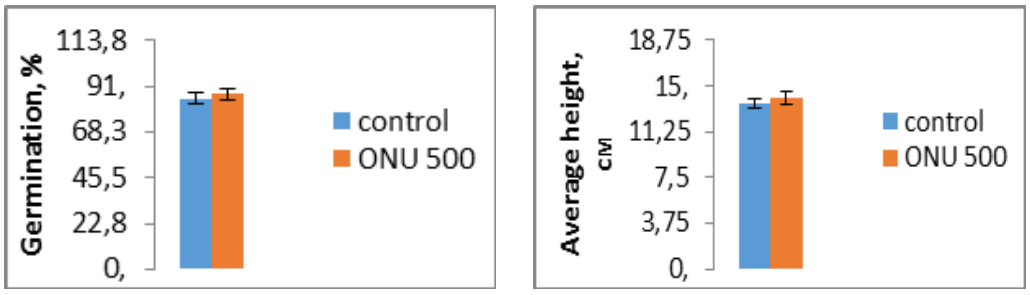


A B

Fig. 1. Sunflowers on the first (A) and third (B) day after sowing

Results and Discussion

Germination of plants from the seeds inoculated with *B. megaterium* ONU 500 did not significantly differ from thecontrol (fig. 2, a). Similarly, inoculation of seeds with bacilli of this strain did not significantly affect the height of sunflower seedlings (fig. 2, b).



a b

Fig. 2. Effect of *B. megaterium* ONU 500 on (a) germination of sunflower seeds; (b) average height of sunflower seedlings (cm)

At the same time, a significant positive effect of inoculation with *B. megaterium* ONU 500 on the root length was observed. The average rootlength increased by 21,08% (fig. 3).

The results of Dahmani et al. (2020) confirmed that strains of *B. megaterium* demonstrated positiveeffect on growth and development of plant roots. These microorganisms also contributed to formation of lateral roots and increased the

lengthof root hairs necessary for collection and transfer of water and minerals from soil to roots [Dahmani et al., 2020].Treatment with a suspension of bacilli increased leaf area (by 21,93%) (fig. 4, a). Phytostimulation effect of inoculation with bacilli was also observed in case of dry weight of seedlings: it increased by 17,8% as compared with the control (fig. 4, b).

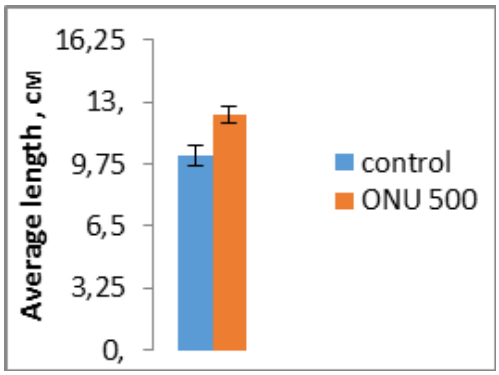
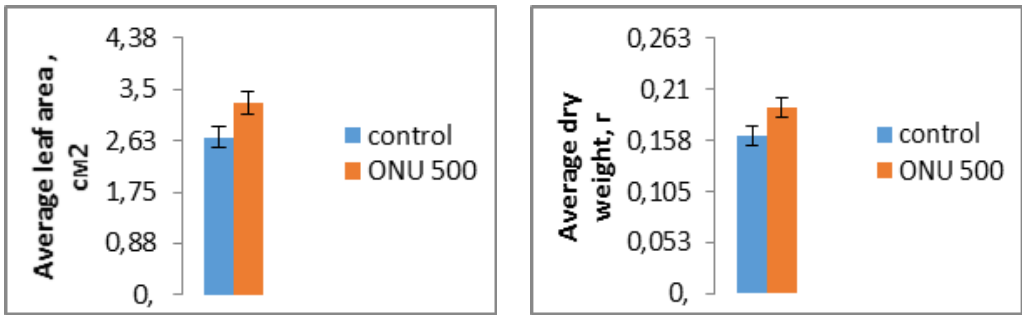


Fig. 3. Average length of sunflower roots (cm)



a **b**

Fig. 4. Effect of *B. megaterium* ONU 500 on (a) average leaf area of sunflower seedlings (cm^2); (b) average dry weight of seedlings (g)

The results of our study coincidewith the literature data. Previous studies of Tverdokhlib et al. (2018), Teslyuk et al. (2019) and Dahmani et al. (2020) indicate thesignificant phytostimulatory effect of *B. megaterium* strains on plant growth, development of roots and vital functions. Promotion of plant growth and visible improvement of morphological characteristics could be explained by the ability of *B. megaterium* tosynthesize phytohormones, especially – indole-3-acetic acid, which is a key regulator of plant growth and development,cytokinins whichplay an important role in cytokinesis regulation, and abscisic acid necessary for environmental stress regulation [Porcel et al., 2014; Tozlu et al., 2018; Wang et al., 2020].

Summarizing all above, it could be emphasized that *B. megaterium* ONU 500 can be used for stimulation of plant growth.

Conclusions

The treatment with *B. megaterium* ONU 500 had a positive impact on the development of sunflower seedlings. An increase in length of seedling roots by 21%, leaf area – by 22%, dry weight of seedlings – by 17,8% was observed.

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BIOTECHNOLOGY OF PRODUCTION OF A SOUR MILK DRINK FERMENTED BY *LACTOBACILLUS PLANTARUM* ONU 355

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Abstract. *A biotechnology of the production of a fermented milk drink based on a strainL. plantarum ONU 355 has been developed.The number of viable lactobacilli cells in the leaven reached (8.0 ± 1.1) x 10^{12} CFU/ml, and in the fermented milk drink - (2.2 ± 0.5) x 10^{12} CFU/ml. No molds were found in the sour milk drink, and the amount of yeast was within the norms (30.0 ± 3.0 CFU/ml). The active and titratable acidity of the leaven was 5.0 and 100 °T, accordingly, and of the ready-made sour milk drink - 5.5 and 110 °T, which corresponds to the current State Standard of Ukraine.*

Key words: *biotechnology, Lactobacillus plantarum ONU 355, fermented milk products*.

The use of probiotics in food is becoming increasingly popular, as these living microorganisms in adequate quantities can benefit health [2]. Benefits of probiotics include improved animal growth, lower serum cholesterol, increased nutrient utilization, andreducing the use of antibiotics etc. [4]. Probiotics used in food usually contain lactobacilli and bifidobacteria [6].

Lactobacillus plantarum is a lactic acid bacterium inhabiting a wide range of ecological niches, including many fermented plant products, as well as some fermented dairy and meat products.

Interestingly, microorganisms considered as probioticsare now traditionally used as starter cultures in fermented foods. Moreover, available probiotics include extremely diverse categories of products, such as pharmaceuticals, various types of food, including juices, food bars, baby food mixes, condiments, sweeteners, water,as well as chewing gum, toothpaste, dietary supplements and cosmetics [2].

Food and Agriculture Organization of the United Nations (FAO) andThe World Health Organization (WHO) defines probiotics as living microorganisms used as food or feed additives that, when consumed in sufficient quantities, cause a beneficial effect on the bacterial microbiota of the host [3].

The health benefits have increased interest in developing new food models with probiotic microorganisms. Modern probiotics reduce the symptoms of lactose intolerance, prevent many types of intestinal diseases and enhance the balance of intestinal microorganisms. Numerous studies have proven the potential ability of probiotics to suppress cognitive deficits and enhance brain activity across the gut-brain-brain axis. In addition, a number of studies have shown that *L. rhamnosus* and *L. plantarum* play a vital role in cognitive impairment: depression, anxiety or stress, and abnormal behavior in autism spectrum disorders. These effects are essentially

attributed to the direction of inflammatory signals along the microbiome-gut-brain axis and the host's metabolism. Despite the exact mechanisms, which are not clear enough, these findings have recognized the high potential of probiotic therapy for complicated behavioral responses [2].

The aim of the work was to develop a biotechnology for the production of sour milk drink based on bacteria of the strain *L. plantarum* ONU 355.

Materials and Methods

The experiments were performed in the Biotechnological Research Center of Odesa I. I. Mechnikov National University, as well as at the Department of Microbiology, Virology and Biotechnology in 2020.

A bacterial strain of *L. plantarum* ONU 355 from the collection of the Department of Microbiology, Virology and Biotechnology of Odesa I. I. Mechnikov National University originally isolated from grapes harvested in France was used as a starter culture.

The strain was stored at -20 °C in 20% glycerol solution. The strain was revived by seeding on MRS medium (deMan, Rogosa, Sharpe) [4]. Petri dishes with MRS agar medium were prepared for culture growth. To prepare the medium, 67.15 g of the powder of the finished MRS medium was stirred in 1000 ml of distilled water, boiled until complete dissolution of the solids, poured into vials and sterilized in an autoclave at 1.1 atm (121 ° C) for 15 minutes.

To obtain bacterial suspension, vials with liquid MRS were inoculated with the colonies of lactobacilli grown on MRS agar. Culture was grown overnight at 37 °C till suspension density of approximately 10^9 CFU/ml.The culture was evaluated for homogenecity, the smear stained with Pfeiffer's fuchsinewas prepared, and observed with immersion microscopy at 1350x.

Bacteria of the strain *L. plantarum* ONU 355 were used directly for the preparation of leaven. First, a wash from a Petri dish of MRS agar medium with anovernight culture of lactobacilli was added to 10 ml of milk. The concentration of lactobacilli *L. plantarum* ONU 355 in the wash was 10^9 CFU/ml, which was detected by spectrophotometry. These 20 ml of wash were after added to 50 ml of boiled cow's milk and left at 37 °C for three days in a thermostat.

The second stage of preparation of the sour milk drink included mixing 50 ml of the obtained leaven and 200 ml of milk, after which the mixture was left at 37 °C overnight in a yogurt maker, the containers of which were pre-sterilized with UV and alcohol. The number of bacteria in the leaven and beverages was detected as follows:

1. To 100 μl of product 900 μl of sterile water was added;
2. Ten-fold dilutions of the product were prepared in sterile ependorfs;
3. 5 μl from each dilution wereplatedper sector of the MRS dish;
4. Incubation overnightat 37 °C was performed;
5. The number of grown CFU was multiplied by the dilution and multiplied by 200.

Active acidity was determined using indicator paper. To evaluate the titratable acidity (in Turner degrees), 10 g of fermented milk beverage was titrated with 0.1M aqueous NaOH solution, and the volume of solution used to neutralize 10 g

of fermented milk beverage was calculated. To do this, we added 20 ml of distilled water to 10 ml of product, then 3 drops of alcohol solution of phenolphthalein (10 g/l)was added to the suspension.The suspension was then titrated until pink colour, which did not disappear for 1 minute. The acidity in Turner degrees was calculated by multiplying by 10 the volume of 0.1 M NaOH solution, which was used to neutralize 10 ml of fermented milk drink according to State Standard of Ukraine 3624-92. The study was conducted in three replications.

Microbiological control of the obtained fermented milk products for the presence of molds and yeasts was carried out by plating on Saburo medium (g/l: dextrose - 40; peptone - 10; agar-agar - 20; autoclaved at 0.5 atm (111 ° C) for 15 minutes).

Ten-fold dilutions starting with 10^{-2} were plated on Saburo medium and cultivated overnight.

Results and Discussion

On the first stage of the study, bacteria of the strain *L. plantarum* ONU 355 were re-cultivated from the frozen state from the museum, where it was stored at 80° C in 20% glycerol for three months. Our goal was to investigate some of the main characteristics of the strain: whether they have changed during storage. Thus, we compared the characteristics of freshly grown culture with the characteristics of the strain known from the strain passport from the Collection of Microorganisms of the Department of Microbiology, Virology and Biotechnology of Odessa I. I. Mechnikov National University. We found that in the MRS broth bacteria of the strain *L. plantarum* ONU 355 grew in uniform turbidity, and homogeneous white sediment at the bottom of the tube rising during shaking was formed (Fig. 1, a).

On a solid MRS bacteria formed convex, opaque, white colonies (Fig. 1, b).

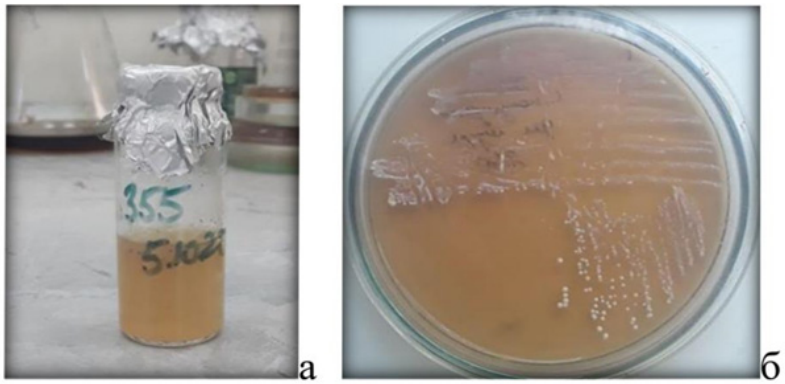

Fig. 1. Growth of *L. plantarum* ONU 355 in liquid (a) and on agarized MRS (b)

Staining of cells with fuchsine red and microscopy with an immersion lens showed the presence of small short rods 0.9-1.2 μm wide and 1.0-8.0 μm long with rounded ends that existed separately, in pairs or in chains (Fig.2).

Thus, the study of the main characteristics of the culture showed that the cultural and morphological properties of *L. plantarum* ONU 355did not change during storage. In the future, this culture was used to prepare a sour milk drink.

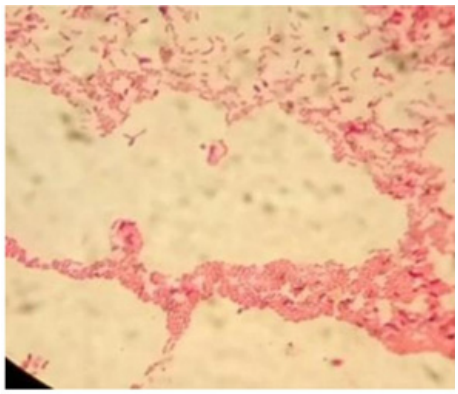

Fig. 2. Microphotography of *L. plantarum* ONU 355 cells stained with fuchsine (1350x)

As the next step, a leaven was made from the boiled domestic cow's milk and the culture of *L. plantarum* strain ONU 355. The resulting leaven was a product of white colour with the consistency of a uniform clot (Fig. 3).

Fig. 3. The leaven after one day of incubation

Table 1

Characteristics of the leaven and milk drink based on *L. plantarum* ONU 355

Product	Colour	Consistency	Aroma	Taste	CFU/ml
Leaven	white	uniformclot	sourmilk, pleasant	sour, pleasant	(8.0±1.1) x 10^{12}
Drink	white	uniformclot	sourmilk, pleasant	sour, pleasant	(2.2 ± 0.5) x 10^{12}

Sour dairy drink made from milk and leavenfermented with*L. plantarum* ONU 355 had a white color and thick consistency: immediately after fermentation there were some small convolutions, but after shaking the consistency was homogeneous.

Active and titratable acidity of both leaven and sour milk drink responded to the State Standards of Ukraine - DSTU 4417:2005 and DSTU 4343:2004.

The taste and aroma were evaluated by five volunteers. The taste was sour, but pleasant with a clear taste of milk.

L. plantarum is traditionally used in production of such sour milk drinks like yogurt and kefir. According to the current State Standards of Ukraine DSTU 4417:2005 and DSTU 4343:2004 the minimum amount of lactic acid bacteria in these ready-to-drink beverages should be 1.0×10^7 CFU/ml, so the strain *L. plantarum* ONU 355, which actively ferments milk and reaches high cell concentrations in the ready product (2.2×10^{12} CFU/ml) can be used in yogurt production.

Also, the combination of positive qualities of the drink, such as - taste, aroma, the presence of viable bacteria - makes it possible to recommend bacteria of this strain for the production of fermented milk products, in particular - the beverages. An additional positive characteristic of the strain *L. plantarum* ONU 355 is its origin: it was isolated from grapes. This makes the strain attractive for the production of organic products. The grapes from which the strain was isolated were cultivated in France, which may indicate that the strain has beneficial characteristics of lactobacilli from this particular geographical region. Therefore, *L. plantarum* ONU 355 can be recommended for leaven production both as a single starter and in mixtures with indigenous strains of lactobacilli isolated in Ukraine. In mixtures, the beneficial properties of strains from different geographical regions can complement each other. But these assumptions require further investigations. In addition, according to previous research performed by scientists of the Department of Microbiology, Virology and Biotechnology of Odessa I. I. Mechnikov National University, bacteria of the strain *L. plantarum* ONU 355 have phytostimulating activity and positively influence plant growth [20]. Therefore, wastes from the production based on this strain can be successfully used for watering and fertilizing plants.

Fermented milk products should not contain undesirable, food spoilage microorganisms, such as molds. Also, the number of yeast cells must be within certain limits (not more than 50 CFU per 1 cm^3). Thus, DSTU 4417:2005 and DSTU 4343:2004 define the maximum permitted concentrations of these microorganisms. Unwanted microorganisms can penetrate the product at different stages of its production - from milk with insufficient heat treatment, from dirty dishes, with polluted air. Lactobacilli are known for their antagonistic properties [25], they can actively inhibit undesirable microbiota, but some microorganisms are resistant to antagonistic compounds of lactic acid bacteria. Therefore, we investigated the presence of some unwanted microorganisms - for example, we inoculated the ready fermented milk drink on Saburo medium to detect molds and yeasts. The results are listed below (Table 2).

Table 2

Analysis of fermented milk drink based on *L. plantarum* ONU 355 on microbial contamination

Microorganism	Presence, CFU/ml
Mold	-
Fungi	30.0 ±3.0

Molds were not detected. Yeasts were isolated, but their number was within the norms of DSTU 33566-2015.Therefore, according to microbiological indicators, the obtained sour milk drink was safe to use.

Steps of milk drink production

1. Biomass oflactobacilli was washed from a Petri dish with MRS medium with 10 ml of boiled cow's milk. The concentration of *L. plantarum* ONU 355 in the wash was 10^9 CFU/ml.

2. Next, these 10 ml of lactobacilli milk was added to 50 ml of boiled cow's milk to make leaven (Fig. 4).

3. Leaven was left at 37 ° C for three days for fermentation.

4. The leaven was added to 200 ml of milk and left at 37 °C overnight (Fig. 5)

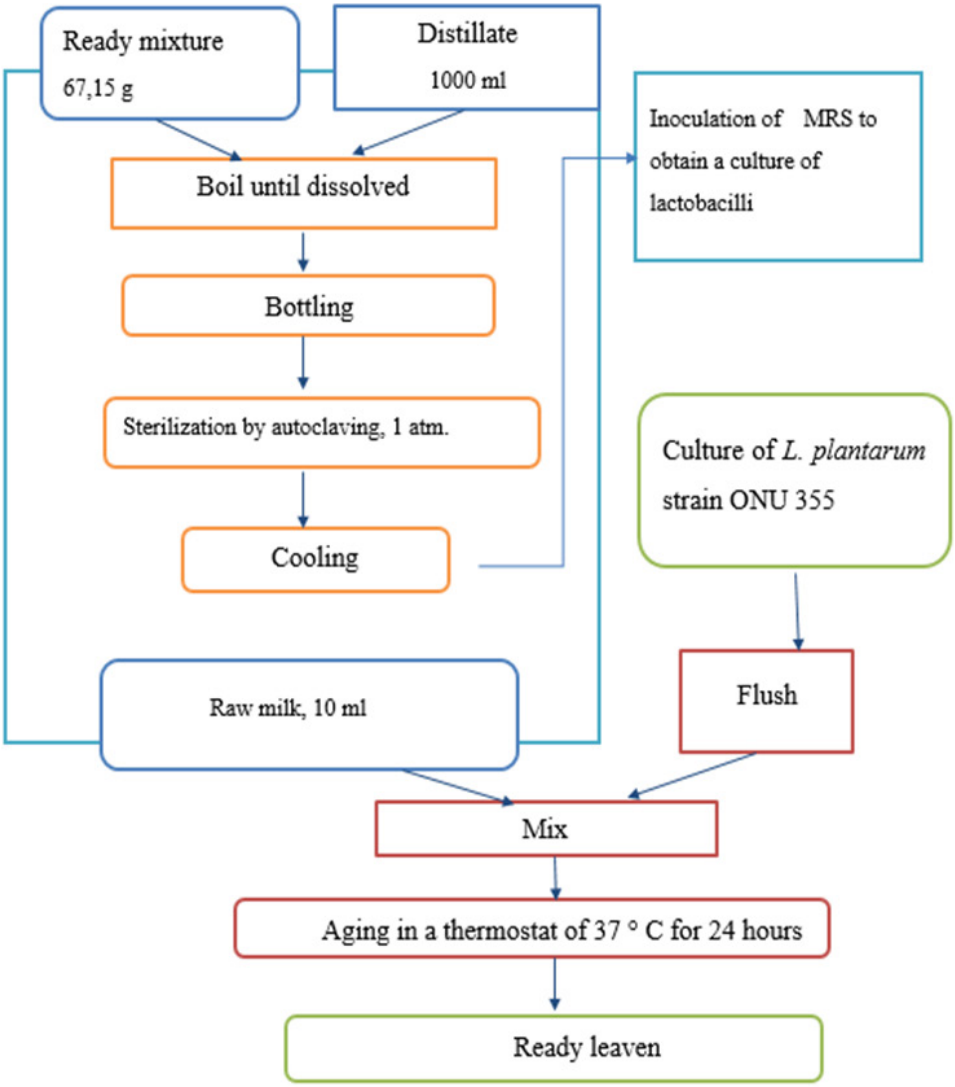

Fig.4. Technological scheme of leaven preparation

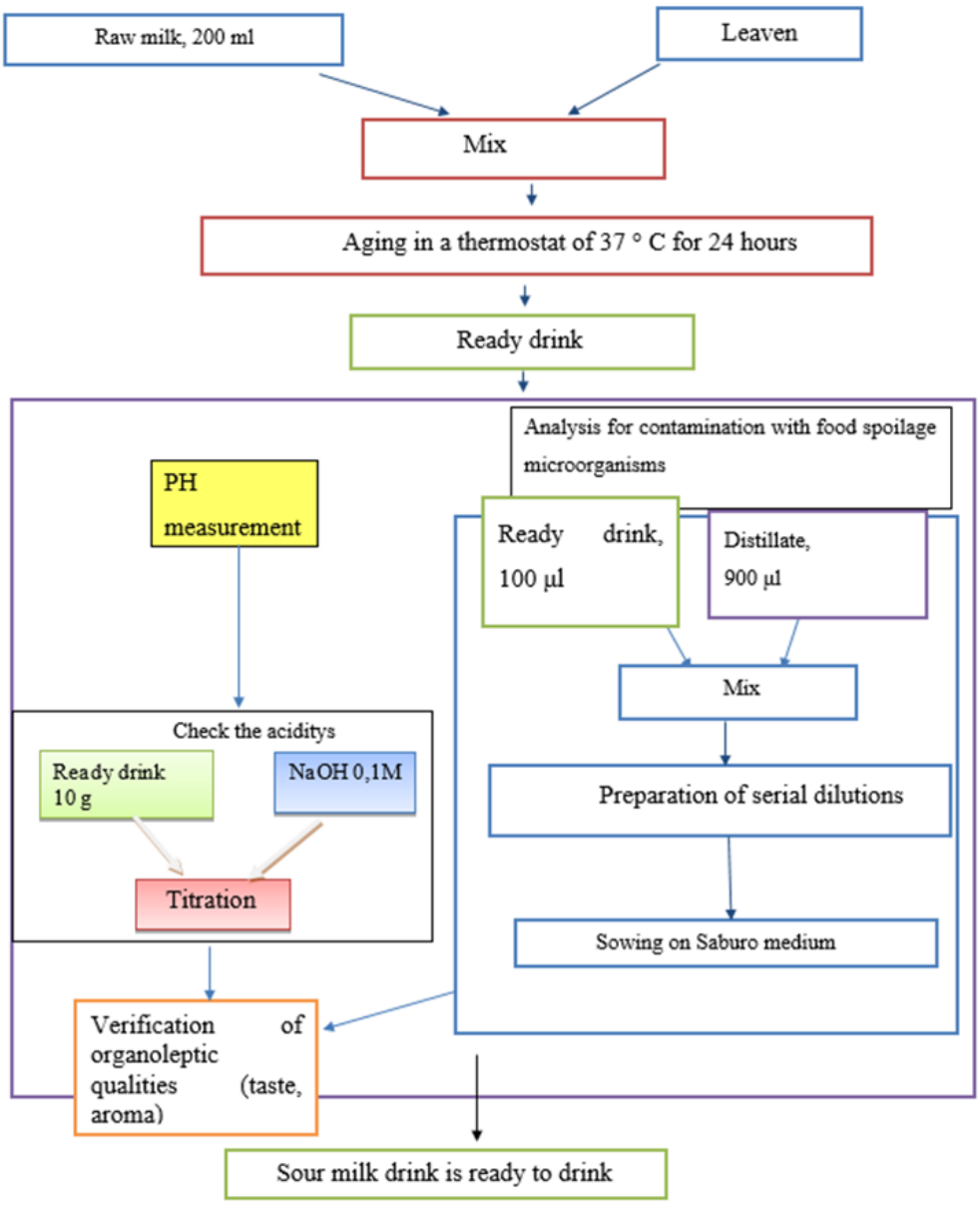

Fig. 5. Technological scheme of preparation of sour-milk drink

Thus, using the culture of the strain *L. plantarum* ONU 355 andthe proposed technological schemes, it is possible to produce a sour milk drink with probiotic properties based on the characteristics of bacteria - components of the drink.

Conclusions

1. Cultural and morphological properties of bacteria *L. plantarum* ONU355 after storage at -80ºC in 20% glycerol did not change andcorresponded to the primary characteristics of this strain.

2. Taste and aroma (pleasant, fermented, milky), consistency (thick rolls), active (pH 5.0 - 5.5) and titratable (100.0 - 110.0 °T) acidity, the leaven and fermented milk drink responded to the current State Standards of Ukraine.

3. The number of viable lactobacilli cells in the leaven was 8.0 x 10^{12} CFU/ml, and in the fermented milk drink - 2.2 x 10^{12} CFU/ml.

4. No molds were detected in the sour milk drink, and the amount of yeast corresponded to the norms (30.0 ± 3.0 CFU/ml).

5. The technological scheme of production of sour-milk drink based on bacteria *L. plantarum* ONU 355 was developed.

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UDC 578

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BACTERIOPHAGES OF THE GENUS *BACILLUS* ISOLATED FROM THE BLACK SEA

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Abstract. *The aim of the work was to determine the presence of bacteriophages in marine strains of Bacillus using lysogenic induction. Strains of Bacillus velezensis (ONU 553), B. pumilus (ONU 554), B. subtilus (ONU 559) were isolated from deep-sea bottom sediments of the Black Sea and researched.To search for prophages, ultraviolet radiation was used as an inductor. The activity of the obtained phagolysates was studied by titration.Biological activity was detected in each studied strain.Ultraviolet radiation can lead to the release of bacteriophages from the state of prophage in marine strains of Bacillus.*

Key words: *bacteriophages, Bacillus, aquatic environment, induction.*

Introduction

Soil microorganisms have long been considered the most promising source of biologically active substances [1]. However, in recent years, their potential has declined markedly. Bacteria isolated from aquatic environments are becoming a new source of biologically active substances [2]. Representatives of marine origin are an effective source for the isolation of natural compounds with new structural characteristics. The bottom sediments of the Black Sea are unique and little studied, which suggests the presence of numerous unique bacterial communities and the substances they produce. *Bacillus* bacteria of marine origin are an effective source for the isolation of natural compounds with new structural characteristics and unique biological properties [3].The bacteria of the *Bacillus* group have a powerful metabolic system and produce a wide range of extracellular enzymes and secondary metabolites [3]. To understand their biology, characteristics, and role in marine microbial communities, detailed attention should be paid to the viruses that can infect them.

Viruses can be the largest reservoir of new genes and play an important role in the evolution of microorganisms and the regulation of population dynamics [4]. It is known that representatives of aquatic *Bacillus* can infect only members of the order *Caudovirales* [5]. It should be noted that among the members of the family *Podoviridae* and *Syphoviridae* predominate temperate, and in the case of *Myoviridae* - lytic bacteriophages. The genomes of temperate phages often carry determinants of the synthesis of toxins or antimicrobial compounds [6]. To date, little remains known about the peculiarities of phages of the genus *Bacillus*, isolated from the aquatic environment. This is of considerable interest given the important role of these bacteria in aquatic biocenoses and the prospects for their application in biotechnology.

Materials and methods

Strains of *B. velezensis* (ONU 553), *B. pumilus* (ONU 554), *B. subtilis* (ONU 559) isolated from deep-bottom Black Sea sediments were used. Lysogenic induction was performed using several methods, using UV as an inducer. All procedures and methods were performed in a semi-dark place to prevent bacterial photoreactivation.In the first method, a daily culture of strains werepassaged on Petri dishes with meat peptone agar (MPA) and dried. After that, for 7 min, ultraviolet light was applied at a distance of 1 m from the UV lamp. The cultures were incubated at 37 °C for 24 hours. The culture grown during the day was distributed on the surface of the nutrient medium, and then exposed to UV at a distance of 1 m from the lamp for 10 minutes. The cultures were incubated overnight in a thermostat at 37 °C. Distribute the culture on the surface of the agar, irradiated it with UV at a distance of 0.5 m for 10 minutes. The cultures were incubated overnight under the same conditions. Growing bacterial colonies were washed from Petri dishes using MPB medium and placed in vials with appropriate non-induced strains. Cultivated overnight at 37 °C. Chloroform was then added to the vials in a volume ratio of 1:10, incubated for 15 min at 37 °C, and then centrifuged at 3,000 rpm for 15 min. The supernatant was examined for biological activity [7].

In the second method, the bacterial culture were passaged for 18 h on Petri dishes with MPA, and then dried in a thermostat at 37 °C for 15 minutes. After that, UV was affected at a distance of 50 cm from the lamp. Exposure time was 5-23 minutes (with an interval of 2 minutes).Washes were performed with sterile saline, centrifuged for 15 min at 3,000 rpm [8]. The supernatant was collected and examined.

The studied cultures, which were in the exponential phase of growth, were diluted in a ratio of 1:100 in phosphate buffer with a pH of 7.6.2 ml of the suspension was transferred to Petri dishes and irradiated for 20 (25, 30, 35, 40) seconds at a distance of 40 (45, 50, 55, 60) cm from the UV lamp. Irradiated cultures were passaged in MPB at room temperature in a ratio of 1:100.Incubate for 5 h at 37 °C and passaged 100 µl on Petri dishes with MPA. The results were taken into account by counting the plaques on the medium [8].

UV induction was performed using 5 ml of bacterial suspension. Open Petri dishes with suspension were placed under a UV lamp at a distance of 1 m and irradiated for 7 minutes. The suspension was then centrifuged at 2000 g for 30 minutes. The supernatant was collected and analysed. To check the biological activity of the obtained lysates, a bacterial suspension of the studied strains was prepared and 1 ml of culture was mixed with 1 ml of phagolysate. Then 0.1 ml of the mixture was passaged on Petri dishes with MPA. Incubate for 48 h at 37 °C. The results were taken into account by counting the plaques on the medium [9].

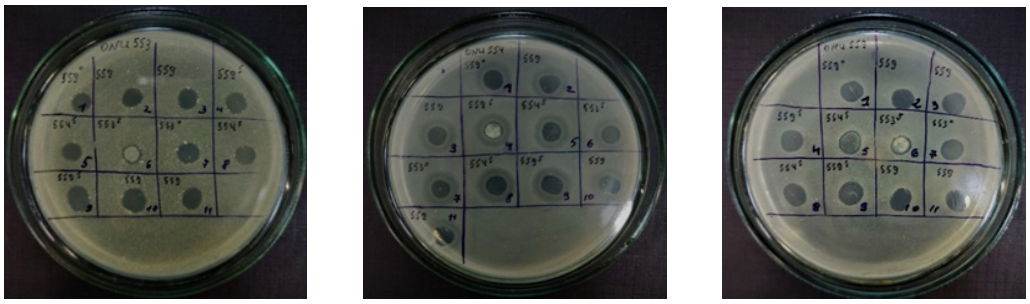
The obtained phagolysates were investigated using the agar layers method. To 0.7% agar was added 25 µl of 3-hour bacterial culture and poured into Petri dishes with MPA. Allow time to harden and drip 10 µl of the obtained phagolysates on a sensitive lawn. Incubate in a thermostat overnight at 37 °C [9].

Studies of the nature of biological activity were performed by titration of the obtained phagolizates in minimal medium A (K_2HPO_4 – 10,5 g, K_2HPO_4 – 4,5 g, $(NH_4)_2SO_4$ – 1,0 g, $Na_3C_6H_5O_7$ – 0,5 g, 1M $MgSO_4 * 7H_2O$ – 1 ml, glucose – 0.2 %)

[10]. The dependence of the manifestation of the biological activity of phagolysates on the incubation time of the strains used for the sensitive lawn was also determined. Infected activity was investigated at 30 min, 2 h, 3 h cultivation.

Results and discussion

As a result, data were obtained indicating the presence of biological activity in all studied strains using the first two methods (Fig. 1). The use of other methods did not lead to the detection of activity.



a **b** **c**

Fig. 1. Plaques on the lawn of sensitive culture: a) ONU 553, b) ONU 554, c) ONU 559

Large transparent plaques were obtained on the lawn of sensitive crops.Most phages formed transparent plaques, which are typical of lytic phages, while turbid plaques may indicate the ability of phages to lysogenize host cells [6].For strain ONU 554, a gala was observed for each phagolysate tested. The average plaque size was 0.79 cm for strain ONU 553, 0.77 cm for strain ONU 554 and 0.88 cm for ONU 559 (Table 1). Large areas may indicate a small size of bacteriophages, which facilitates their movement in the upper agar layer [9].

It should be noted that the ability and effectiveness of infection of bacterial cells with phages might depend on the growth phase of the host.

Establishing the dependence of the infection activity of the obtained phagolysates on the time of incubation of strains used as a sensitive lawn, allowed to establish that the most active infection occurs after 2 h of incubation of sensitive strains (Table 2).

The average size of plaques for strains ONU 553, ONU 554, ONU 559, with incubation for 30 min was 0.86 cm, with incubation for 2 h - 1.1 cm, for 3 h - 0.95 cm.

It is known that members of the genus *Bacillus* are characterized by the presence of defective bacteriophages in the genome.As a result of induction of such bacteriophage, tails or capsids can be formed. Tails have killer activity and can form plaques on the lawn of sensitive cultures. Titration was performed to establish the nature of the plaques obtained. (Table 3).

Determination of the titer of the obtained phagolysates noted that the maximum dilution of phagolysates, which led to the production of plaques, was 10^{-3}. The low titer can be explained by the production of defective phages due to induction.

Table 1

The diameter of plaques on the lawns of the studied strains

№	Sensitive strains Phagolysate	ONU 553	ONU 554	ONU 559
1	ONU 559[1]	0,6 cm	0,7 cm	0,8 cm
2	ONU 559[1]	0,6 cm	0,7 cm	0,8 cm
3	ONU 559[1]	0,8 cm	0,8 cm	0,9 cm
4	ONU 559[2]	0,8 cm	-	0,8 cm
5	ONU 554[2]	0,7 cm	0,7 cm	0,7 cm
6	ONU 553[2]	-	-	-
7	ONU 553[1]	0,9 cm	0,7 cm	0,9 cm
8	ONU 554[2]	0,7 cm	0,8 cm	0,9 cm
9	ONU 559[2]	1 cm	0,8 cm	1 cm
10	ONU 559[1]	1 cm	0,9 cm	1 cm
11	ONU 559[1]	0,8 cm	0,8 cm	1 cm

Note: 1 – phagolysates obtained by the first method, 2 – phagolysates obtained by the second method.

Table 2

Investigation of the dependence of the manifestation of biological activity on the time of incubation of sensitive strains

№	Sensitive strains Phagolysate	ONU 553			ONU 554			ONU 559		
		30 min	2 h	3 h	30 min	2 h	3 h	30 min	2h	3 h
1	ONU 559[1]	0,7	0,9	1,0	0,6	0,9	0,9	0,8	1,0	1,0
2	ONU 559[1]	0,8	1,0	0,9	0,6	0,9	0,9	0,8	1,1	1,0
3	ONU 559[1]	0,9	1,1	1,0	0,9	1,0	1,0	0,9	1,1	1,0
4	ONU 559[2]	0,9	1,0	0,9	-	-	-	0,8	1,2	1,1
5	ONU 554[2]	1,1	0,8	0,8	0,6	0,8	1,1	1,2	1,2	1,0
6	ONU 553[2]	-	-	-	-	-	-	-	-	-
7	ONU 553[1]	0,8	1,1	0,9	1,0	1,0	1,0	1,0	1,2	1,1
8	ONU 554[2]	0,7	1,0	0,9	0,6	0,8	1,0	1,0	1,2	1,0
9	ONU 559[2]	1,1	1,5	1,1	1,2	1,3	0,6	0,9	1,5	1,0
10	ONU 559[1]	1,0	1,3	1,0	1,1	1,3	0,8	0,8	1,4	0,9
11	ONU 559[1]	0,8	1,0	0,9	0,8	1,1	0,9	0,7	1,1	1,0

Note: measurements are given in centimeters.

Table 3

Titration of phagolysates obtained on sensitive cultures after cultivation for 2 hours

№	Sensitive strains Phagolysate	ONU 553			ONU 554			ONU 559		
		10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
1	ONU 559[1]	-	-	-	-	-	-	-	-	-
2	ONU 559[1]	+	-	-	+	-	-	+	+	-
3	ONU 559[1]	+								
4	ONU 559[2]	+			-	-	-			
5	ONU 554[2]	+	+	-						
6	ONU 553[2]	-	-	-	-	-	-	-	-	-
7	ONU 553[1]	-	-	-	-	-	-	-	-	-
8	ONU 554[2]	+	+	-						
9	ONU 559[2]	+	+	-						
10	ONU 559[1]	+	-	+	+	-	-	+	-	-
11	ONU 559[1]	+	+	-	+	+	-	+	+	-

Conclusions

Lysogenic induction of bacteriophages of the genus *Bacillus* isolated from deep-bottom Black Sea sediments was performed. Ultraviolet radiation was used as an inductor. Fagolysates were obtained, which showed large transparent plaques on sensitive lawns. Such plaques are more characteristic of lytic bacteriophages. The optimal incubation time of the cultures to obtain a high level of bacteriophage infection was set at two hours.Determination of the titer of the obtained phagolysates was marked by the highest titer of 10^{-3}. This result can be explained by the presence of a large number of defective prophages in the genus *Bacillus*. The data obtained indicate the presence of bacteriophages in deep-sea bacteria.

Bacteriophages play an important role in the evolution and adaptation of host cells. Further detection and study of bacteriophages of aquatic microorganisms is promising. This will allow us to understand their role in functioning and features. The knowledge gained as a result of further research will be able to help determine the prospects for their use in biotechnology [1].

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MODULATING EFFECTS OF FERULIC ACID AND ALPHA-KETOGLUTARATE ON CAFETERIA DIET-INDUCED OXIDATIVE STRESS IN THE BLOOD PLASMA AND LIVER OF MICE

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Abstract. *Chronic overeating of high-calorie cafeteria food leads to the development of metabolic syndrome – a complex of metabolic abnormalities that has an adverse effect on quality of life. In the present study, we studied the ability of two natural compounds – ferulic acid (FA) and alpha-ketoglutarate (AKG) to modulate the oxidative stress and inflammatory processes in the liver and blood plasma of one-year-old female C57BL/6 mice fed by a cafeteria diet (CD). The cafeteria diet did not affect the levels of lipid peroxides and activities of NADPH-quinone dehydrogenase, glutathione-S-transferase, and catalase but increased the activity of glutathione peroxidase in the liver. In the blood of CD-fed mice paraoxonase activity did not differ from control one, whereas myeloperoxidase activity was lower compared with the control. It suggests that CD might induce low intensity oxidative stress. Consumption of ferulic acid led to higher the activity of glutathione peroxidase and glucose-6-phosphate dehydrogenase but lowered the activity of catalase. Alpha-ketoglutarate decreased the activity of catalase but increased the activity of glucose-6-phosphate dehydrogenase. Thus, we showed the possible reduction of oxidative stress and up- and downregulation of the activity of antioxidant enzymes in mice fed by the cafeteria diet supplemented with FA or AKG.*

Key words: *cafeteria diet, obesity, antioxidant defence.*

Introduction

Overweight and obesity are tightly related to a number of metabolic complications collectively called "metabolic syndrome". Metabolic syndrome is accompanied by pro-inflammatory processes, which are closely related to free radical processes in the body [1].

The advantage of using mice as a model in the study of metabolic disorders, such as obesity, is their relatively short life, and, therefore, changes caused by the experimental diet will be reflected after a few weeks, while in humans, the effect may be difficult to detect even during several years [2]. In addition, mice eating a high-calorie diet are known to have some typical features of metabolic syndrome, such as insulin insensitivity, increased glucose level and blood pressure, as well as increased abdominal fat amount and increased intensity of oxidative stress [3].

In the present study, we investigated the ability of two natural compounds, ferulic acid (FA) and alpha-ketoglutarate (AKG), to modulate the oxidative and

inflammatory processes in the liver and blood plasma of mice fed by a cafeteria diet (CD). We chose FA and AKG due their antioxidant and anti-inflammatory abilities. Ferulic acid is able to neutralize free radicals due to the phenolic groups in its structure [4]. Alpha-ketoglutarate is able to simulate the effect of calorie restriction and has anti-inflammatory and antioxidant effects [5].

Materials and methods

Female C57BL/6 mice were maintained at 22 ± 2 ° C, humidity 50-60% with a 12-hour light/dark cycle. Mice were fed a balanced feed for laboratory animals ("Vita", Ukraine) of the following composition: fat content – 4.8%, protein – 21.8%, fiber – 3.9%. All experimental protocols were approved by the Animal Experimental Committee of Vasyl Stefanyk Precarpathian National University.

For the experiment, one-year-old mice were randomly separated in two groups. During eighth weeks the first group continued to consume a standard diet, whereas the second group consumed a cafeteria diet. The cafeteria diet included products of the human diet: corn sticks, chocolate, hard cheese, ham, sausages, sweet and salty peanuts, cookies, chips, and crackers [6]. At the eighth week of the experiment, mice were further divided into six groups of 3-5 mice per cage as follows:

1) Control group (C) continued to consume a standard diet throughout the experiment.

2) Group "Control + ferulic acid" (C + FA) consumed a standard diet with the addition of ferulic acid (40 mg / kg body weight / day).

3) Group "Control + alpha-ketoglutarate" (C + AKG) consumed a standard diet with the addition of disodium salt of AKG to drinking water at a concentration of 1%.

4) Group "Cafeteria diet" (CD) consumed a cafeteria diet throughout the experiment.

5) Group "Cafeteria diet + ferulic acid" (CD + FA) consumed a cafeteria diet with the addition of ferulic acid.

6) Group "Cafeteria diet + alpha-ketoglutarate" (CD + AKG) consumed a cafeteria diet with the addition of AKG at a concentration of 1%.

Mice were fed with the respective diets for four next weeks. At the end of the experiment, mice were euthanized using by light carbon dioxide anesthesia. To collect a blood sample, the right retro-orbital sinus was punctured with heparinized glass capillary. Blood was centrifugated (1500 g, 15 min, 4 °C) to obtain plasma. Liver was removed, washed with ice-cold 0.9% NaCl, and then quickly frozen in liquid nitrogen.

For measurement of lipid peroxides (LOOH), lipids were extracted by homogenization of frozen liver in cold 96% ethanol (1:10 w/v). LOOH content was examined with xylenol orange by ferrous-xylenol orange method using cumene hydroperoxide as a positive standard [7]. To determine the activities of antioxidant enzymes, frozen livers were homogenized 1:10 (w:v) in medium containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM EDTA and 1 mM of protease inhibitor phenylmethylsulfonyl fluoride. Activities of catalase, glutathione peroxidase (GPx), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase

(G6PDH) were measured as described previously [8]. The activity of NAD(P)H quinone dehydrogenase (NQO1) was determined by recording the recovery of dicumarol-sensitive 2,6-dichlorophenolindophenol [9]. Paraoxonase activity was determined by registering the decrease 4-nitrophenyl acetate, which degrades under the action of paraoxonase [10]. Plasma myeloperoxidase activity was measured by the oxidation 3,3',5,5'-tetramethylbenzidine in the presence of hydrogen peroxide to brown oxybenzidine.

Statistical analysis was performed using Graphpad Prism 7 (GraphPad Software Inc). Differences between groups were analyzed using one-way analysis of variance followed by "Original FDR method of Benjamini and Hochberg".

Results and discussion

All experimental diets did not affect the content of lipid peroxides (Fig. 1).

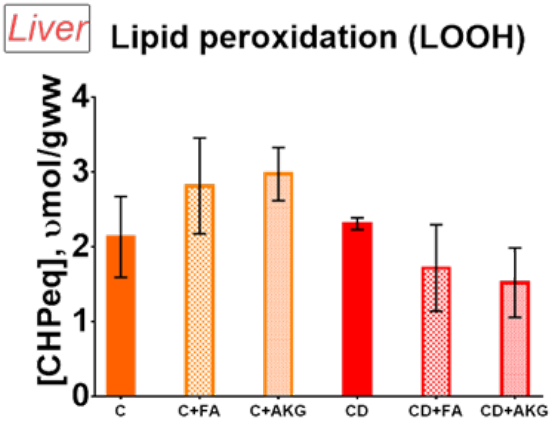

Figure 1. The content of lipid peroxides in the liver of female C57Bl/6 mice fed a basic food (C) or a cafeteria diet (CD) with the addition of alpha-ketoglutarate (AKG) or ferulic acid (FA) to drinking water. Data are presented as mean ± SEM from 3-5 mice in each group

The cafeteria diet did not affect the catalase activity in the mouse liver compared with the control one (Fig. 2A). Mice fed by CD with FA had lower catalase activity than the control group. It should be noted that addition of FA to the standard diet also led to lower catalase activity as compared with the control group. The addition of AKG to the CD reduced catalase activity by 40% compared with the control group. All experimental diets did not affect the activity of glutathione-S-transferase (GST) (Fig. 2B), although it is well-known that the increase in the activity of this enzyme may be linked to the oxidative stress development [11].

Glutathione peroxidase is an enzyme that is important in the regulation of free radical processes because the enzyme is able to reduce lipid peroxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [12]. Both, control and CD, diets with the addition of FA and CD alone increased the activity of GPx compared with the control group (Fig. 2C). A 2.5-fold increase in the activity was observed in diets with FA, whereas CD alone and with FA increased

GPx activity by approximately twice compared to the control group. Control diet with FA reduced catalase activity and increased GPx activity. CD alone and with FA increased the activity of G6PDH by 35%. The CD with AKG increased G6PDH activity by 65% (Fig. 2D).

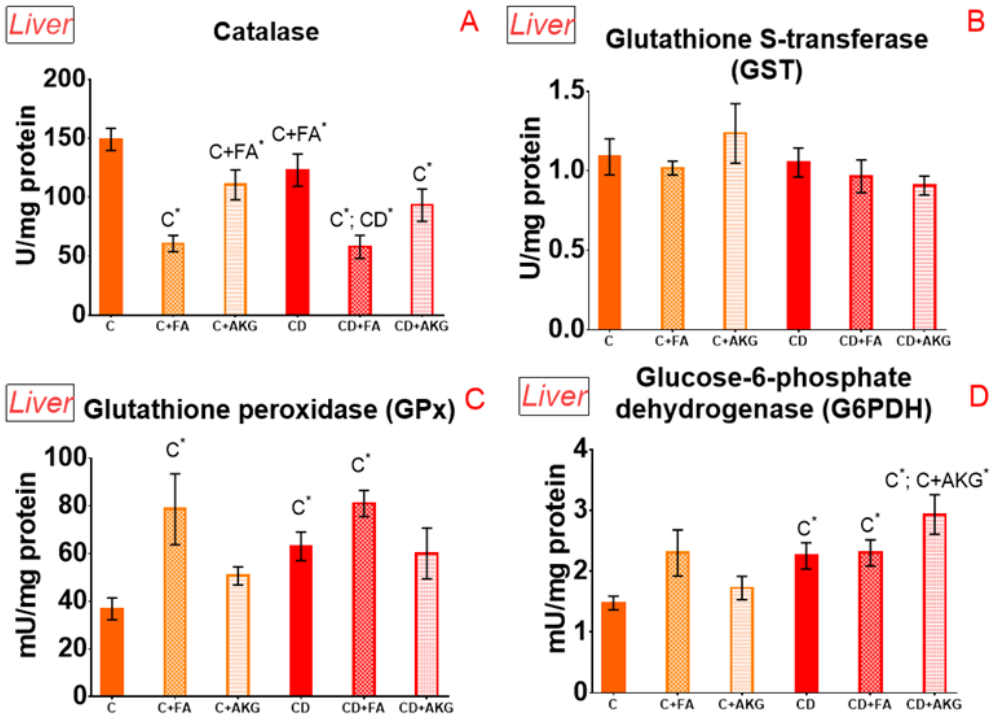

Figure 2. The activity of main antioxidant enzymes in the liver of female C57Bl/6 mice fed by a basic food (C) or a cafeteria diet (CD) with the addition of alpha-ketoglutarate (AKG) or ferulic acid (FA) to drinking water. *Significantly different (P < 0.05) between groups. Data are presented as mean ± SEM from 3-5 mice in each group

All experimental diets did not affect the activity of NAD(P)H quinone dehydrogenase 1 (NQO1) (Fig. 3A), which is an enzyme involved in the neutralization of toxic compounds in liver [13] and the activity of serum paraoxonase (Fig. 3B), which is considered a factor in preventing atherosclerosis by preventing the oxidation of low-density lipids [14].

The standard diet with FA, CD alone and with AKG reduced the activity of the myeloperoxidase enzyme by 2.5 times compared with the control group (Fig.4). Mice fed by CD with FA had 2-fold higher myeloperoxidase activity than CD-fed ones.

Conclusions

Cafeteria diet induces mild oxidative stress reflected by higher activities of several antioxidant enzymes (GPx and G6PDH) in the liver of middle-aged mice. Alpha-ketoglutarate added to CD showed mild modulating effects, such as increase

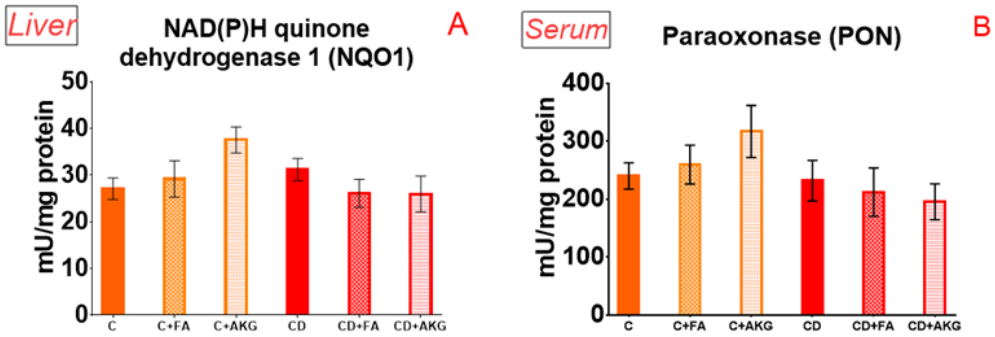

Figure 3. The activity of NQO1 and PON in the liver and serum of female C57Bl/6 mice fed by a basic food (C) or a cafeteria diet (CD) with the addition of alpha-ketoglutarate (AKG) or ferulic acid (FA) to drinking water. Data are presented as mean ± SEM from 3-5 mice in each group

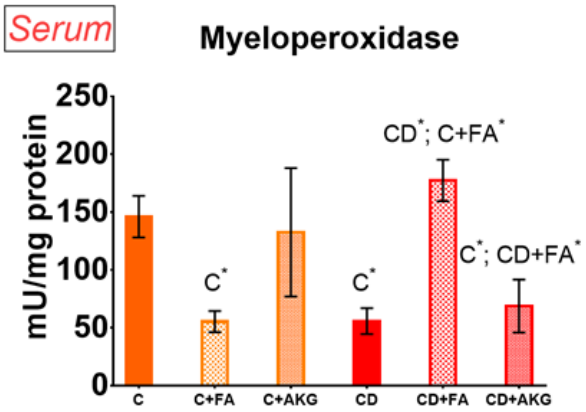

Figure 4. The activity of myeloperoxidase in serum of female C57Bl/6 mice fed by a basic food (C) or a cafeteria diet (CD) with the addition of alpha-ketoglutarate (AKG) or ferulic acid (FA) to drinking water. *Significantly different (P < 0.05) between groups. Data are presented as mean ± SEM from 3-5 mice in each group

in liver G6PDH activity and decrease in myeloperoxidase activity as comparted with the control group. At the same time, ferulic acid had more pronounced effects on CD-induced changes in mice that included decrease in catalase activity and increase in serum myeloperoxidase activity.

Acknowledgements

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Yavorska A.S., Kurasova V.O., Vasylieva N.Yu., Zinchenko O. Yu.

ISOLATION OF HALOPHYLIC ACTINOBACTERIA FROM THE BLACK SEA AND KUYALNIK ESTUARY

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Abstract. ***Aim:*** *to evaluate the efficacy of different media and sample pretreatment approaches for isolation of actinobacteria from saline and hypersaline environments.* ***Materials and Methods.*** *Bottom sediments from the Black Sea and Kuyalnik estuary were used as a source of actinobacteria. Sediment samples were pretreated by heating under different conditions (50 °C, 15 min; 50 °C, 50 min; 70 °C 15 min), freezing (−20 °C, 24 h) and UV (distance 20 cm, λ=254 nm, 15 W, 50 sec), diluted by 10^{-1}, plated on M1 and SCA media and incubated at 30 °C within 2-6 weeks.* ***Results.*** *The highest rate of actinobacteria from the Black Sea (5.1%) was isolated on M1 medium after pretreatment of samples by freezing. The highest efficacy of actinobacteria isolation from bottom sediments of Kuyalnik estuary was observed on M1 medium after freezing pretreatment (2.8% of actinobacteria colonies among the total number of isolated variants). The number of actinobacteria was higher in sediment samples from the Black Sea compared to Kuyalnik estuary.* ***Conclusions.*** *The optimal conditions for isolation of actinobacteria from the Black Seaand Kuyalnik estuary was pretreatment of samples by freezing with subsequent plating on M1 medium containing seawater or estuary water respectively.*

Key words: *Black Sea, Kuyalnik estuary, actinobacteria, saline and hypersaline environments.*

Development of new antimicrobials remains one of the most actual research directions due to increasing resistance of microorganisms to existing antibiotics and chemotherapeutical drugs. Actinobacteria are known as one of the most valuable sources of antibiotics – over 90% of antimicrobial preparations, used in medical practice, are produced by actinobacteria [Demain and Sanchez, 2009]. At the same time, antimicrobial potential of soil as the traditional source of actinobacteria is considered well studied despite of some last discoveries of new classes of antimicrobial metabolites of soil actinomycetes. On the other hand, biosynthetic activity of actinobacteria from "non-traditional" environments, such as seawater and salt lakes, is far from its deep exploration [Bohringer at al., 2017; Fiedler at al., 2008; Jensen et al., 2005; Lam, 2006, Tivari and Gupta, 2012]. For now, different approaches to increase the isolation rate from saline and hypersaline sources have been developed [Lee et al., 2014; Lee et al., 2015, Subramani and Sipkema, 2019; Takizawa et al., 1993], but there is the need for adjustment of these approaches in each individual case.

In Ukraine, the Black Sea and hypersaline estuaries can be considered perspective sources of actinobacteria with unknown metabolic activity. Thus, the objective of this study was to evaluate the efficacy of different media and sample

treatment approaches for isolation of actinobacteria from saline (Black Sea) and hypersaline (Kuyalnik estuary) environments.

Materials and Methods

The study was conducted at the Department of Microbiology, Virology and Biotechnology of Odessa I.I. Mechnikov National University (ONU) in 2021-2122. Sediment samples from the Black Sea were collected at the depth of 6 m near Hydrobiology station of ONU. Sediment samples from Kuyalnik estuary were collected at the depth of 20-30 cm near the "Kuyalnik" sanatorium. Samples were collected using sterile 0.5 l containers. Containers were filled by 60% with the sediments, and 40% of total volume marine or estuary water were added to conserve samples. Samples were processed the same day.

Isolation of actinobacteria was performed using two nutrient media – M1 and SCA [Lee et al., 2014]. M1 was prepared using seawater or Kuyalnik estuary water respectively. SCA medium was prepared using tap water.

Samples were previously processed as described in table 1.

Table 1

Methods of pretreatment of sediment samples

№	Pretreatment regime	Reference
1	50 °C, 50 min	[Takizawa et al., 1993; Subramani and Sipkema, 2019]
2	50 °C, 15 min	[Lee et al., 2014; Lee et al., 2015; Subramani and Sipkema, 2019]
3	70 °C, 15 min	[Naikpatil et al., 2011; Subramani and Sipkema, 2019]
4	UV– distance 20 cm, ƛ=254 nm, 15 W, 50 sec	[Takizawa et al., 1993; Subramani and Sipkema, 2019]
5	−20 °C, 24 h	[Jensen et al., 2005; Subramani and Sipkema, 2019]

Isolation of actinobacteria was performed by serial dilution method with subsequent plating on agar media. Considering the data on the low number of actinobacteria among marine microorganisms 10^{-1}dilution was used for isolation.

After pretreatment 10 g of sediments were diluted in 90 ml of 9% NaCl in flasks and shaken at 120 rpm during 30 min. 0.1 ml of obtained suspension were pouredon M1 and CSA media in Petri dishes, spread by Drigalski spatula and incubated at 30 °C for 2-6 weeks. Viable count of all colonies and actinobacteria colonies was performed after incubation and the share of actinobacteria was calculated.

Results and discussion

The data on actinobacteria share among the total number of the coloniesisolated from the Black Sea is shown on fig. 1. The highest rate of actinobacteria (5.1%) was isolated on M1 medium after pretreatment of samples by freezing. In

general, actinobacteria share among the total number of colonies was higher on this medium than on SCA.

The highest efficacy of actinobacteria isolation from bottom sediments of Kuyalnik estuary was observed after pretreatment by freezing with the following plating on M1 medium. The share of actinomycetes under these conditions was 2.8%. At the same time, in contrast to the previous experiment, in this case correlation between the number of isolated actinobacteria and composition of nutrient medium was not observed. Thus, actinobacteria growth on M1 after UV-pretreatment was not detected but their share on SCA was 1.3% (fig. 2). And, after sample pretreatment at 50 °C during 50 min the growth was not observed on both media.

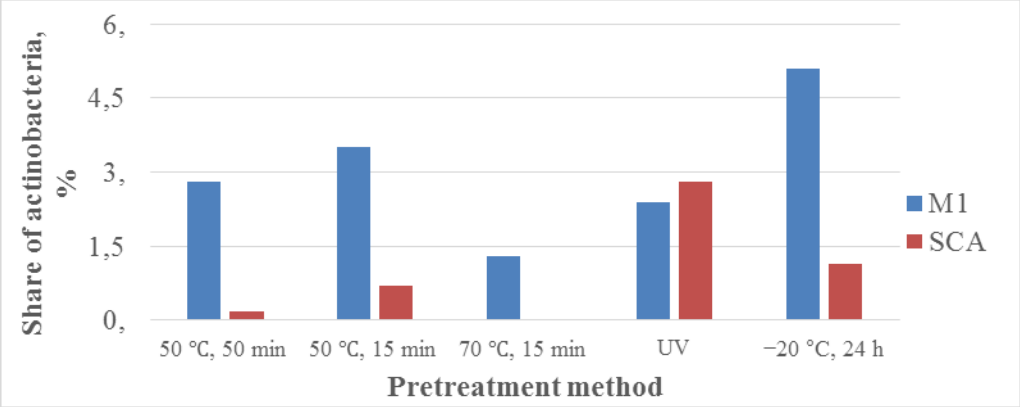

Fig. 1. The share of actinobacteria in sediment samples from the Black Sea

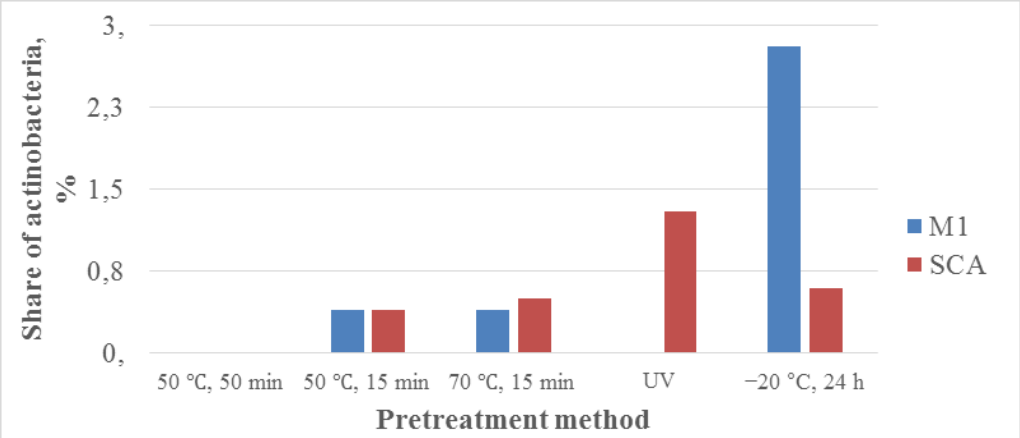

Fig. 2. The share of actinobacteria in sediment samples from Kuyalnik estuary

The comparison of efficacy of different ways of pretreatment for isolation of actinobacteria from bottom sediments of the Black Sea and Kuyalnik estuary demonstrated that, in general, the number of actinobacteria strains was higher in the samples from the sea compared to estuary (table 2). At the same time, the most effective pretreatment regime for both sample sources was freezing with the subsequent plating on M1.

Table 2

The number of actinobacteria in samples of bottom sediments of the Black Sea and Kuyalnic estuary under different conditions of isolation

Nutrient medium	Pretreatment regime	Source of isolation	
		Black Sea	Kuyalnik estuary
M1	50 °C, 50 min	2.8*	0
	50 °C, 15 min	3.5	0.4
	70 °C, 15 min	1.9	0.4
	UV– distance 20 cm, ƛ=254 nm, 15 W, 50 sec	2.4	0
	−20 °C, 24 h	5.1	2.8
SCA	50 °C, 50 min	0.2	0
	50 °C, 15 min	0.7	0.4
	70 °C, 15 min	0	0.5
	UV– distance 20 cm, ƛ=254 nm, 15 W, 50 sec	2.8	1.3
	−20 °C, 24 h	1.1	0.6

Apparently, M1 medium containing the water from natural habitats of marine and estuary actinobacteria imitates better the usual environment for these microorganisms. At the same time, the absence of strict correlation between medium composition and the number of isolated under different pretreatment conditions actinobacteria from Kuyalnik estuary illustrates the necessity of detection of biodiversity of isolated actinobacteria because different species can require different approaches to their isolation. Takizawa et al. (1993) showed that the share of actinomycetes constituted 0.15 to 8.63% of the total number of culturable microbial strains isolated from Chesapeake Bay, which salinity is about 25-30 PSU, what can be considered saline environment [Rich and Maier, 2015].The studies on the hypersaline environments demonstrated that the share of actinobacteria ranged between 0.001% and 15% [Arasu et al., 2016]. The salinity of the Black Sea near Odessa ranges from 15 to 15 PSU, the salinity of Kuyalnik estuary can reach 200 PSU. Thus, the Black Sea can be considered as saline environment and Kuyalnik estuary – as hypersaline one [Rich and Maier, 2015]. The share of isolated actinobacteria is comparable to the data of other researchers in both caseswhat makes used approaches suitable for isolation of actinobacteria from mentioned above reservoirs.

Conclusions

The highest share of actinobacteria (5.1%) from the bottom sediments of the Black Sea was isolated on M1 medium after pretreatment by freezing at −20 °C during 24 h. The highest efficacy of isolation of actinobacteria (2.8% among the to-

tal number of isolated strains) from the samples of sediments collected in Kuyalnik estuary was observed on M1 medium after pretreatment by freezing. The lowest efficacy was detected after UV-pretreatment regardless the composition of nutrient medium. The number of actinobacteria isolated from the Black Sea was higher than from Kuyalnik estuary.

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Zhylovska K., Tytarenko N., Tesliuk N.

DETERMINATION OF THE INFLUENCE OF ACTINOBACTERIA ON SEED GERMINATION AND MORPHOMETRIC PARAMETERS OF MAIZE SPROUTS

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Abstract. *Today in the world it is very important to find ways to reduce the use of chemical fertilizers and maintain sustainable agriculture. Actinobacteria with growth-stimulating properties are a potential source for the development of biopreparations that can help solve this problem. Since soil actinomycetes are already widely studied, it is particularly interesting to isolate and study strains from other environments that may be producers of new or rare metabolites.* ***The aim*** *of the study was to establish the growth-stimulating properties of actinomycetes strains isolated from the Black Sea on maize sprouts.* ***Methods.*** *Strains of actinobacteria were inoculated on sterile maize seeds at a concentration of 10^5 CFU/ml, and after 5 days of cultivation, the germination rate and morphometric parameters of sprouts were evaluated.* ***Results.*** *Strains Myt7b, Conc18, Conc4, Lim4 showed astimulating effect on the germination rate of seeds and sprouts growth of maize plants and can be recommended for further extensive research.*

Key words: *PGPB, actinobacteria, plant growth promotion, Zea mays L.*

Introduction

In recent years, a very important global problem of the excessive use of chemical fertilizers causes special concerns. In addition to large financial losses, in the long run it also leads to adverse environmental consequences, unsuitability of soils for growing plants and reducing sown areas. The group of plant growth-promoting bacteria (PGPB) is one of the main areas of research to promote sustainable agriculture nowadays [4]. Some microorganisms are already successfully used as an alternative to chemical fertilizers in agricultural production. But there is always a need to find new strains that have growth-promoting or antagonistic to phytopathogens properties. In this regard, a group of actinobacteria has a great potential [7].

Actinobacteria are filamentous gram-positive microorganisms that abundantlyinhabit the rhizosphere of various plants. They are active producers of antibiotics: more than 10,000 compounds with antibiotic properties have been identified in actinobacteria. In addition, about 45% of commercial antibiotics in the world are derived from this group of bacteria [2]. This property allows them to effectively carry out biocontrol of phytopathogens in the soil.

Actinobacteria have both direct and indirect mechanisms of impact on plants. Among the currently known mechanisms: synthesis of phytohormones, siderophores, ACC-deaminase, antibiotics (including volatile compounds) and enzymes

that cause hydrolysis of the cell wall of phytopathogenic fungi, hyperparasitism, promotion of symbiosis of plants with other microorganisms, solubilization of phosphates in soil [6].

Because actinobacteria are natural inhabitants of the soil, they often form symbiotic relationships with plants in the rhizosphere,colonizing the interior of plants. It enhances their potential as biological agentsfor plant growth protection and stimulation.

Additionally, actinomycetes isolated from unusual environments are interesting for their potential for the synthesis of new metabolites and changes in biochemical properties, which may be unexpectedly useful in protecting and stimulating of the plant growth [5].

The aim of this study was to establish the growth-stimulating properties of actinomycetes strains isolated from the Black Sea on maize plants by measuring therate and characteristics of seed germination, and morphometric parameters of sprouts.

Materials and methods

In the experiment strains of actinobacteria, which were isolated by researchers-fromOdesa I.I. Mechnikov National Universityfrom biological overgrowth of concrete structures (Conc strains), shell rock (Lim strains) and mussels (Myt strains) of the Odesa Bay of the Black Sea, were used. Previously, the main biological properties of those strains were studied, including the possibility of growth on different media, in different ranges of temperatures and concentrations of NaCl, the ability to form melanoid pigments, and to utilize various carbon sources [1].

For the experiment of plant growthstimulation, 10 strains of actinomycetes were selected - Conc4, Conc18, Conc24, Conc32, Myt4b, Myt5, Myt7b, Myt7ch, Lim4, Lim6.1.

Strains of actinobacteria were cultured for 14 days on Bennett medium in anincubator-shaker at 180 rpm and 28 ˚C [3]. For the experiment the liquid culture of bacteria, adjusted with sterile distilled water to a concentration of 10^5 CFU/ml, was used.

The material for the study was maize seeds of the hybrid DCS 1541 Bayer. Surface sterilization of plant material was carried out in three stages: 1) commercial preparation Horus 1.4 g/l - 1 hour; 2) 5% solution of sodium hypochlorite - 10 minutes; 3) hydrogen peroxide 3% - 10 minutes. After sterilization, the seeds were washed three times with sterile distilled water and immersed in a suspension of cells of a certain strain of actinobacteria for 1 hour. The control was kept in distilled water the same time. After exposure, the grains were placed on filter paper soaked with 10 ml of sterile water in a Petri dish, and cultured in a growth chamber at + 22-24 ° C, light intensity 2500 lux, relative humidity 55-70% and photoperiod 16/8 (day/night) hours per day. Observations were performed on the 5th day: the number of germinated seeds was registered, as well as the percentage of seeds that formed roots and/or shoots, also the average length of shoots and roots, and the average number of roots were measured. For the each strain 20 seeds were tested, the experiment was repeated three times. Statistical processing of the results was performed using Microsoft Excel.

Results and discussion

It was found that inoculation with different strains had different effects on the germination of maize seeds. On the 5th day in variants with strains Conc18, Myt7b, Conc4, Lim4, by 7-11% more germinated seeds were observed than in the control (Fig. 1).

Inoculation with strains Myt4b and Conc24 caused a decrease in the number of germinated seeds - by 16 and 41%, respectively. This may be due to both the phytotoxic effect of metabolites of these microorganisms and their concentration (if so, there is the necessity to research the effects of less concentratedsuspensions).

Regarding the formation of roots, on the 5th day most of sprouts, on average, correlated with the control. Sprouts inoculated with Conc4 strain showed the highest percentage of root formation - 94%, and in the controlit was, on average, 83%. Shoot formation in our experiment was stimulated by seed treatment with Myt7b, Conc4 and Lim4 strains - on average, by 8-11%.

Treatment with actinobacteria also shown an effect on the average length of shoots and roots of sprouts (Fig. 2).

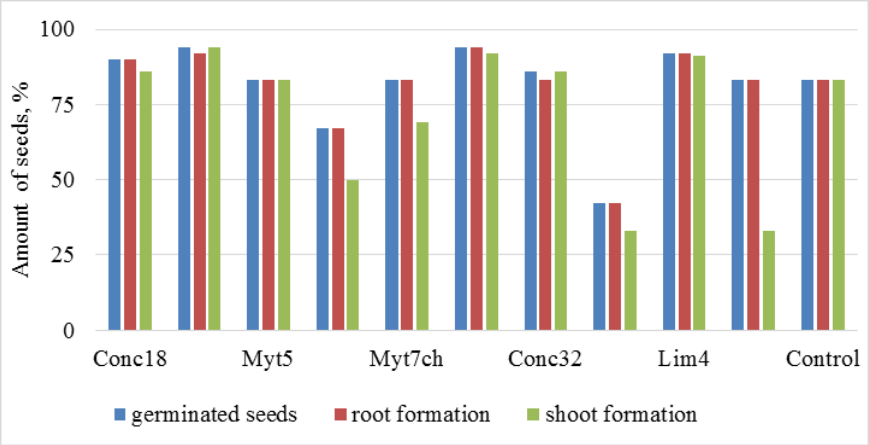

Fig. 1. Germination of maize seeds on the 5th day after inoculation with strains of actinobacteria

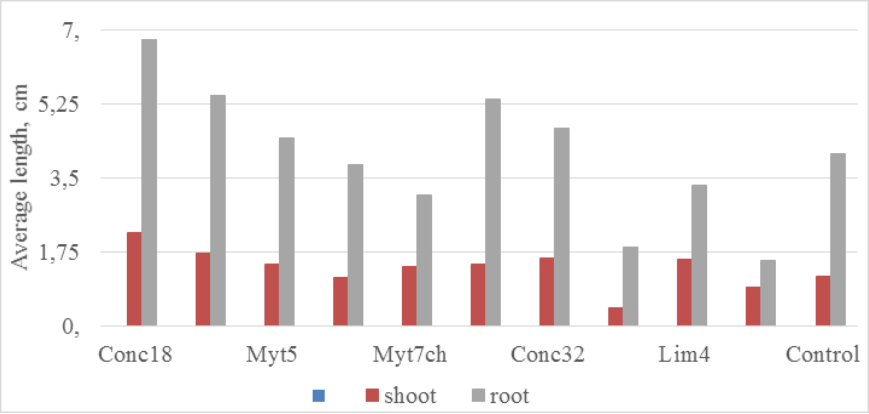

Fig. 2. The average length of shoots and roots of maize sprouts on the 5th day after inoculation with strains of actinobacteria

Statistically significant increase in shoot length on the 5th day was observed by inoculation with strains Conc18, Myt7b, Conc32, Lim4. Treatment with Conc24 and Lim6 strains reduced the average shoot length, and other strains showed no difference with control. The average root length increased when exposed to seeds with strains Conc18, Myt7b, Conc4, Conc32, and decreased relatively to control when treated with strains Myt7ch, Conc24, Lim4, Lim6.1

Since maize is a monocotyledonous plant and has a fibrous root system, in addition to the average length of roots, we also estimated their number per sprout (Fig. 3).

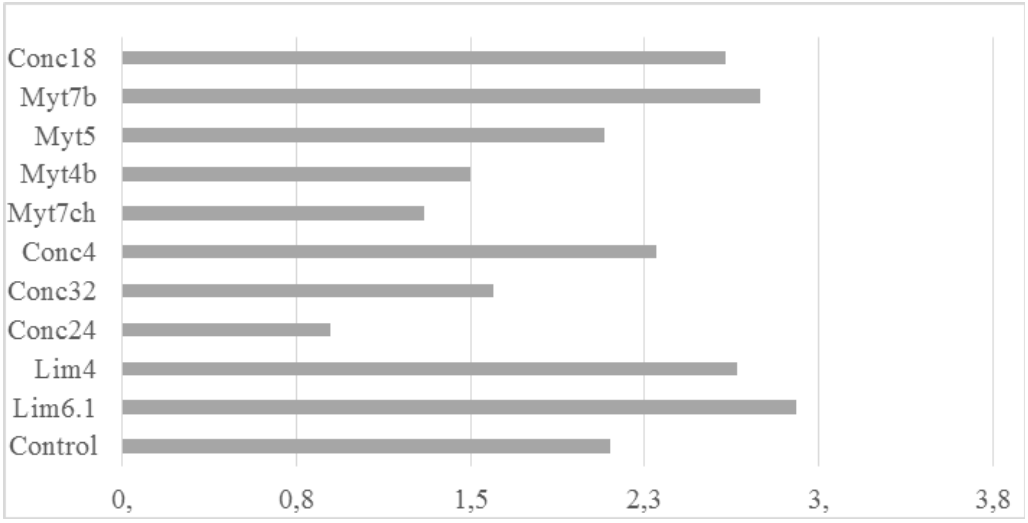

Fig. 3. The average number of roots in a maize sprout on the 5th day after inoculation with strains of actinobacteria

On the 5th day, sprouts inoculated with strains Lim6.1, Lim4, Myt7b, Conc18, showed by 0.5-0.8 roots per sprout more than control. Interestingly, from the obtained data we can observe how treatment with certain strains (Lim6.1, Lim4) leads to a decrease in the average length of the roots, but at the same time - to an increase in the average number of roots per sprout.

In general, after analysis of all measured parameters, we can identify 4 strains of actinobacteria, exposure to cultures of which had the most positive effect on maize sprouts: Myt7b, Conc18, Conc4, Lim4. On the 5th day, exposure to strain Myt7b showed an increase in all researched characteristics. The same with strain Conc18, except for the formation of shoots by seedlings, which remained at the level of control.

Treatment with Conc4 culture suspension increased germination, shoot and root formation, and root length, but the results did not differ significantly from control in the number of roots and shoot length. Exposure to Lim4 strain increased all experimental parameters, but decreased the average length of roots. Some strains showed a phytotoxic effect: Conc24, Myt4b, Myt7ch, Lim6.1, and treatment with Myt5 strain showed no changes in germination and external characteristics of plants compared to control.

The diverse manifestations of the strains impact indicatedifferent possible mechanisms that provide the observed effects of bacteria on plants. First of all, the increase in germination rate, the formation of roots and shoots in sprouts, as well as their average length and number can be explained by the production of substances of phytohormonal origin: auxins, cytokinins and gibberellins. These phytohormones affect plant tissues in different ways depending on the concentration and ratio. Also, some actinobacteria are able to colonize the interior parts of plants, which can also be a factor of growth stimulation. It is possible that some strains are nitrogen fixers, phytopathogen antagonists, phosphate solubilizers, producers of ACC-deaminase or new plant-stimulating compounds - however, the determination of exact mechanisms will be the aim of future research.

Conclusions

It was found that inoculation with strains Myt7b, Conc18, Conc4, Lim4 showed a growth-promoting effect on seed germination and morphometric parameters of maize sprouts. Other studied strains either did not have such activity, or showed phytotoxic effect in the experiment. The diversity of thestimulation traits suggests different possible mechanisms involved in these processes. Given the results of the screening, it is possible to recommend further studies of strains Myt7b, Conc18, Conc4, Lim4 in other conditions to confirm the growth-promoting activity withsubsequent development of a biological preparation for plant protection.

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