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Ainietdinova H., Burlaka V., Dreus A.

LYSOGENIC CONVERSION AND IT'S ROLE IN BACTERIAL EVOLUTION AND EXPRESSION OF VIRULENCE FACTORS

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Abstract. *Genomics data obtained in the last decade indicate that a plenty of bacterial genomes have multiple prophage elements. Understanding the molecular genetic mechanism of the occurrence, maintenance and transmission of lytic and lysogenic viral infections in bacterial populations will be useful to establish its role in bacterial evolution and expression of virulence factors. And lysogenic conversion resulting from the integration of prophages encoding powerful toxins is probably the most determinant contribution of prophages to the evolution of pathogenic bacteria.*

Keywords: *bacteriophage, prophage, lysogenic conversion, toxins, virulence, horizontal gene transfer*

Introduction

Lysogeny is a genetically determined ability of bacteria to lyse with the release of a bacteriophage [1]. It is determined by the presence of a prophage, a special form of intracellular viral DNA that replicates coordinated with the bacterial chromosome and then transmitted to daughter cells. Bacteriophages that can exist as prophages and lysogenize cells (lysogenic conversion) are called moderate. The development strategy for all moderate phages includes a choice between lysis or lysogenization of the host cell. In many respects, this choice depends on the physiological state of the host bacterium [2].

Most molecular knowledge of lysogeny has been derived from a handful of E. coli phages, such as λ and Mu, which integrate into the bacterial chromosome by dint of site-specific recombination [3] or random transposition [4], respectively. By contrast, other phages are maintained extrachromosomally with either circular (for example, P1, [5] or linear (for example, N15, [6]) genomes. Several temperate phages, such as satellite phage P4, needs other temperate phages, such as P2, to complete whole infection cycles [7]. Other temperate phages, for example, Vibrio cholera phage CTXphi, chronically infect their host during productive cycles and integrate during lysogenic cycles [8]. Even though infection details could be different, lysogeny generally proceeds through the three main steps: (i) establishment, (ii) maintenance and, potentially, (iii) induction of productive cycles. Lysogenic conversion constitutes the phenotypic effects of prophage carriage to its host cell [9].

Horizontal gene transfer should be considered as the fast mode of evolution (from years to decades). New sets of genes are acquired by transduction, transposition, transformation, and, last but not least, lysogenization with phages. It has been

quite an exciting discovery that phages play an important role in the emergence of pathogens. This fact was first noted in relation to toxins of *Corynebacterium diphtheriae* (diphtheria), *Streptococcus pyogenes* (scarlet fever), *Staphylococcus aureus* (food poisoning), *Clostridium botulinum* (botulism) and *E. coli* (Shiga toxin), which are all phage encoded. However, these genes do not directly play a role in the life cycle of the phages, the list of phage-encoded fitness factors is rapidly increase and now made up a wide range of different genes.

E. coli lysogenic conversion

Enterohemorrhagic *E. coli* strains cause serious hemorrhagic colitis and hemolytic uremic syndrome due to the production of Shiga toxins (Stx), Stx1 and Stx2, and enterohemolysins (Hly), all of which are encoded by lambdoid phages (H-19B) [10,11]. Stx is an AB5 toxin with one active A subunit and five identical B subunits. B subunits bind to glycolipids on the host cells, and the A subunit is taken up by the cell and retrograde trafficked to its active site. In this way, after entering a cell via a macropinosome, [12] the protein (A subunit) cleaves a specific adenine nucleotide from the 28S RNA of the 60S subunit of the ribosome, which leads to halting protein synthesis [13]. Depending on the strain, stx genes can be encoded on unrelated phages and produce very different amounts of toxin. Genomic sequences of *E. coli* O157 strains Sakai and EDL933 showed the existence of 18 and 12 prophages, respectively, many of which are lambdoid that encode genes related to virulence [14]. Moreover, other phage-encoded virulence genes present in *E. coli* strains include the Bor and Lom proteins, which confer cell adhesion and serum resistance [15].

The Cholera Toxin Filamentous Phage CTXф

CTXф filamentous bacteriophage, specific in Vibrio *cholerae* is one of the most extensively studied with respect to the development cycle, the way of integration into the chromosome, genetic organization, replication and influence on the properties of the host bacteria in moderate filamentous phages. Was first identified in 1996 by M.K. Waldor and J.J. Mekalanos [16]. Phage genome contains genes that encode cholera toxin secreted by cholera vibrion cells[17]. The activity of the cholera toxin is responsible for the development of profuse diarrhea, which is a hallmark of cholera. Only those strains that carry CTXф cause cholera epidemics and pandemics, i.e. phage acquisition plays a key role in the emergence of pathogenic strains of *V. cholerae*. In order for the CTXф phage to penetrate cholera vibrion cells, toxic-controlled saws (TCP), on which the phage is adsorbed, are a necessary factor. Genes encoding TCP are part of the VPI1 pathogenicity island, which is about 40 TCP, flanked on both sides by sequences of 20 bp. In addition to the tcp genes encoding the saws, the island contains a number of key regulatory pathogenicity genes, as well as genes supposedly integrations and transposases. *V. cholerae* strains that do not produce TCP saws are insensitive to CTXф phage infection [16]. The integration of CTXф DNA occurs in a specific chromosomal dif site by recombination between att (dif) sites on the bacterial chromosome (attB site on chrI, the larger of the two chromosomes of the cholera vibrion) and the phage genome (attP). A phage does not encode a protein with the homology of any

of the known recombinases. It uses recombinases XerC and XerD encoded with the chromosome the host bacteria [18,19]. The integration of phage DNA into the $attB^+$ genome, CTXф strain of the cholera vibrio of El-Thor biovar leads to the formation of a single CTXф prophage or, more often - tandem prophages flanked by 17./18 bp, so-called end repetitions (ERs). Toxigenic strains of El-Tor cholera vibrion often contain a 2.7 tbp genetic element related to CTXф, known as RS1 [20]. The formation of infective CTXф phage particles requires the presence of either two CTXф prophages or a series of alternating CTXф-RS1 prophages. If there is only one phage, no phage is formed. Unlike most well-characterized phages that can integrate with the chromosome of the host, as well as being cut out of it, excision of the CTXф prophage from the chromosome never occurs [21].

Lysogenic Conversion of *Mycobacteria*

Because of the importance of mycobacterial diseases such as tuberculosis (TB) and leprosy, mycobacteriophages were studied for a long time, the first of which were isolated in the 1940s [22,23]. Mycobacteriophages therefore reflects the specific evolutionary. Most *M. tuberculosis* strains carry one or both of two small (10 kbp) prophage-like elements wRv1 and wRv2, but it seems unlikely they contribute to virulence. Some mycobacteriophages (in Cluster D) do encode a vegetative insecticidal protein (VIP2)-like insect toxin genes that could confer virulence to a bacterial host, although it is unclear what that host might be, or what it might infect; they do not infect *M. tuberculosis*. *M. tuberculosis* is challenging to grow because of its slow growth rate (24-hour doubling time) and its pathogenicity. It was intractable to genetic manipulation until breakthroughs in the late 1980s that took advantage of mycobacteriophages to bootstrap methods for transfection and transformation, electroporation, plasmid vectors, and selectable markers [24]. Furthermore, phages have continued to be key players in developing a more facile genetic system. For example, several applications rely specifically on the ability of phages to inject their DNA into essentially every cell within a mycobacterial population, making phages ideal for transposon delivery and preparation of complex transposon libraries [25], gene replacement using specialized transduction, and tuberculosis (TB) diagnosis by inclusion of a reporter gene [26]. For mycobacteriophage Bxb1, the consequences of integration for host physiology are well established [27]. Bxb1 uses a serineintegrase to integrate into an attB site located within the groEL1 gene of *M. smegmatis* [20]. GroEL1 serves as a dedicated chaperone for regulation of mycolic acid biosynthesis and is required for the formation of mature biofilms. Thus, lysogens of Bxb1 are defective in forming biofilms, perhaps providing a selective advantage as cheaters within a broader population of non-lysogenic biofilms [28].

Lysogenic Conversion of *Ralstonia solanacearum*

An example of ambivalent influence of lysogenic bacteriophages is *Ralstonia solanacearum* (former Pseudomonas solanacearum) - a soilborne Gram-negative bacterium, that causes bacterial wilt and damages economically important plant species. Different kinds of bacteriophages that infect Ralstonia solanacearum were isolated, characterized and classified by Yamada et. al. into six types (RSS phages

(lysogenic), RSM phages (lysogenic), RSB phages (lytic), RSA phages (lysogenic), RSL phages (lytic), RSC phages (lysogenic), and others) on the basis of morphology, integration mechanisms (integration of RSS0 at a dif site, similarly to CTX of Vibrio cholerae [29] or using of phage-encoded recombinases (ORF14 of RSM1) by RSM phages [30]. and effects on the host cell.

RSS-type phage ϕRSS1 enhanced virulence [31], as well as ϕRSY1 that lysogenized into the host cells and converted their features to form large and rough-margined colonies, enhanced twitching motility, and a high aggregation frequency [32]. At the same time RSM-type phage ϕRSM1 caused loss of virulence of *R. solanacearum* on tomato plants [33], while establishing a persistent association between the host and phage, releasing phage particles from the growing and dividing host cells [34]. R. solanacearum cells infected by lysogenic ϕRs551 produced significantly lower amounts of EPS (71.5±3 mg/10 ml) than the wild-type cells (107±5 mg/10 ml), displayed lower swimming activity and possessed lower virulence level (until five days after inoculation no symptoms were observed; inability to cause any disease symptoms on some inoculated plants even 21 days after soil drenching inoculation) [35]. *R. solanacearum* lysogenic bacteriophages along with lytic ones can be seen as prospective agents for lowering the damages of bacterial population.

Lysogenic Conversion of *Corynebacterium diphtheria*

Toxin genes encoded by a prophage are responsible for turning bacteria into toxinogenic forms as it happens with *Corynebacterium diphtheriae* under the influence of tox+ corinephages (including the tox+ phages α, β, δ, L, P, and π). [36]. A variety of studies have shown that the integration manner of phage β into the chromosome of *C. diphtheriae* is similar to integration of the λ-phage genome into the chromosome of E. coli.

The integration of corynephage β appears to occur via site-specific recombination between a phage attachment site (attP) and one of two functionally equivalent bacterial attachment sites (attB1 and attB2) in the chromosome of *C. diphtheriae* [37]. Despite a long history of research new tox^{+} corynephages responsible for phage conversion are constantly being discovered, such as corynephage β-similar bacteriophage that has homologous structural components to a different cryptic prophage from *C. ulcerans* [38].

Moreover a focus on diphtheria toxin-producing isolates of *C. ulcerans* is observed, because they are not only capable of causing infections in humans indistinguishable from classical diphtheria caused by C. diphtheriae [39], but also can cause zoonotic infections [40].

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Barabash M., Melnyk M., Iungin O.

THE EFFECT OF AHLS ON WINTER WHEAT ENDOPHYTIC MICROFLORA

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Abstract. *We investigated whether the acyl-homoserine lactone N-hexanoyl-L-homoserine lactone (C6-HSL) impacted winter wheat (Triticum aestivum L.) seed germination and microflora profile. In vitro germination experiments indicated that C6-HSL seed priming had a positive impact on germination levels and increase the number of nitrogen-fixing and phosphate-mobilizing bacteria. Moreover, a number of highly effective morphotypes of phosphate-mobilizing bacteria for further studies was isolated as a result of 300 μM C6-HSL application.*

Key words: *AHLs, priming, biofilm, quorum sensing, winter wheat, endophytic bacteria*

Introduction

Plants and bacteria coexist in a tightly-bonded manner. Complex interaction of plants with the microbial communities in their own phytosphere (sets of rhizomes, endo- and philospheres) is crucially important for both partners of this coexistance [8]. Assimilation nutrients by plants, their development and resistance to stress depend on this interactions [6, 7]. Effective coexistence is mediated, in particular, by special communication systems - signaling pathways of plants and microorganisms, which are implemented at the individual intracellular and extracellular levels. There are several types of mediator molecules for remote signal transduction known by now. The signaling system (quarum sensing, QS) with the participation of acylhomoserinlactone molecules (AHLs) is the most deeply studied. The nitrogen-containing heterocyclic hydroxyketones, systems of peptide nature are less studied compounds [15, 17]. So, one of the mediators of the signaling system of phytospheric bacterial community and plant is acylhomoserine lactone system. It seems that AHL mediators form the most numerous group of substances taken participation in the transmission of signals from micro- to macro-organism [10].

The processes of plant growth and development occur under close coordination of cell division, stretching growth and differentiation. The synchronization of these processes involves the exchange of signaling molecules between the root and the shoot, which can be affected by both biotic and abiotic factors. The interaction between plants and associated microorganisms requires special regulation. Plants produce a number of organic compounds, including sugars, organic acids, vitamins and phytohormones, which microbial populations use as nutrients and regulatory substances. At the same time, microorganisms synthesize compounds that can ac-

tivate the plant's immune system, regulate its growth and morphogenesis directly or indirectly.

The urgent task of agricultural sciences is the limitation of the use of synthetic pesticides and its replacement with environmentally friendly chemicals that will effectively protect crops without destructive effects to the environment. Therefore, searching for and developing new substances that would meet these requirements is a priority task for biotechnology. Priming (pre-sowing seed treatment) is considered as an effective biotechnological approach that increases viability and resistance of the seed itself, and later on in seedlings and plants [14]. Priming is discussed as a promissing agricultural biotechnology due to its possible prolonged combined effect not only on primed seeds and plants but due to the effect on plants of next generations [13]. High seeds quality is an important prerequisite for a stable harvest. However, high quality seeds can differ in the degree of maturity, which leads to asynchrony of germination and heterogeneity of seedling development. Priming seeds induces repair processes in wheat germs, and the cell cycle is started afterwards. Moreover, it was shown that compounds of the QS system are able to increase the efficiency of nitrogen fixation by bacteria of the genus Rhizobia, as the formation of nitrogen-fixing nodules in legumes, the development of symbiotic relationships, synthesis of exopolysaccharides and adaptation to stressors are regulated by QS [5]. Intensification of AHL formation in Rhizobia sp. and the use of bacterial inocula activates the processes of growth and development, nitrogen fixation, increases stress resistance, reduces the use of chemical fertilizers and plant protection products.

Bacterial liquid cultures of soil plant growth promoting microorganisms (PGPR group) are already used today as a component in complex mixtures in environmentally friendly agriculture [2]. For example, such perspectives of the use of rhizospheric bacteria (*Bacillus, Pseudomonas, Serratia* sp.) for the production of new natural plant protection products were discussed [1, 12]. However, the disadvantages of such chemicals are the complexity of application, seasonal dependence and high cost. It is proved that the production of bacterial AHL of the PGPR group is generally higher than that of soil bacteria of similar genera and species.In particular, it is shown that pea seeds during germination are released into the soil substances that mimic the action of AHLs. Probably, it improves seed germination, increase the stability of seedlings, model the plant-bacterial interaction in the phytosphere.

The main aim of our research was to study the effect of acyl-homoserine lactone N-hexanoyl-L-homoserine lactone (C6-HSL) on winter wheat (*Triticum aestivum* L.) seeds germination and microflora.

Material and methods

Seeds varieties

Winter wheat plants *Triticum aestivum L.* of new genotypes of Ukrainian selection were used. Podolyanka and Smuglyanka varieties provided by V.M. Remeslo Myronivka Institute of Wheat NAAS of Ukraine were selected.

Podolyanka (medium-early variety) was characterized by high tillering, frost

resistance exceeds the average, is grown in the steppe, forest-steppe and Polissya of Ukraine.

Smuglyanka (medium-early variety) was a short-stemmed, high-intensity type, drought-resistant, grown in the Forest-Steppe, Polissya and Steppe zone of Ukraine.

Seed germination assay

The effects of seed priming on germination were investigated according to the International Seed Testing Association (ISTA) standards. Three samples of 20 seeds of each variety and treatment were germinated in the dark at 24˚C for 24 h in Petri dishes containing Knop's nutrient solution [3] to determine germination levels. Non-primed control seeds were treated with sterile water.

Microbiota test

Seed treatment was performed with an aqueous solution of the L-isomer of acylhomoserine hexanoyl lactone (C6-HSL), consentrations of 100, 150 and 300 μm in three replicates of 10 seeds. Seeds treated with sterile distilled water were used as a control. Dried after treatment and prepared seeds were germinated under sterile conditions with the addition of saline in serial tenfold dilutions (10^{-2}−10^{-8}). Aliquots (0.1 ml) of dilutions were inoculated on pre-prepared Petri dishes with Nutrient agar (HiMedia Ltd.), Ashby and Muromtseva media (Sinbias, Ukraine). The media were prepared according to standard protocols [4]. These media were used to determine the total number of chemoorganoheterotrophic bacteria, nitrogen-fixing and phosphate-mobilizing microorganisms, respectively. Inoculated plates were cultivated for 7 days at 24 °C. After that, the CFU and morphotypes were counted. Statistical data processing was performed using the Excel 2010 software package (p<0.05).

Results and discussion

Priming with C6-HSL had an effect on microflora and seedlings length in both studied varieties of winter wheat (Figure 1).

Priming the seeds with C6-HSL has increased the length of seedlings. In the variant with Podolyanka variety, the increase in seedling length ranged from 31 to 41%, Smuglyanka 98−123% (Fig.1). This difference in growth between varieties may be explained by the physiological characteristics of the varieties itself. But the fact of positive impact of C6-HSL use is remarkable. As it was shown in recent reports the positive impact of AHLs could range from growth promotion to induced resistance of plants [15]. It also should be noted that there was no significant difference within the experimantal sets: seedling length of primed seeds remained within the experimental error in 100, 150 and 300 μM C6-HSL. There was no correlation between the increasing of active substance concentration and the wheat seedlings length. The number of germinated seeds in all variants were within the limits indicated in the passport of varieties. There was no toxic effect observed on seed germination even at the highest applied concentration of C6-HSL. So the necessary and sufficient concentration for winter wheat seeds length increasing was 100 μM. Our results agreed with the previous studies that AHLs with a short acyl chain, like C4 or C6, were shown to increase the growth rate and primary root elongation [15].

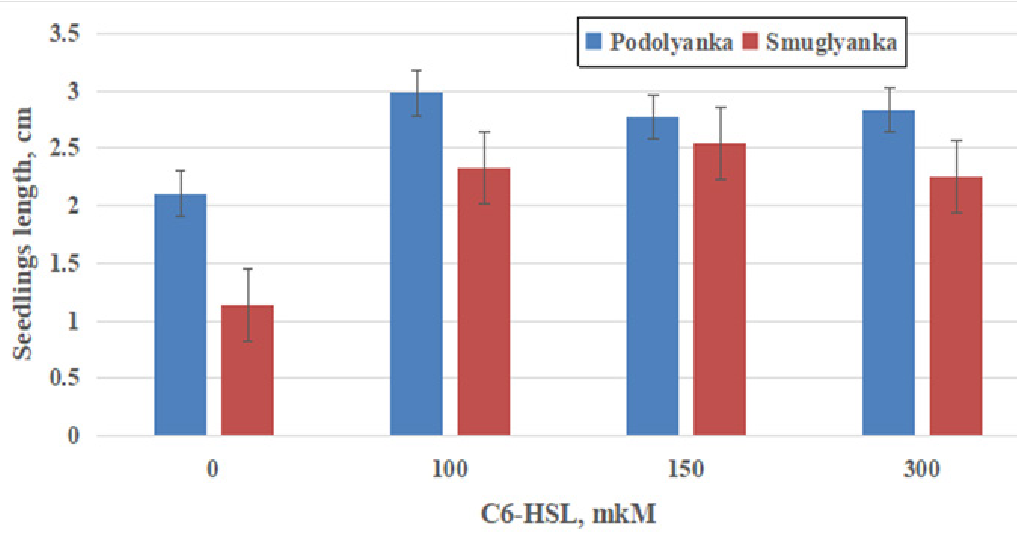

Figure 1. Seedlings length of winter wheat with C6-HSL application

Agricultural biotechnologies is focused often on arranging of plant-microbial interractions in rhizosphere in order to rainforce the uptake of nitrogen and to set up a biological defense barrier. Our previous results have shown that number of Nitrogen cycle bacteria (amylolytic, denitrifying, nitrifying, N-fixing, and oligonitrophilic bacteria) determined from plants growing from primed samples were found to be dependent on seed treatment. This preliminary result suggests that the seed priming effect alters the functional composition of bacterial communities around wheat roots [11].

As a result of the current studies it was shown that seeds priming with C6-HSL had an concentration-depended effect on seed microflora. Thus, the total number of all studied bacterial physiological groups increased by at least one order of magnitude in both varieties at the concentrations of 100 and 150 µm C6-HSL. At the same time, this index was reduced by an order of magnitude (up to 10^4 CFU/ cm^3) for chemoorganoheterotrophic and nitrogen-fixing bacteria at the concentration of 300 µM C6-HSL. Although the number of phosphate-mobilizing bacteria variety increased by 2 orders of magnitude compared to unprimed seeds. Moreover, there was observed the increasing of morphotypes number, so we can indicate an biodiversity deversification [9] under C6-HSL application. There is a need for further studies of these results.

The highly effective morphotypes of phosphate-mobilizing bacteria were isolated for further studies. Using the term “highly-effective” one means productivity of isolates to dissolve insoluble phosphate compounds. There is a great interest in phosphate-mobilizing agricultural biotechnologies develop ment in Ukraine in general and in South Ukraine particularly. It is due to enormous deposits of insoluble phosphates in South Ukraine soils [16] which could be used as a free fertiliser for crops growing in case of applying highly effective phosphate-mobilizing bacteria.

Conclusions

Priming of winter wheat seeds with acylhomoserine hexanoyl lactone had an effect on the seed microflora and significantly increased the length of seedlings. The necessary and sufficient concentration for seeds length and number of seed beneficial bacteria increasing was 100 μM. The magnitude of the effect was varietal-dependent. The obtained results can be used for further development of biotechnologies to stimulate the growth of cereals.

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SYNTHESIS OF BIOSURFACTANTS BY STRAIN *ALCALIGENES FAECALIS* M1 ISOLATED FROM BLACK SEA WATER

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Abstract. *Among 30 isolates of mesophilic chemoorganoheterotrophic, aerobic / facultative-anaerobic bacteria of sea water, 1 strain of Alcaligenes faecalis (A. faecalis M1) was identified. Using a drop test, it was shown that A. faecalis M1 is capable of synthesizing biosurfactants that effectively reduce surface tension and the activity of the corresponding products of the collection strain A. faecalis 8/1. It was found that biosurfactants of A. faecalis M1 are superior to the comparison strain in terms of emulsifying activity against various hydrophobic substances. The E24 index is in the range of 41.5–72.3%, while the comparison strain is in the range of 25.9–46.3%. Biosurfactants of A. faecalis M1 more efficiently emulsified hydrocarbons, abiosurfactants of A. faecalis 8/1 - sunflower oil. It has been shown that A. faecalis M1 biosurfactants form more stable emulsions than A. faecalis 8/1 biosurfactants.*

Key words: *Alcaligenes faecalis, biosurfactants, emulsifying activity index*

Introduction

The study of the properties of the microorganism *Alcaligenes faecalis* has a high relevance associated with its use in the synthesis and production of biotechnologically useful products, as well as in the processes of bioremediation and biodegradation of certain specific compounds.

The study of the microorganism has been actively conducted over the past 30 years, mainly by specialists from Japan and India, where the biotechnology industry is well developed, although this microorganism was first described only in 1919 [9].

Potential uses are quite wide and varied in their direction: decomposition of bioplastics, oxidation of arsenic compounds, transformation and utilization of a number of petroleum products, production of polysaccharides - components of gels and biofilms, also known for high heterotrophic nitrifying potential bring practical benefits to industry [1].

A. faecalis are gram-negative rods or cocobacilli 0.5–1.2 × 1–3 microns in size that do not form spores [6]. Move with the help of flagella, in the amount of 1 to 9, which are located peritrichially.

Usually this bacterium can be found in the aquatic environment and soil, very rarely it can be found in human clinical samples such as blood, urine, sputum, pus and feces [2].

The species *A. faecalis* is an opportunistic pathogen, it causes disease only when the human immune system is suppressed or when exposed to various negative factors, such as chronic diseases, alcoholism, addiction to smoking or drugs [8].

A. faecalis can be valuable for the synthesis of some organic substances, it can also be used for biodegradation and transformation of certain compounds, due to its strong denitrifying properties under aerobic conditions. Also promising is the production of polysaccharides specific for this genus of bacteria [10].

Like most bacteria, *A. faecalis* is capable of forming a biofilm. The biofilm is grown on a slide placed in PNB and an Erlenmeyer tube. All this is placed in the shaker at 30 ºC for 24 hours. In a shaken environment, the biofilm develops faster due to the constant washing of cells with nutrients. Producing polysaccharides, provided strong adhesion to the surface due to the glycocalyx [10].

Materials and methods

Samples of sea water were taken near the pier near the Mechnikov ONU hydrobiological station.

1 ml of sea water was diluted in the eight tubes with 9 ml of distilled water, pre-inoculated into IPA and incubated at 37 ºC for 24 hours. After incubation, Gram-stained smears were made from the obtained cultures. Cultures in which gram-negative short rods were detected in smears were seeded on IPA Petri dishes to obtain individual colonies.

The resulting colonies were seeded on Endo medium and were selected red colonies. To confirm the belonging of the isolated isolates to the genus *Alcaligenes*, a number of biochemical parameters were studied.

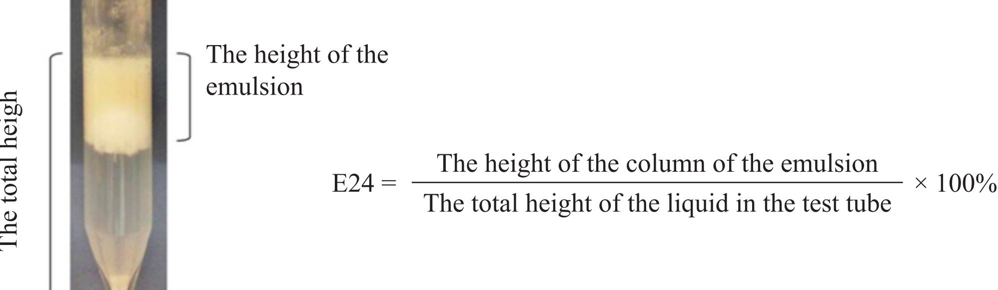
The properties of the selected strains were compared with the collection strain *A. faecalis* 8/1 from the collection of cultures of the Department of Microbiology, Virology and Biotechnology of Mechnikov ONU.

Cultivation of *A.faecalis* strains was performed in LB medium (g/l): peptone 10.0; NaCl 10.0; yeast extract 5.0 during the day. Upon completion of the cultivation, the number of cells was determined spectrophotometrically at 600 nm and the suspensions were diluted with LB medium to a concentration of 10^9 cells in 1 ml. To obtain cell-free supernatants, the samples were centrifuged for 20 min at 3000 rpm. The supernatants were collected in clean tubes and used to detect biosurfactants in them.

The presence of surfactants was determined in a drop test [5]. To do this, 5 µl of a 1% solution of methylene blue was added to 200 µl of the supernatant and 20 µl was applied to the center of the square (1 × 1 cm) on the surface of the parafilm. The control was a sample that contained distilled water instead of the supernatant.

After the drops dried, the parafilm strips were scanned using the computer program AltamiStudio

Emulsifying activity was evaluated by the method of [4]. 5 ml of hydrophobic liquid was added to the graduated tubes and 5 ml of supernatant was added. The samples were shaken vigorously for 2 minutes and left at room temperature for 24 hours. After 24 h, measure the height of the emulsion and calculate the emulsification index - E24, by the formula:



Results and discussion

At the first stage of work, bacterial isolates were allocated from seawater and identified. There were 14 gram-negative isolates, 6 were selected, which by their morphological, cultural and biochemical properties could be assigned to the family *Pseudomonadaceae*.

These 6 isolates were tested for growth ability at 42 °C. Given that only isolates 3 and 4 did not show the ability to grow at 42 °C, they were selected for further research as possible representatives of the genus *Alcaligenes*, and other isolates were assigned to the genus *Pseudomonas*.

After initial identification, the ability of all 6 isolates to utilize a number of carbon sources in the variegated Gis series was investigated. The allocated isolates were compared with the reference strain *A. faecalis* 8/1.

The obtained results showed that *A. faecalis* 8/1, in the conditions of the variegated series of Gis did not show the ability to grow on a medium containing glucose, as well as limited ability to grow on xylose. However, there was a significant ability to utilize citrate. Measurement of the pH of the medium after 24 days of incubation in all cases showed a shift to the alkaline side of ~ 8–8.5 Among the allocated isolates, only isolate 3 showed similar properties, while isolate 4 showed the ability to grow on glucose and cause acidification of the medium (pH ~ 5.0). Based on the results obtained, isolate 3 was identified as *A. faecalis* M1.

The presence of biosurfactants in the supernatants of daily cultures of *A. faecalis* was evaluated in a drop test. The results are presented in the table 1.

Studies have shown that the supernatants of daily cultures isolated from sea water and reference strains according to the results of the drop test contain compounds that can reduce surface tension, but the supernatant of *A. faecalis* M1 contains much more such compounds. Thus, in comparison with the control, the diameter of droplet spread of *A. faecalis* M1 supernatant by the hydrophobic surface of the parafilm was 3.4 times larger, while for the supernatant of the reference strain *A. faecalis* 8/1 – only approximately 2.1 times. Thus, it can be assumed that in general the supernatant of the *A. faecalis* strain isolated from the marine environment contains 1.5 times more compounds having biosurfactant properties.

The emulsifying activity of the cell-free supernatants of the studied strains was evaluated in relation to sunflower oil, n-hexadecane, n-hexane, diesel and toluene. The obtained results show that the biosurfactants of the collection strain *A. faecalis* 8/1 are inferior to the marine isolate in terms of emulsifying activity and differ from the latter in relation to various hydrophobic substances. The values

Table 1

The *Alcaligenes faecalis* supernatants activity in the drop test

Variant	*Alcaligenes faecalis* 8/1	*Alcaligenes faecalis* M1	Control, water
Parafilm photo with supernatants			
Diametr, mm	4,38 ± 0,12*	7,25 ± 0,17*,**	2,13 ± 0,07

Note: photos are in full size, the side of the square is 1 cm;
* - the difference is significant in comparison with the control;
** - the difference is significant in comparison with the strain A. faecalis 8/1

of E_{24} emulsified *A. faecalis* 8/1 substances are as follows: sunflower oil > n-hexadecane > diesel > n-hexane > toluene. In the case of *A. faecalis* M1, this series is somewhat different: diesel > n-hexadecane > n-hexane > sunflower oil > toluene.

To determine the stability of the obtained emulsions, measurements of the height of their columns were performed after 72, 120 and 168 hours.

The results showed that against the background of higher emulsifying activity, *A. faecalis* M1 supernatants showed the ability to give significantly more stable emulsions than *A. faecalis* 8/1 supernatants with all of the used hydrophobic compounds and mixtures. Thus, for comparison with the first day (fig. 1) the height of the columns of emulsions was almost equal to the indicators of the first day in the case of *A. faecalis M1* and *A. faecalis* 8/1, which indicates the stability of the obtained emulsions.

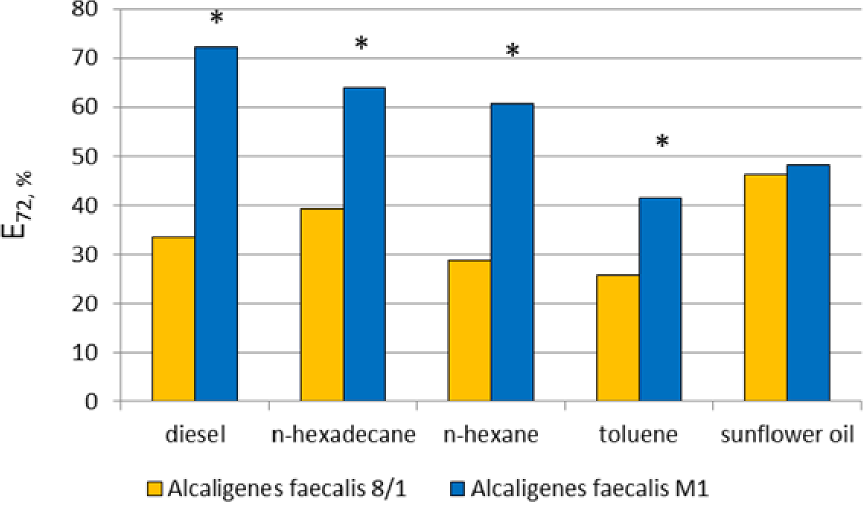


Figure. 1. Emulsifying activity of supernatants of the studied strains of *A. faecalis* after three days

Note: * - the difference is significant compared to the strain *A. faecalis* 8/1

However, from 4 to 5 days (fig. 2), the height of the emulsion columns began to gradually decrease.

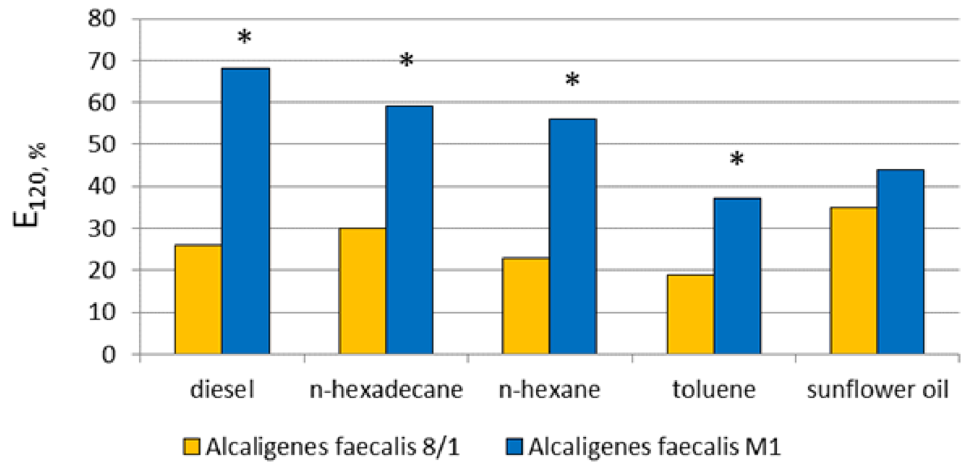


Figure. 2. Emulsifying activity of supernatants of the studied strains of *A. faecalis* after five days

Note: * - the difference is significant compared to the strain *A. faecalis* 8/1

This decrease was observed up to 7 days, but it was stronger in the case of strain *A. faecalis* 8/1 (fig. 3).

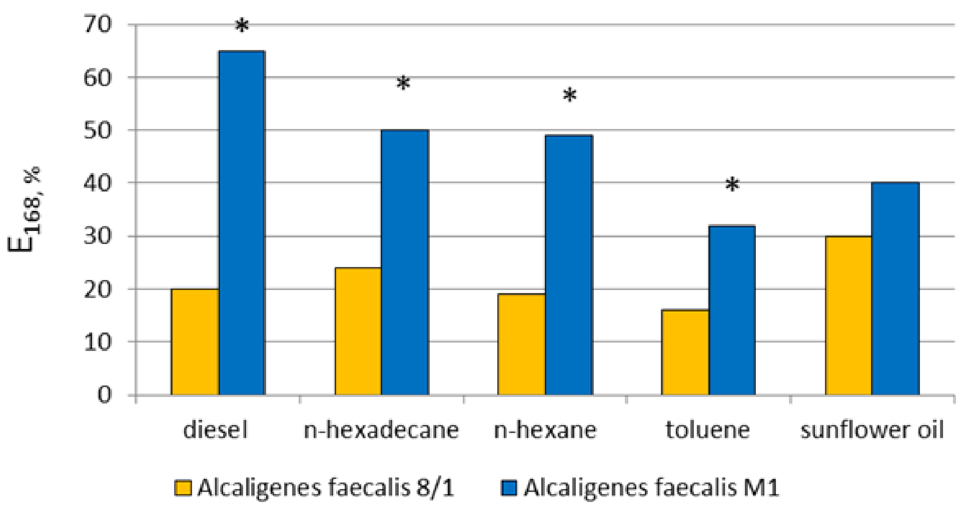


Figure. 3. Emulsifying activity of supernatants of the studied strains of *A. faecalis* after seven days

Note: * - the difference is significant compared to the strain *A. faecalis* 8/1

Thus, on the fifth day, the height of the emulsion columns for supernatants of *A. faecalis* strain 8/1 decreased by 7% for diesel, 9% for hexadecane, 5% for hexane, 6% for toluene and 11% for vegetable oil. At the same time for *A. faecalis* M1 these indicators decreased by approximately 4% in all cases. In the next two days there was also a decrease in the height of the columns of emulsions, which was also more pronounced in the case of *A. faecalis* 8/1. On the seventh day, the column height decreased by a total of 13% for diesel, 15% for hexadecane, 9% for hexane, 9% for toluene and 16% for vegetable oil, respectively, in the case of the reference strain. At the same time, for *A. faecalis* M1 from the third to the seventh day, the height of the emulsion columns was generally reduced by only 7–8%.

Conclusions

The obtained results allow us to conclude that the strain of *A. faecalis* M1 isolated from the marine environment has the ability to produce compounds that have the properties of emulsifiers and biosurfactants and to release them into the culture medium. This is indicated by the positive results of the drop test, as well as the significant emulsifying activity of the culture supernatants of this microorganism. From the literature [3, 7] it is known that the fractions of the supernatant, which show emulsifying activity by almost 90% consist of compounds which include 84% of sugars (of the total mass of fractions) and 7% of lipids. Thus, we can assume the glycolipid nature of *A. faecalis* biosurfactants and, as a consequence, their higher viability compared to lipopeptide and polymeric biosurfactants produced by other marine mesophilic microorganisms (for example, the genus *Bacillus*), due to lighter and cheaper production technologies.

Compared with the reference strain *A. faecalis* 8/1, the naturally isolated strain showed a greater ability to synthesize biosurfactants, which may be explained by the significant distribution in nature of difficult-to-access poorly soluble sources of nutrients and / or adaptation of the isolated strain to man-made marine pollution.

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CORRELATION BETWEEN THE LEVEL OF ANTAGONISTIC ACTIVITY AND THE COMPOSITION OF NUTRIENT MEDIA

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Introduction

Antagonistic properties of lactic acid bacteria associated with the production of organic acids, thermolabile and thermostable high and low molecular weight antibacterial substances and antibiotics. Moreover, some substances produced by lactic acid bacteria are characterized by high antagonistic activity even at low concentrations in the medium. This category includes antibiotic substances (lactocil, lactobrevin, nisin, lactobacillin, etc.) [Стоянова, 2012].

Due to the widespread occurrence in nature and the ability to form a wide range of metabolites with valuable properties, according to many microbiologists and biotechnologists, lactic acid bacteria can become a real alternative to substances harmful to the health and life of macroorganisms, such as antibiotics, pesticides, fungicides, preservatives, etc. [Стоянова,2012; Асташкина, 2010].

The research aim was determining the effect of medium composition on the level of antagonistic activity.

Materials and methods

In our study, we used strain *L. vaccinostercus* ONU 2 which was isolated from sea sponges. Determination of antagonistic properties was carried out *in vitro* by a hole-diffuse method [Presti et al., 2015; Servin, 2004; Schillinger, 1989] in relation to opportunistic microorganisms.The following indicator strains were used in the work *Staphylococcus aureus* ATCC 25923*, Salmonella enteric* NCTC 6017*, Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6896*, Klebsiella pneumonia* ATCC 10031*, Pseudomonas aeruginosa* ATCC 27853*, Bacillus subtilis* ATCC 6633.

To order to determine the components of culture media that enhance the antagonistic activity of strains of lactic acid bacteria, we used a number of culture media that were different in composition in quantitative composition (Table 1).

Results

Order to determine the components of culture media that enhance the antagonistic activity of strains of lactic acid bacteria, we used a number of culture media that were different in composition in quantitative composition (Table 2).

When we analyzed the obtained data on the indicators of antagonistic activity, it was shown that only when using medium No. 1, medium No. 4 and medium No. 8, the indicators of antagonistic activity against most opportunistic microorganisms were significant (Tab.2).

Table 1

The composition of the nutrient media used in the design of the experiment (g/l)

	peptone	trypton	MPB	glucose	maltose	lactose	saccharose	Yeast extract	Sodium acetate ($C_2H_3O_2Na$)	Potassium dihydroorthophosphate KH_2PO_4	Potassium hydroorthophosphate K_2HPO_4	Ammonium citrate	Tomato juice (ml)	casein	gelatin	NaCl	Ascorbic acid	cysteine	$(NH4)_2SO4$	tween 80 (ml)	Etanol (ml)
Medium № 1	15,0			20,0				5,0	5,0	5,0		2,0	100,0							1,0	
Medium № 2				5,0		5,0	5,0	5,0	1,5					20,0	2,5	4,0	0,5				
Medium № 3		5,0	5,0	5,0				2,5	3,0					5,0			0,5				
Medium № 4	10,0		2,0	20,0				5,0	5,0		2,0	2,0								1,0	
Medium № 5			5,0	20,0				5,0					100,0	10,0		5,0		0,3			
Medium № 6							7.0	3.0		13.6				3.0					4.0	1,0	
Medium № 7			2,0	20,0				5,0	20,0	0,5	0,5			10,0			0,3			1,0	40,0
Medium № 8		10,0	3,0	4,0	4,0			5,0			2,6	2,0						0,5		1,0	
Medium № 9			5,0	10,0				5,0			2,0			10,0		5,0		0,5		1,0	

Table 2

Indicators of antagonistic activity of the strain *L. vaccinostercus* ONU 2 against culture-pathogenic strains when using different composition media

Indicator strain	Diameter of the zone of lack of growth (mm)								
	Medium № 1	**Medium № 2**	**Medium № 3**	**Medium № 4**	**Medium № 5**	**Medium № 6**	**Medium № 7**	**Medium № 8**	**Medium № 9**
S. enterica NCTC 6017	9	0	0	9	0	0	0	12	1
St.aureus ATCC 25923	1,5	1	1	2	2	0	0	3	1
P. vulgaris ATCC 6896	10	0	0	9	0	0	0	13	1,5
K. pneumoniae ATCC 10031	11	0	1	12	0	1,5	0	15	1,5
E. coli ATCC 25922	11	1	0	11	1	1	1,5	12	10
B. subtilis ATCC 6633	10	0	1	9	0	0	0	9	1,5
P. aeruginosa ATCC 27853	13	1	0	11	0	0	0	15	1,5

That is, we can draw a preliminary conclusion that the indicators of antagonistic activity depended on the composition of the medium.

The one-way analysis of variance according to Kruskal-Wallis confirmed that the composition of the medium affects the level of antagonistic activity. The Kruskal-Wallis H-test is a nonparametric analogue of one-way analysis of variance for comparing independent groups [Медик и др., 2007; Унгуряну идр., 2016]. The obtained calculated values of the Kruskal-Wallis criterion are 31.63 at a significance level of p-value = 0.0001, which indicates the adoption of an alternative hypothesis (Fig. 1).

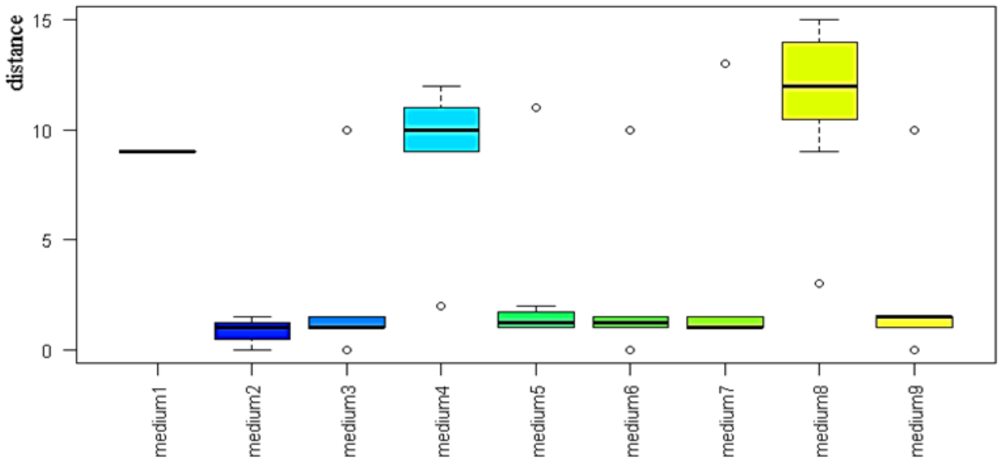

Fig. 1. The results of one-way analysis of variance in terms of antagonistic activity (Kruskal-Wallischi-squared = 31,63, df = 8, p-value = 0,0001)

After analyzing table 1, we excluded from it those components that were encountered in isolated cases and conducted a correlation analysis in order to determine the presence and level of connection between the signs of "antagonistic activity" and "environment component". As a result, the values of the Pearson correlation coefficients (r) between the parameters were calculated, and the concentrations of the components of the nutrient media were also studied. The results are presented in graphical form (Fig. 2).

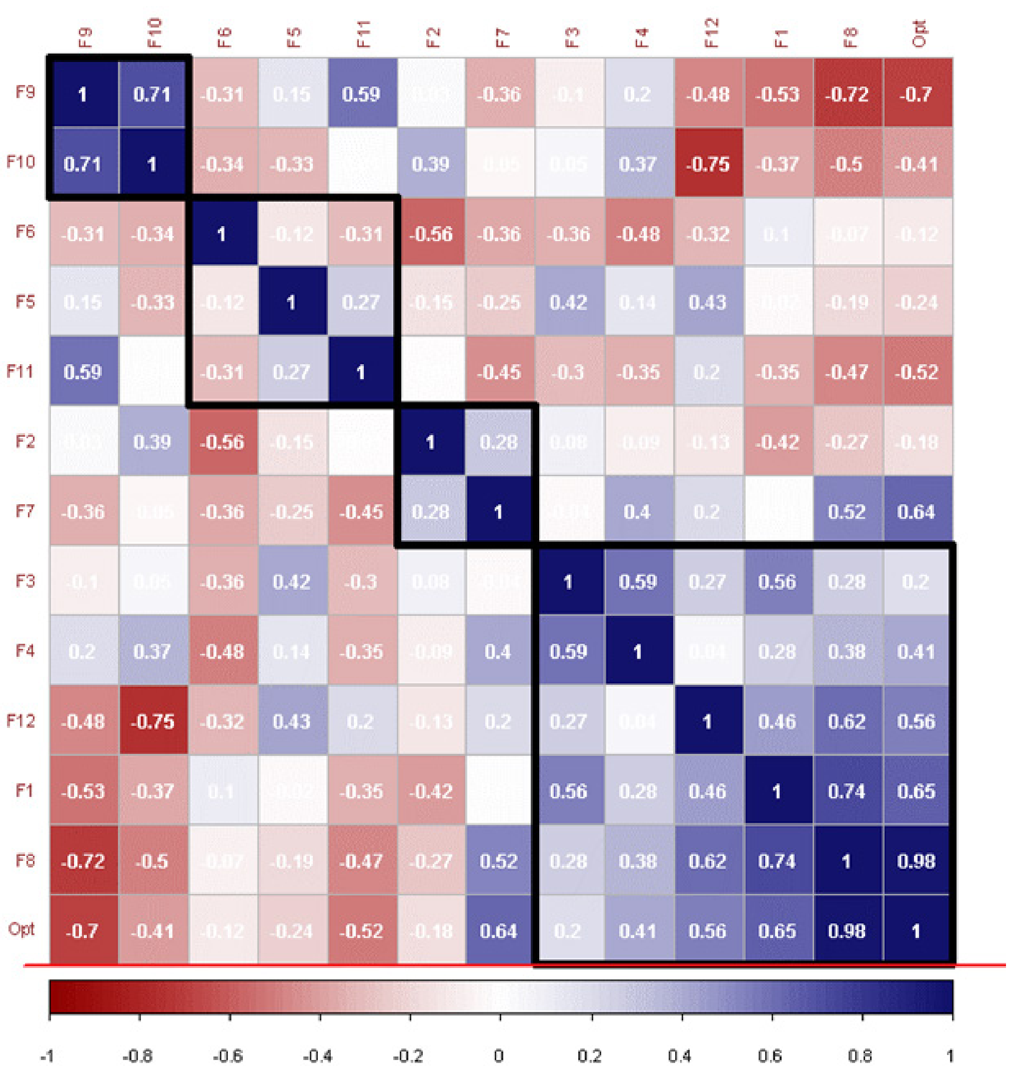

Fig. 2. Pearson correlation coefficients (r) between the antagonistic activity indicators of the strain *L. vaccinostercus ONU* 2 and the composition of the nutrient medium

Designations: F1 - peptone, F2 - MPB, F3 - glucose, F4 – yeast extract, F5 – sodium acetate, F6 – potassium dihydroorthophosphate, F7 – potassium phosphate, F8 – ammonium citrate, F9 - casein, F10 - NaCl, F11 – ascorbic acid, F12 – ethanol and Opt – an indicator of antagonistic activity

Based on the results, we determined that the following factors positively affect the level of antagonistic activity: peptone (r = 0.65), ammonium citrate (r = 0.98), potassium phosphate (r = 0.64), ethanol (r = 0.56), yeast extract (r = 0.41), glucose (r = 0.21), ascorbic acid (r = -0.52) and NaCl (r = -0.41). The obtained Pearson correlation coefficients indicate that in order to increase the level of antagonistic activity, the necessary organic factors that contribute to the rapid increase in cell concentration in the medium, and inorganic components are more necessary for maintaining the achieved concentration, are a source of nitrogen and phosphorus.

Conclusion

As is known, most lactic acid bacteria are known for their antagonistic properties [Бабич и др., 2015; Sharmaet al., 2017]. Therefore, it is very important to fully reveal the potential of the strains that can be used as producers or as the basis of a bacterial preparation against opportunistic bacteria.

During the work, we used the method of one-way analysis of variance (ANOVA) and correlation analysis, which allowed us to determine the components of the media that provided the maximum effect of the antagonistic activity of the strain *L. vaccinostercus* ONU 2. So among those that were "most influential" were peptone, glucose, yeast extract, ammonium citrate, KH_2PO_4 and ethanol.

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EFFICIENCY OF PHENOL-CONTAINING WATER PURIFICATION BY IMMOBILIZED DESTRUCTIVE BACTERIA

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Abstract. *As a result of screening evaluation of phenolic oxidative activity of 4 strains of bacteria isolated from pharmaceutical wastewater, the most biochemically active strain of Bacillus subtilis 13 was selected, which after 6–13 days utilized 78–97 % of phenol (phenol concentration in water decreased from 200 mg / l to 6 ± 2 mg / l). Among the 7 studied carriers of different origins, activated carbon, peat and a synthetic carrier of the «VIIA» type had sorption activity against phenol. Immobilization of Basillus subtilis 13 bacteria on sorbent carriers led to increased efficiency and acceleration of the process of water purification from phenol, while desorption of the pollutant did not occur.*

Key words: *immobilized bacteria-destructors, carriers-sorbents, water purification, phenol*

Introduction

Among the harmful substances that pollute the environment, phenol is an extremely dangerous pollutant due to its high toxicity and widespread distribution. Phenol enters water bodies with industrial wastewater from chemical-pharmaceutical, petrochemical enterprises, as well as fecal effluents from medical institutions [1, 2]. Phenol belongs to the 2nd class of danger of chemical products. The toxicity of phenols and their ability to accumulate in living organisms depends on the length and number of alkyl groups in the nucleus. Phenol and its derivatives have a low threshold of smell and taste in water [3].

An important role in the process of detoxification of phenols in the environment is played by microorganisms whose enzymes break down aromatic rings, thus forming non-toxic compounds that are included in the cycle of elements in the biosphere [4, 5]. An urgent problem is the development of biotechnological methods for the purification of phenol-containing waters using immobilized destructive bacteria [6, 7].

The aim of the work is to evaluate the efficiency of phenol-containing water purification by destructive bacteria immobilized on different sorbent carriers.

The task of the study included:

- to conduct a screening assessment of phenolic oxidative activity of bacterial strains isolated from pharmaceutical wastewater;
- select carriers with sorption activity against phenol;
- to evaluate the efficiency of purification of phenol-containing waters immobilized on different carriers by destructive bacteria.

Materials and methods

The material for the study were strains of microorganisms isolated from wastewater of Ukrainian pharmaceutical production.

To isolate bacteria - phenol destructors, a sample of wastewater in a dilution of 1: 10^3 - 1: 10^9, was sown on agar nutrient medium M-9 of the following composition: Na2HPO4 - 6.78 g / l, KH_2PO4 - 3 g / l, NH_4Cl - 1 g / l , NaCl- 0.5 g / l, which contained as the only source of carbon and energy phenol at a concentration of 50.0 mg / l. Cultivated on Petri dishes for 3 days at a temperature of 300 C.

For screening evaluation of phenolic oxidative activity, bacterial strains were grown in liquid nutrient medium M-9 with the addition of phenol at a concentration of 200 mg / l.

Immobilization of destructive bacteria was performed on sorbent carriers of different origins - zeolite, mussel shells, activated carbon, ceramic tubes, sand, peat and synthetic carrier "Via". Equal volumes (50 ml) of carriers were added to the vials and 50 ml of water containing phenol at a concentration of 200 mg / l was added. To immobilize the bacteria, 50 ml of the daily bacterial culture was added to these vials. Inoculation was performed in an Innova'40 shaker (rotor speed 70 rpm at 28 ºC) for 2 days. To determine the residual concentration of phenol used a photometric method using 4-aminoantipyrine [8].

Results and Discussion

As a result of studies from wastewater from pharmaceutical production, 4 strains were isolated, which gave abundant growth on agar nutrient medium M-9, which contained phenol in the amount of 200.0 mg / l (table 1).

From the presented data it is seen that the most biochemically active destructor of phenol was strain Fs13. When the concentration of bacterial cells in phenol-containing medium M-95.5 × 10^4 CFU / ml, temperature 25 ± 2 ^{0}C, pH = 7.0-7.2 for 3 days of exposure, the residual concentration of phenol decreased to 156 ± 7 mg / l, and on the 6th day - up to 44 ± 5 mg / l, while the degree of destruction of phenol reached 78%.

Table 1

The results of screening evaluation of phenol oxidative activity of bacteria isolated from wastewater from pharmaceutical production

Strain	Residual concentration of phenol, mg/l				
	Exposition, day				
	3	6	8	10	13
Фс1	200±1	162±28	138±8	128±14	112±23
Фс11	200±5	92±10	74±7	58±1	44±6
Фс13	156±7	44±5	6±2	7±1	6±1
Фс15	190±19	130±11	80±17	56±9	28±4

After 8-13 days, the degree of destruction of phenol was maximum - reached 97%, the residual concentration of phenol did not exceed 6 ± 2 - 7 ± 1 mg / l. According to the biological properties of the strain Fs13 was identified as *Bacillus subtilis 13.*

The choice of sorbent carriers for the immobilization of phenol-oxidizing bacteria *B. subtilis* 13 was due to their availability, cheapness, specific surface area. Biologically positive natural and synthetic carriers were used: mussels, horse peat, zeolite, sand, activated carbon, ceramic tubes, synthetic carrier type "VIIA". The results of the evaluation of the sorption capacity of the carriers relative to phenol are presented in table 2.

Table 3 shows that sand, zeolite, mussel shells, ceramic tubes did not adsorb phenol. Peat and a synthetic carrier of the VIIA type had the adsorption capacity for phenol. Activated carbon most efficiently sorbed phenol - the efficiency of the process of extracting phenol from water reached 45%, the residual concentration of phenol in water - 90.0 ± 8.7 mg / l.

Table 2

Sorption of phenol by natural carriers-sorbents

Carrier	The concentration of phenol, mg / l
Zeolite	199,8±1,0
Sand	200,0±1,5
Peat	160,0±15,2
«VIIA »	125,0±10,5
Ceramic tubes	178,0±15,8
Activated carbon	90,0±8,7
Mussel shells	195,0±1,5

Note: M ± m at P <0,05; the initial concentration of phenol in water before processing - 200.0 mg / l; pH 6.8 - 7.2; exposition of 11 days.

However, the sorption capacity of these carriers in relation to phenol is limited, so use them for water purification for a long time is not possible. In this regard, biological modification of sorbent carriers was performed by immobilization of phenol-destroying bacteria *Bacillus subtilis* 13 isolated from the wastewater of the pharmaceutical company.

Immobilization of *B. subtilis* 13 bacteria on the surface of sand, zeolite and mussel shells (which in a sterile state practically do not sorb phenol) led to an increase in the degree of phenol extraction from water to 16–25% during the first day of exposure, to 6 days - up to 27 -39%, and on the 11th day 98–99% of phenol was utilized by bacteria (table 3). 1 day after the start of the experiment, the most effective process of extracting phenol from aqueous solutions was using *B. subtilis* 13 immobilized on a carrier with the largest sorption capacity - activated carbon - the degree of extraction of phenol from water reached 60%, the residual concentration of phenol in water 34.0 ± 2.8 mg / l.

Table 3

Extraction of phenol from water by bacteria *Bacillus subtilis* 13 immobilized on sorbent carriers of different nature

Carriers-sorbents with immobilized bacteria-destructors	Exposition, day		
	1	6	11
	Residual concentration, mg / l		
Zeolite	163,5±12,5	119±12,4	2,0±0,1
Mussel shells	145±12,0	136±11,2	20±1,2
Peat	118±10,5	28±2,5	16±0,8
Activated carbon	34,0±2,8	9,0±0,6	0,001±0,0001
Sand	163,5±15,6	9,0±0,8	0,001±0,0001
Ceramic tubes	173±14,1	100,0±9,7	0,001±0,0001
«VIIA »	113±12,2	6,0±0,5	0,001±0,0001

Note: M ± m at P <0,05; the initial concentration of phenol in water before processing - 200.0 mg / l; pH 6.8 - 7.2.

With increasing exposure to 6 days, the active process of phenol utilization was carried out by immobilization of bacteria *B. subtilis* 13 sand and synthetic carrier type "VIIA" - the degree of biodegradation of phenol was 94 - 95%, while the concentration of phenol in water decreased from 200 mg / l to 6,0 ± 0.5 - 9.0 ± 0.8 mg / l. Immobilized on activated carbon, sand, ceramic tubes, synthetic carrier type "VIIA" bacteria *Bacillus subtilis* 13 after 11 days reduced the concentration of phenol in water to the level of the maximum allowable concentration for water bodies for drinking and cultural use (0.001 mg / l). It is known from the literature that such a high degree of biodegradation of phenol can provide a complex mechanism of interaction of bacteria with this pollutant, which includes a combination of several biochemical reactions and is accompanied by the formation of catechol at the initial stage of phenol biodegradation [6]. The next stage of microbial metabolism is associated with the cleavage of the aromatic ring of diatomic phenol [5]. Created diatomic phenol - subject to intradiol o-cleavage or extradiol m-cleavage of catechol under aerobic conditions. These pathways of aromatic ring cleavage are catalyzed by various dioxygenases [7]. M-pathway ring cleavage catalyzes 2,3-dioxygenase, and o-pathway ring cleavage catalyzes 1,2-dioxygenase [4].

Conclusions

1. As a result of screening evaluation of phenolic oxidative activity of 4 strains of bacteria isolated from pharmaceutical wastewater, the most biochemically active strain of *Bacillus subtilis* 13 was selected, which after 6-13 days utilized 78 - 97% of phenol (phenol concentration in water decreased from 200 mg/l to 6 ± 2 mg/l).
2. Among the 7 studied carriers of different origin, activated carbon, peat and a synthetic carrier of the VIIA type had sorption activity against phenol.

3. Immobilization of cells of the bacillus *Bacillus subtilis* 13 on sorbent carriers led to increased efficiency and acceleration of the process of water purification from phenol, while the desorption of the pollutant did not occur.

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CLONAL MICROPROPAGATION OF *PAULOWNIA TOMENTOSA IN VITRO*

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Abstract. *Paulownia tomentosa is a fast-growing species of wood that has significant economic potential (valuable wood, high biomass production rate, increased resistance to stress, etc.). A tree 15–20 m high, sometimes up to 25 m and a diameter of 0.6 m, sometimes up to 1 m.*

The method of clonal micropropagation is based on the unique ability of plants to regenerate from somatic cells and allows the reproduction of plants with complicated seed or vegetative propagation, to heal the planting material and to increase the rate of its receipt several times. Also, the method of clonal micropropagation allows to renew and stabilize the number of disturbed populations of rare species of plants.

Key words: *Paulownia tomentosa, clonal micropropagation, introduction, nutrient medium*

Clonal micropropagation is an important biotechnological trend that allows the mass reproduction of plants in aseptic culture. This approach is productive for the massive, rapid reproduction of valuable, unique, recruited genotypes or rare, endangered species and varieties for the propagation of plant species or unique plant species for which reproduction in nature as a seed and vegetatively is complicated. The method of clonal micropropagation is based on the induced phytohormones of the extension of the apical and axillary meristems. The essence of the method is to cultivate plants in sterile conditions with controlled parameters of the medium, on artificial nutrient media.

Today there are many different methods of clonal micropropagation. They are based on four principles:

1) activation of the development of plant meristem
2) the formation of an adventitious bud from the tissues of the explant;
3) induction of somatic embryogenesis;
4) differentiation of the adventitious buds in the primary and transversal cullus tissue [Bhojwani, 2013].

Each type of plant requires correction in the classic propagation technique.

Relevance - reproduction with help the method of tissue culture is gaining popularity. For *P. tomentosa* there is no precise mineral composition of the medium, which consistence is the optimal; it has not been determined which medium is the best.

The aim of work was to stude the process of introduction into culture *in vitro P. tomentosa*.

Materials and methods

The work was performed at the department of Microbiology, Virology and Biotechnology of the Odesa I.I. Mechnikov National University. Mechnikov.

For introduction in culture in vitro we take shoots with activated lateral buds of plants. Shoots were taken from a donor plant in February. The material was obtained by cultivating plants in vitro in a nutrient medium of Murashige and Skoog (MS) with addition of 20 g/l of sucrose, 9 g/l of agar, and 1 mg/l of 6-benzylaminopurine (6-BAP). The next step is the growth of initial explants in a media with different consistency (on the solid nutrient media [8.0 g/l] and semi-liquid nutrient media [4.0g/l]). Registration of the explants' survivability, time of the beginning of axillary buds proliferation, and amount of obtained shoots was conducted [Zelenanska, 2009].

Results

The technology of clonal micropropagation of *P.tomentosa*, includes the following main stages [Carmen, 2014]:

1. Selection and sterilization of primary explants.
2. Introduction of explants into culture in vitro (fig, 1,).
3. Rooting and reproduction of microclones on nutrient media (fig,2).
4. Adaptation of plants from in vitro conditions to in vivo conditions.

Fig. 1. Introduction of explants into culture *in vitro*

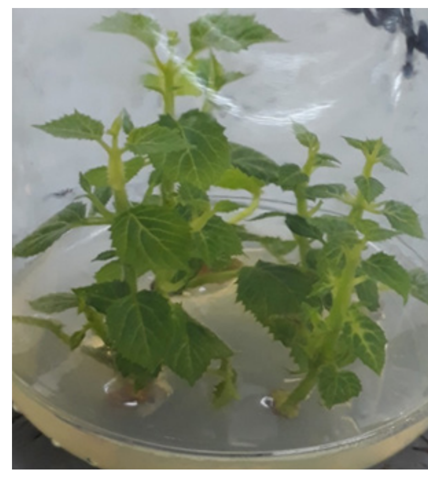

Fig. 2. Rooting and reproduction of microclones on nutrient media

The micropropagation of *P. tomentosa* was carried out through direct morphogenesis, using the shoots with axillary buds, since it is known that the plants regenerated in this way are mostly genetically homogeneous, identical to the parent form.

Semi-liquid nutrient media were used. The advantage of using semi-liquid media in comparison with solid nutrient media is revealed. The search of the optimal nutrient medium for Paulownia tomentosa shoots induction in vitro was successfully done.

Modified semi-liquid MS was determined as the optimal nutrient media. Its application contributed to better survivability, differentiation, and regeneration of *Paulownia tomentosa* shoots (Tabl.1).

Table 1

Average survivability performance of *P.tomentosa* microclones during the introduction process using different consistency nutrient media

Time passed from the planting, days	Consistency nutrient media	Type of explants	Average survivability of the microclones, %
3th	MS(solid)	shoots	90
		Shoots*	100
	MS(semil-iquid)	shoots	80
		Shoots*	100
6th	MS(solid)	shoots	40
		Shoots*	70
	MS(semi-liquid)	shoots	60
		Shoots*	90
10th	MS(solid)	shoots	0
		Shoots*	50
	MS(semi-liquid)	shoots	60
		Shoots*	60

shoots* - plant donor is seedlings obtained by microclone method

Total vitality on MS (solid) is 40%, total vitality on MS(semi-liquid) is 60%. On MS(solid) there was a proliferation of buds for 6 days. On MS(semiliquid) there was only swelling of the buds.

Conclusions

1. *P. tomentosa* is a great choice for greening the cities.
2. The stage of introduction into the culture in the wind and adaptation of the plants grown in vitro to the environment are some of the most problematic stages.
3. On solid nutrient media, the percentage of liveliness is less than that of on semiliquid nutrient media.
4. Proliferation on the solid nutrient media occurred earlier.
5. Use material from the plants obtained by in vitro gave better results.

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UDC 62_

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SPECIAL FEATURES OF THE USE OF TELEMEDICINE TECHNOLOGIES IN DERMATOLOGY

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Abstract. *The article focuses on the application of telemedicine technologies in dermatology. The usage of telemedicine approach makes it possible to study certain skin pathologies remotely and monitor the progress of the treatment in the dynamics. The use of equipment and telemedicine services for the study of skin diseases is explained. The dermatoscopic telemedicine system is described. The possibilities of using mobile telemedicine in dermatology are analyzed.*

Keywords: *telemedicine, image processing, dermatology, mobile dermatology, dermatoscopy*

Introduction

Due to the Covid-19 epidemic, telemedicine technology is gaining widespread acceptance. In fact, the relevance of screening and teleconsultation in isolation is unusually high. Such technologies can be applied in various fields of medicine, for example, when testing fine motor skills [1, 2], in otorhinolaryngology [3-5], in the analysis of data of radiation research methods [6-10]. With the advent of this approach and appropriate technologies, dermatologists can expand access to health care by reducing the time spent on traditional outpatient care. Dermatological telemedicine services offer reduced waiting time, increased flexibility of the schedule and sufficient patient satisfaction.

It is known that at the moment telemedicine is suitable for realization of remote consultations and monitoring the dynamics of dermatological diseases such as acne, pigment lesions, atopic dermatitis, skin tumours [11, 12]. Of course, the effectiveness of the traditional interaction with the doctor remains effective, but in the context of self-isolation, technology helps to ensure that diagnostic information is recorded and analyzed at a distance and that quality counselling is provided [13, 14].

It is therefore advisable to develop a telemedicine system for video dermatoscopy and to analyze its components.

Materials and Methods

Such a system should contain a digital video dermatoscope, which allows you to record diagnostic images with adjustable optical magnification from 10 to 200 times and a resolution of at least 5 Megapixels with a matrix size of at least 1 / 2.5 inches to provide an acceptable dynamic range, as well as a integrated lighting unit. The device shall be capable of recording digital images on a memory card

and transmitting them by means telemedicine services for analysis. The inventive method consists in producing images in formats (for example, TIFF), which are devoid of specific artifacts from compression of images resulting in distortion of diagnostic information. The specialist should have specialized software for storing and processing the obtained diagnostic images, taking into account the analysis of the color components of the areas of interest specific to specific pathologies [15, 16]. At the same time, the processing of recorded dermatoscopic images and the application of already existing approaches to their analysis are of primary importance [17, 18]. For example, in the case of atopic dermatitis, it is relevant in dynamics to observe changes in skin color during treatment [17]. Given that dermatoscopic images are recorded by the patient at home, remote monitoring of the conditions for obtaining diagnostic images and the validity of the method and the repeatability of the measurement results shall be mandatory [19, 20]. The received data should be transmitted through the channels of communication between the patient and the specialist to ensure qualified teleconsultations.

It is also advisable to develop specialized certified equipment and dedicated communication channels for the rapid and safe transmission of not only diagnostic images, but also essential medical information (data of anamnesis, sanitary and hygienic characteristics of workplaces, etc.), which allows a highly qualified specialist to monitor the treatment of patient with dermatological diseases with the help of telemedical consultations. These systems have become particularly relevant in the context of the COVID-19 pandemic, forced quarantine and self-isolation, when it is not recommended to visit diagnostic centers for routine treatment. The possibilities of mobile teledermatology may reduce time and financial costs in the process of monitoring a number of chronic skin diseases.

Conclusions

In the present circumstances, the benefits of using telemedicine services for primary diagnosis and control of treatment of certain dermatological diseases are obvious. The development of the approach is the development of intelligent methods of analysis of dermatoscopic images, which, with additional a priori information, can improve the effectiveness of detection of certain skin pathologies and control the treatment process remotely. The possibilities of mobile teledermatology make it possible to reduce time and financial costs in the process of monitoring the treatment of certain chronic skin diseases.

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ROTAVIRUS DISTRIBUTION IN MEDICAL WASTEWATER AND SEAWATER OF THE BLACK SEA COAST IN ODESA

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Abstract. *A comparative analysis of a rotavirus detection rate in medical wastewater, taken from the collectors of infectious unit in hospitals and seawater of the Black Sea Coast in Odesa, showed an increase in 1.25 times the rotavirus antigen detection rate in the wastewater than in the seawater.*

Keywords: *rotaviruses, wastewater, seawater, morbidity on rotavirus intestinal infections*

Introduction

One of the most problematic nosological forms in the structure of acute intestinal infections is rotavirus infection, in the distribution of which a water factor plays an important factor [1-8].

Rotaviruses infect almost every child during first 3-5 years of life, and it is the most common cause of severe dehydrating diarrhea in children under the age of 5[9]. The urgency of the problem of viral acute intestinal infections, in particular, rotavirus, is due to their prevalence, high morbidity, and significant socio-economic costs. According to the WHO estimation, around 453000 fatal cases associated with rotavirus gastroenteritis are reported among children worldwide.

The mortality rate due to rotavirus infection is 86 per 100000 cases among children under the age of 5 years. About 90% of all deaths associated with rotavirus infection are observed in low-income countries, like in Africa and Asia, due to medical care lack. National mortality rates for this reason range from 474/100 000 (Afghanistan) to less than 1/100 000 (63 countries); In 4 countries (Afghanistan, Burundi, Somalia and Chad) the mortality rate exceeds 300/100000 [10].

In Ukraine, rotavirus infection accounts for 35-75% of all cases of acute intestinal infections [1]. Rotavirus surveillance epidemiological data are important at the state, regional and global levels; it contributes to the decision on the appropriateness of a rotavirus vaccine inclusion (RVI) to the national vaccination calendar and are also used to confirm the impact of such a vaccine after its introduction.

Since 2008, a The Global Rotavirus Laboratory Network (GRLN) has been established, which included seven member states of the WHO European Region: Azerbaijan, Armenia, Georgia, Republic of Moldova, Tajikistan, Uzbekistan and Ukraine.

Such a burden of illness has necessitated vaccination. Wide dissemination of intestinal infections of viral etiology, including rotavirus infection, and their high mortality determine the need for continuous epidemiological surveillance of this group of infections, for a search for optimal preventive services [10].

The aim of the current research was to study the rotavirus detection rate in medical sewage and seawater of the Black Sea Coast in the city of Odesa in 2015 to 2017.

Materials and methods

The material for the study was 1033 sewage samples (unclean), selected from the collectors of infectious units of hospitals, sewage treatment facilities and 604 samples of seawater of the Odesa Black Sea in 2015 to 2017.

Enzyme-linked immuno sorbent assay and molecular genetic research methods were used. Enzyme-linked immuno sorbent assay (ELISA) was used to detect the rotaviruses on the diagnostic systems of "Rotavirus - antigen - ELISA - Best" and "Vector-Best".

In order to detect an RNA rotavirus, a molecular genetic method (polymerase chain reaction) was used on the “Ampli-Sens” test systems. The concentration of viruses in water samples was carried out by adsorption method using hydroxyl methyl silicic acid.

Results and discussion

A total of 704 wastewater tests and 505 seawater samples were tested by ELISA method for the presence of rotavirus in the period from 2015 to 2017. Were detected 25 positive wastewater samples, which equal to almost 4% and 16 positive seawater samples, which was 3.2%. 65 samples of wastewater and 96 samples of seawater were investigated by PCR method. 17 (2.6%) positive results were found in the wastewater study and 10 (10.5%) positive results in the study of seawater.

The rotavirus detection rate of the wastewater of infectious hospitals reached its maximum in 2016.

Information on the disease burden received in the framework of surveillance for rotavirus infection, contributed to the decision to include the monovalent rota vaccine to national vaccine calendars in 5 of the 7 countries GRLN participating. In the absence of financial support in Ukraine, rota vaccine has not been introduced.

In the virology laboratory of Odesa a retest and genotype of rotaviruses from patients were conducted during 2017. The following genotypes were identified: G1, Q1P8, Q4P8, Q2P4, Q9P4, Q3P8, Q9P8, Q2P8, and the Q9P4 genotype, which was first determined in 2017 in patients in the Odesa region.

Conclusion

The results of our research indicate that in the city of Odesa the epidemiological situation of rotavirus infection remains tense; therefore, it is necessary to carry out preventive services aimed at improving the sanitary and municipality services of the city of Odesa, ensuring strict compliance with the sanitary norms of water supply and a sewerage, as well as an improvement of disinfection measures at the treatment facilities of the infectious disease hospitals, biological wastewater treatment plants, and in the enterprises for the food production and sale.

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PROPERTIES OF ANTARCTIC ISOLATES THAT GROW ON WASTE WATERS

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Aim: *to characterize morphological, cultural physiological and biochemical properties of Antarctic isolates that are able to grow on wastewaters.* **Materials and Methods**. *In the investigation the wastewater from a distillery and infiltrates of the Lviv solid waste landfill were used. Isolates of microorganisms were isolated from samples obtained during the Ukrainian Antarctic expedition (2019). Morphological (shape, cell size, Gram stain), cultural (growth characteristics in liquid and solid Tryptic Soy agar), physiological and biochemical activities (catalase, oxidase, amylolytic, lipolytic, proteolytic, cellulase activities, ability to use different carbon and nitrogen sources) were studied. The resistance of isolates to* $CoCl_2 \times 6H_2O$ *(0.17 mM) or* $FeSO_4 \times 7H_2O$ *(0.54 mM), or* $MnCl_2 \times 4H_2O$ *(0.18 mM), or* $K_2Cr_2O_7$ *(0.96 mM), or* $CdCl_2 \times 2.5H_2O$ *(0.9 µ) or* $CuCl_2 \times 2H_2O$ *(1.57 mM) was studied.* **Results.** *Among the obtained isolates, 12 grew on the corresponding wastewater at a dilution of 1:5. Isolates 88A_103, 79A_103 were the most metabolically active. Three isolates are resistant to all investigated metal ions – 88A_103, 95A_109 and 95A_110.* **Conclusions.** *Isolates from different Antarctic substrates, that grow in wastewater of different origins, are characterized by metabolic diversity and belong to different groups. Most of them are adapted to life in a wide temperature range, are resistant to Co (II), Fe (II), Mn (II), Cd (II), so the study of their properties can be the basis for the development of various biotechnologies, including bioremediation of wastewater.*

Key worlds: *Antarctic microorganisms, waste waters, metabolic activity, psychrotrophs*

The Antarctic microbiota is characterized by unique properties such as multiresistance to antibiotics, heavy metal ions, UV radiation and many other toxic compounds (e.g. phenols). Antarctic microorganisms are able to synthesize cold shock proteins, antifreeze-nucleating proteins, unsaturated fatty acids. They possess systems of counteraction to osmotic and oxidative stress and the ability to biofilm formation. These adaptations ensure survival in the extreme conditions of this habitat [8, 14, 16, 21]. Among these properties, in particular, almost the main – the formation of enzymes that are active at low temperatures ("cold enzymes") and that are by 10 times more active than mesophilic enzymes at this conditions, easily inactivated by increasing temperature [6; 8, 12]. Under conditions of lack of nutrients, microorganisms are able to form enzymes that break down complex organic compounds [20]. Some bacteria, fungi, algae, isolated from Antarctic habitats, are able to break down aliphatic and aromatic hydrocarbons [8]. Isolated microorganisms from Antarctic oasis located near Ukrainian Antarctic station Academic Vernadsky

were resistant to a wide range of concentrations of extreme factors (UV radiation, toxic metals (Hg^{2+},Cu^{2+}, Cr(VI), Co^{2+}, Cd^{2+}, Ni^{2+})), including bactericidal [18].

Infiltrates of the Lviv solid waste landfill are characterized by a large variety of organic and inorganic pollutants, the content of which is 2–6240 times higher than the maximum permissible concentrations (MPC) of these substances [3]. Distillery waste waters have a significant organic loading (reduced sugars, proteins, organic acids and polysaccharides) [13], contain a large amount of phenols, heavy metal ions, ammonium, phosphates, sulfates [5, 17]. The content of heavy metals, according to various sources, exceeds the MPC for water by 2–65 times, and phenols – 4200–10000 times [5, 9].

The unique properties of Antarctic microorganisms, metabolic plasticity, as well as multiresistance to various toxic compounds give grounds for finding strains that can potentially be used for bioremediation of wastewater and will be economically and environmentally beneficial for use in biotechnology.

The aim of the study was to investigate the cultural, morphological, physiological and biochemical properties of the isolates (from Antarctic substrates) that grow on waste water of distillery and infiltrates of the Lviv solid waste landfill for further selection of the most biochemically active.

Materials and Methods

Microorganisms were isolated from samples obtained during the Ukrainian Antarctic Expedition (February-April 2019) by Dr. Pavlo Khoetski. The material for the study was 22 samples of soil, moss, mossy soil, *Deschampsia antarctica* (№ 1 Ant 2019, № 2 A 2019, № 3 2019, 4 A 2019, 5 A 2019, 6 A 2019, 8 A 2019, 9.9 A 2019, 10 A 2019, 11 A 2019, 12 A 2019, 14 A 2019, 17 A 2019, 64 A 2019, 67 A 2019, 68 A 2019, 75 A 2019, 79 A 2019, 88 A 2019, 89 A 2019, 95 A 2019, 96 A 2019), selected from: Booth Island; Cape Tuxen, Antarctic Peninsula; Darboux Island; Barselot Island; Yalour Island; Rasmussen Point, Antarctic Peninsula; Roca Islands; Skua Island; Weller Island; Galindez Island; Reservoir on Galindez Island; Pitterman Island; Irizar Island; Forge Islands. To calculate the number of colony-forming unitis (CFU) and for isolates obtaining, the suspensions of each samples were sown on Tryptic Soy agar (TSA), agarized waste water of distillery and infiltrates of the Lviv solid waste landfill (dilution of 1:10) and grown during 10 days at temperature +20 °C. Isolates that grew in wastewater at a dilution of 1:10 were sown into wastewater at a dilution of 1:5. Isolates that formed characteristic colonies on selective media (Chromocult *Listeria* agar OTTAVIANI and AGOSTI (Merk, USA), ENDO agar (Merk, USA), Kligler's agar (Merk, USA), bile-esculin agar (Merk, USA), Baird-Parker Agar (Oxoid, UK), Brilliance *Bacillus cereus* agar (Oxoid, UK)) were not used in the work.

Morphological properties of isolates (Gram staining, cell shape, size) determined using a Carl Zeiss Axio Lab.A1 binocular microscope. The motility of isolates was determined in the TSA column after inoculation by injection [1]. As a positive control were used *Proteus vulgaris*, and as a negative – *Staphylococcus albus*. The need for O_2 of the isolates was determined by seeding in a thioglycollate medium (Merk, USA). The ability of isolates to grow at different temperatures was determined after 5 days of cultivation on TSA at temperatures +4, +20, +29 and +37 °C.

The catalase activity of the isolates was determined by addition a drop of 10% solution of H_2O_2 to the cell suspension [1]. Oxidase activity was determined using strips with N,N-dimethyl-p-phenylenediamine oxalate and α-naphthol (Millipore, USA). The ability of isolates to form ammonia and hydrogen sulfide during metabolism of sulfur containing amino acids was determined by the change in color of the indicator papers (litmus and lead acetate) during the cultivation of microorganisms in TSB (Tryptic Soy Broth) with 0.01 % of cysteine [1]. As test cultures were used: *Proteus vulgaris* – for positive control, *Escherichia coli* – for negative. The ability of isolates to fix molecular N_2 was determined after 5 days of cultivation on Ashby medium. The ability of isolates to reduce nitrates was determined after their growth in a medium with 0.2% sodium nitrate (g/l: glucose – 20.0; K_2HPO_4 – 1.0; $MgSO_4 \times H_2O$ – 0.5, NaCl – 0.5; distilled water – 1 l) and Bubble Durham tube. Determination of nitrites was revealed by a qualitative reaction of azo compound formation with Griss reagent. The formation of N_2 is indicated by the accumulation of gas in the Bubble Durham tube [1]. Amylolytic activity was determined by the ability of isolates to grow on starch-ammonia agar. The degree of hydrolysis of starch was determined after 5 days of growth by applying Lugol's reagent to the surface of the medium. The formation of transparent zones around the colonies indicates complete hydrolysis of starch. Lipolytic activity was determined by the formation of an opaque zone of calcium salts of fatty acids around colonies of microorganisms in the medium of this composition (g/l): peptone – 10.0; NaCl – 5.0; $CaCl_2 \times 6H_2O$ – 0.1; agar – 20.0; distilled water – 1 l, pH 7.4. After sterilization, an aqueous solution of tween-40 (polyoxyethylene sorbitan monopalmitate) was added to the medium to the final concentration 10g/l. The proteolytic activity of the isolates was determined by the ability to hydrolyze gelatin after 5 days of cultivation in TSB with gelatin. Cellulase activity was determined by the ability to grow in Hutchinson's medium.

The ability of microorganisms to assimilate organic carbon sources was determined by growth on Hiss medium with arabinose, glucose, dulcite, inosit, xylose, lactose, maltose, mannose, rhamnose, sucrose, sorbit and by changing the color of the medium.

The resistance of isolates to heavy metal ions was investigated by growing on TSA medium with the addition of $CoCl_2 \times 6H_2O$ (0.17 mM) or $FeSO_4 \times 7H_2O$ (0.54 mM), or $MnCl_2 \times 4H_2O$ (0.18 mM), or $K_2Cr_2O_7$ (0.96 mM), or $CdCl_2 \times 2{,}5H_2O$ (0.9 μ) or $CuCl_2 \times 2H_2O$ (1.57 mM). These concentrations of heavy metal ions exceed the MPC for water by 100 times [10].

All research results are presented as average with mean error (M±m). The reliability of the obtained results was calculated using the Student's *t*-test in Microsoft Excel 2003 [2].

Results and Discussion

The number of cultivated microorganisms in 22 samples of moss, soil, mossy soil, lichens, *Deschampsia antarctica*, benthos, which were selected during the Ukrainian Antarctic expeditions in 2019, was analyzed. The largest number of CFU/g of the sample ($4.6 \times 10^7 \pm 6.9 \times 10^6$) was found in the sample 9.9 A 2019 (soil, moss, mushrooms selected from Rasmussen Point, Antarctic Peninsula), in samples

№3 2019 (lichen from Cape Tuxen, Antarctic Peninsula), 5 A 2019 (soil, *D. antarctica*, moss from Barselot Island), 6 A 2019 (soil, *D. antarctica*, moss from Barselot Island), 12 A 2019 (mossy soil from Weller Island) the number of CFU/g of the sample was within $1.1–2.6×10^6$.

To obtain isolates promising for bioremediation of wastewater, suspensions were sown on agarized wastewaters. In samples 8 A 2019, 10 A 2019, 11 A 2019, 68 A 2019, 75 A 2019, 88 A 2019, 89 A 2019, 95 A 2019, 96 A 2019, the number of microorganisms growing on wastewater did not differ from the number of microorganisms on TSA. The number of CFU in samples 6 A 2019, 12 A 2019, 67 A 2019 on wastewaters was by 2–3 times lower.

From investigated samples, 20 isolates that grow on distillery wastewater and 18 that grow on infiltrates of the Lviv solid waste landfill in the dilution of 1:10 were obtained. These isolates retained the ability to grow in the studied wastewater after repeated resown. The physiological and biochemical properties of 12 isolates (4A_104, 8A_103, 10A_106, 14A_102, 14A_103, 88A_103, 79A_103, 95A_109, 95A_110, 96A_108 – isolated from the wastewater of the distillery; 9.9A_105, 17A_103 – from infiltrates of the Lviv solid waste landfill), that grew on the relevant wastewater in dilution of 1:5, were investigated.

From cold habitats mainly isolate gram-negative bacteria belonging to Alpha-, Beta-, Gammaproteobacteria, and the phylum *Cytophaga-Flavobacterium-Bacteroides.* Among the isolated gram-positive bacteria predominate are species of genus *Arthrobacter* and *Micrococcus* [4, 6]. In the works of Barrientos-Díaz L. [4], Duarte A.W.F. [7], in addition to bacteria, the isolation of microscopic fungi from Antarctic habitats is mentioned. Microscopic fungi from these habitats are representatives of phyla *Ascomycota*, *Basidiomycota*, *Zygomycota*, *Chytridiomycota*, and *Glomeromycota* [15]. Mainly pigmented yeasts, that are isolated from Antarctic substrates, belong to phylum *Basidiomycota* (predominant genera *Cystofilobasidium*, *Dioszegia*, *Sporobolomyces*, *Collophora*, *Cryptococcus* and *Rhodotorula*) [7].

Among the isolates obtained by us are those that form pigmented colonies of different shape and consistency. Isolate 8A_103 forms large milk viscous colonies, 14A_103 – beige large viscous colonies, 4A_104, 14A_102, 88A_103, 95A_109, 95A_110, 96A_108 – beige viscous colonies of medium size, 9.9A_105, 79A_103 – beige small colonies. Isolate 10A_106 forms large orange colonies, and isolate 17A_103 – fine-grained pink colonies. According to Gram, they are stained positively (4A_104, 10A_106, 95A_110, 96A_108) or negatively (95A_109, 79A_103, 88A_103, 8A_103, 14A_102, 14A_103).

Cells of all isolates are rod-shaped, except 17A_103 and 9.9A_105 – that are microscopic fungi.

Isolates 88A_103 та 79A_103 are able to use urea, reduce nitrates to nitrites, and amino acids are metabolized with the release of hydrogen sulfide and ammonia. Among all isolates that reduce nitrates, none performed complete reduction to molecular nitrogen.

The most intensive process of nitrate reduction is carried out by isolates 14A_102, 88A_103, 79A_103 and 9.9A_105.

The isolated microorganisms are psychrotrophs, as they are able to grow at low temperatures, but grow better at temperatures higher than +20 °C, which is not

typical for psychrophiles [6]. Isolates 96A_108 and 14A_103 are able to grow in a wide range of temperatures, but the optimal for growth is +20 °C (table 1).

Isolates 10A_106, 88A_103, 79A_103, 17A_103 – mesophilic microorganisms. The obtained data indicate that microorganisms from Antarctic substrates are well adapted to life in a wide temperature range.

Table 1

Physiological and biochemical properties of Antarctic isolates

Property	Isolate											
	96A_108	**10A_106**	**8A_103**	**4A_104**	**14A_102**	**14A_103**	**88A_103**	**95A_109**	**95A_110**	**79A_103**	**17A_103**	**9.9A_105**
Ammonia formation	+	+	+	+	+	+	+	+	+	+	–	+
H_2S formation	–	–	–	+	–	–	+	–	–	+	–	–
Usage of urea	+	–	+	–	–	+	+	–	–	+	–	–
NO_3^- reduction	+	+	+	–	+	+	+	+	–	+	–	+
N_2 fixation	+	+	+	+	–	–	+	+	+	+	+	+
Usage of:												
Arabinose	+	+	+	+	+	+A	+	+	+	+	+	+
Glucose	+A	+	+	+A	+	+	+A	+	+A	+	+	+
Dulcite	+	+	+	+	–	+	+	+	+	+	+	+
Inosit	+	+	+	+	+	+	+	+	+	+	–	+
Xylose	+A	+	+A	+	+	+	+	+A	+A	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	–
Maltose	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+A	+	+	+	+	+	+	+	+	–	+
Rhamnose	+	+	+	+	–	+	+	+	+	+	–	+
Sucrose	+	+A	+	+	+	+	–	+	+	+	–	+
Sorbit	+	+	+	+	+	+	+	+	+	+	+	+
t, °C	4–37	20–37	4–29	4–29	4–29	4–37	20–29	4–29	4–29	20–29	20	4–29

Note: A – acid production.

All isolates are aerobic, have catalase activity, oxidase activity is not detected in isolates 17A_103 and 9.9A_105. The most diverse enzymatic activities possess isolates 95A_109, 95A_110 and 79A_103 (table 2). Cellulase activity was detected only in the isolate 17A_103. Among the yeasts isolated from Antarctic substrates, extracellular cellulases form *Leuconeurospora* sp., *Cryptococcus* sp., *Dioszegia* sp.and *Mrakia* spp. [21].

Table 2

Enzymatic activity of Antarctic isolates

Isolate	Catalase activity	Oxidase activity	Amilase activity	Lipase activity	Cellulase activity	Proteolitic activity (gelatin hydrolysis)
96A_108	+	+	+	+	–	–
10A_106	+	+	–	+	–	–
8A_103	+	+	+	+	–	–
4A_104	+	+	–	+	–	–
14A_102	+	+	–	–	–	–
14A_103	+	+	–	–	–	+
88A_103	+	+	+	+	–	–
95A_109	+	+	+	+	–	+
95A_110	+	+	+	+	–	+
79A_103	+	+	+	+	–	+
17A_103	+	–	–	+	+	–
9.9A_105	+	–	–	+	–	–

Because isolates grow on agarized wastewater and are characterized by different enzymatic activities, they are likely to use toxic compounds contained in these waters. Multiresistance to heavy metals is one of the features of Antarctic isolates. Therefore, as one of the first steps in the study of resistance of isolates to heavy metal ions, we sow them on a medium containing $CoCl_2 \times 6H_2O$ (0.17 mM) or $FeSO_4 \times 7H_2O$ (0.54 mM), or $MnCl_2 \times 4H_2O$ (0.18 mM), or $K_2Cr_2O_7$ (0.96 mM), or $CdCl_2 \times 2{,}5H_2O$ (0.9 μM) or $CuCl_2 \times 2H_2O$ (1.57 mM). The ion content of each of the metals was by 100 times higher than the MPC [10]. All studied isolates are resistant to 0.18 mM of Mn^{2+}. Three isolates are resistant to all studied metal ions – 88A_103, 95A_109 and 95A_110. Microorganisms that have been isolated from Antarctic habitats are resistant to much higher concentrations of heavy metal ions than we studied. In particular, in the study of G. Kan [11] minimum inhibitory concentrations for *Rhodotorula mucilaginosa* AN5 were defined as 50 mM of Hg (minimum) and 1000 mM of Cd (maximum); other investigated heavy metal ions were within these limits according to the scheme Cd>Pb=Mn>Cu>Cr>Hg. Tashyrev O. et al. isolated from Antarctic substrates microorganisms resistant to 100–1000 ppm of Cr(VI), Cu^{2+}, Cd^{2+} and 10–50 ppm of Hg^{2+} [19].

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Conclusions

Microbiological research in Antarctica is of great importance both in the field of basic biological knowledge and for biotechnology. Isolates from different Antarctic substrates, that grow in wastewater of different origins, are characterized by

metabolic diversity and belong to different groups. Microorganisms from Antarctic substrates are well adapted to life in a wide temperature range. Because the isolates are able to grow in wastewater, and are characterized by a combination of different enzymatic activities, resistance to pollutants, including heavy metal compounds, the study of their properties can be the basis for the development of various biotechnologies, in particular, bioremediation of wastewater.

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ANTIBACTERIAL PROPERTIES OF CHITOSAN ELECTROSPUN MEMBRANES TREATED WITH ALKALI NEUTRALIZATION METHOD

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Abstract. *Chitosan is biodegradable and biocompatible polymer, which possesses antimicrobial potential. Objective of the study was to assess the antimicrobial activity of electrospun chitosan nanofibrous membranes with dichloromethane(DMC) and trifluoroacetic acid (TFA) in different ratios (7:3 and 9:1) as solvents.1M sodium hydroxide (NaOH) was used for fiber cross-linking. Scanning electron microscopy (SEM) applied to provide fiber characterization. The antibacterial properties of different membranes formulations against Staphylococcus aureus and E. coli was studied.TheTFA elevation in solution improved the antibacterial properties whereas higher proportions of DCM stabilizedthe morphology of the electrospunnanofibers. Overall, chitosan-TFA/DCM electrospunmembranes retained morphology of nanofibers and antimicrobial effects against both microorganisms depending onrelation of solvents and are applicable as an antimicrobial remedy.*

Key words: *chitosan, electrospinning, antimicrobial properties, alkali treatment*

Introduction

Diverse natural and syntetic polymer solutions, or a mixture of these polymers are used inelectrospinning – one of the most effective technique for nanofibrous membranes manufacturing [1].

Chitosan (Ch), deacetylated derivative of chitin, is anamino based polysaccharide with vital biological properties such as non-toxicity, biocompatibility, biodegradability and antibacterial effect [2, 3].The amino groups on chitosan provide its cationic nature and potential antimicrobial properties[4]. It is provedthat chitosaninterrelate with the negatively charged bacterial cell wall and cause membrane perforation leading to leakage of intracellularcomponents [5].

The electrospinningprocess is highly controllable via managing solution properties and processing conditions that allows to produce nanofibers with diameters ranging from few tens of nanometers to micrometers [6, 7].However, obtaining pure chitosan nanofibers by electrospinning is problematic mainly due to the very rigid structure and high viscosity of chitosan chains [8]. Dichloromethane (DMC) and trifluoroacetic acid (TFA)as spinnable polymers have been blended to facilitate nanofiber formation [7]. Addition of solvent system to chitosan solution enlarged the charged density of the polymer blend that lead to stronger stretch forces on the ejected fiber jet and decrease the diameter of nanofibers [9].

Dissolution of chitosan in TFA with/without DCM as the modifying cosolvent result in damage of the fibrous structure of nanofibersand high solubility in aqueous media ofNH3+CF3COO–salt residues formed as a result. Neutralization with a NaOH aqueous solution resolves this issues but preserves fibrous structureincompletely[10].

Despite various researches on the antibacterial efficiency of electrospun chitosan nanofibers, impact of solvents utilizing and neutralization methods on morphology and properties of fibers are still require clarification.

The aim of this study was to manufacture electrospunnanofibrous membranes from chitosan with different TFA:DCM solvents system ratios and investigate their structure after alkali treatment, and assess antimicrobial properties against Gram-positive and Gram-negative bacteria.

Materials and Methods

Chemicals and polymers

Chitosan powder was bought from Glentham Life Sciences (Corsham,Unighted Kingdom), Dichloromethane (DCM) andTrifluoroacetic acid (TFA) –from Honeywell Fluka(Loughborough,Wiltshire,Unighted Kingdom). NaOH was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Membrane preparation

Chitosan solution was prepared by dissolving of Ch powder in TFA/DCM solution (Table 1). Components were mixed under magnetic stirring overnight at room temperature.

Table 1

Parameters of Ch-TFA/DCM solutions

Parameters	*Solution 1*	*Solution 2*
MW, kDa	890	
DD, %	≥90	
Viscosity, cP (centipoise)	100-300	
Polymerconcentration, %	3.5	
Solventsratio TFA:DCM	7:3	9:1

Electrospinning system (LinariEngi-neerings.r.l., Italy) was used to produce membranes from the prepared solutions. The electrospinning processing conditions are listed in Table 2.

Neutralization treatments of chitosan nanofiber membranes

Ch-TFA/DCM membranes were neutralized in alkali solution – 1M NaOH (70% ethanol/30% water solution) in 24-well plastic plate for 24 h with repeatedly washingwith distilled water and drying for 1 day at room temperature.

Table 2

Electrospinning parameters of Ch-TFA/DCM solutions

Processing Parameters	
Flowrate, mL/h	5.0
Voltage, kV	30–35
Tip-collector distance, cm	15
Volume of the syringe, mL	10
Inner diameter of the needle, mm	0.69
Rotating speed, rpm	800-1000
Temperature, °C	21–24
Relative humidity, %	<35

Electron Microscopy (SEM)

The fiber morphology, diameter, and homogeneity of the produced fiber mats were evaluated using a scanning electron microscope (FEI Inspect S50B, Brno, Czech Republic). Average fiber diameter was determined by measuring 100 unique nanofibers from the SEM images, for each chitosan membrane. Membranes were previously dried in a desiccator with silica for 24 hours, and then coated with a thincarbon layer using the vacuum set up VUP-5M (SELMI, Sumy, Ukraine). The average diameter of the electrospunnanofibers was measured from SEM images by using ImageJ 1.50c software (https://imagej.nih.gov/ij/).

Antimicrobial properties assessment

Gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria from the laboratory of microbiology (Center for the collective use of scientific equipment, Medical Institute of Sumy State University) were used as model bacteria in this study. The samples were cut to a size of 5×5 mm and sterilized underUV-lamp for 24 hours. Then, samples were immersed into sterile 24-well plastic plate with 2 mL of bacterial suspension in concentration of 10^5 CFU/ml for 2, 4, 6 and 8 h. After incubation with nanofibrousmembranes, 20 µL of medium was taken from the wells at all time points of experiment and inoculated on Mueller-Hinton's agar plates. The plates were incubated in a 37°C for 24 hours. Then, the colonies were counted and the results were verified in CFU/ml. The controls were wells with microbial growth without influence of chitosan membrane (positive control) and wells contained sterile, microbe-free nutrient broth medium (negative control). The average values of triplicated controls and samples were calculated.

After final time point of cultivation membranes were prepared for SEM study. The samples were washed in phosphate buffer saline (PBS; pH=7.4) to remove the protein excess. Then, membranes were fixed in 2,5 % glutaraldehyde (0.1M PBS) twice for 40 min, followed by PBS-washing (2 times for 15 min). After that, the samples were dehydrated by immersing into alcohols of rising concentrations (50°, 70°, 90° and 96°) for 30 min at each concentration and left into 96° spirit overnight. After alcohol aspiration the samples were dried overnight at room temperature.

Results and Discussion

Well-structured nanofibers with very few beads were formed in both cases Solution 1 and Solution 2 (Figure 1). Samples had few beads indicating that the tested chitosan electrospinning conditions provide sufficient chain entanglement for fiber formation. The addition of dichloromethane (DCM) to the chitosan-TFA solution improves the homogeneity of the electrospun chitosan fibers without interconnected fibrous networks [10].

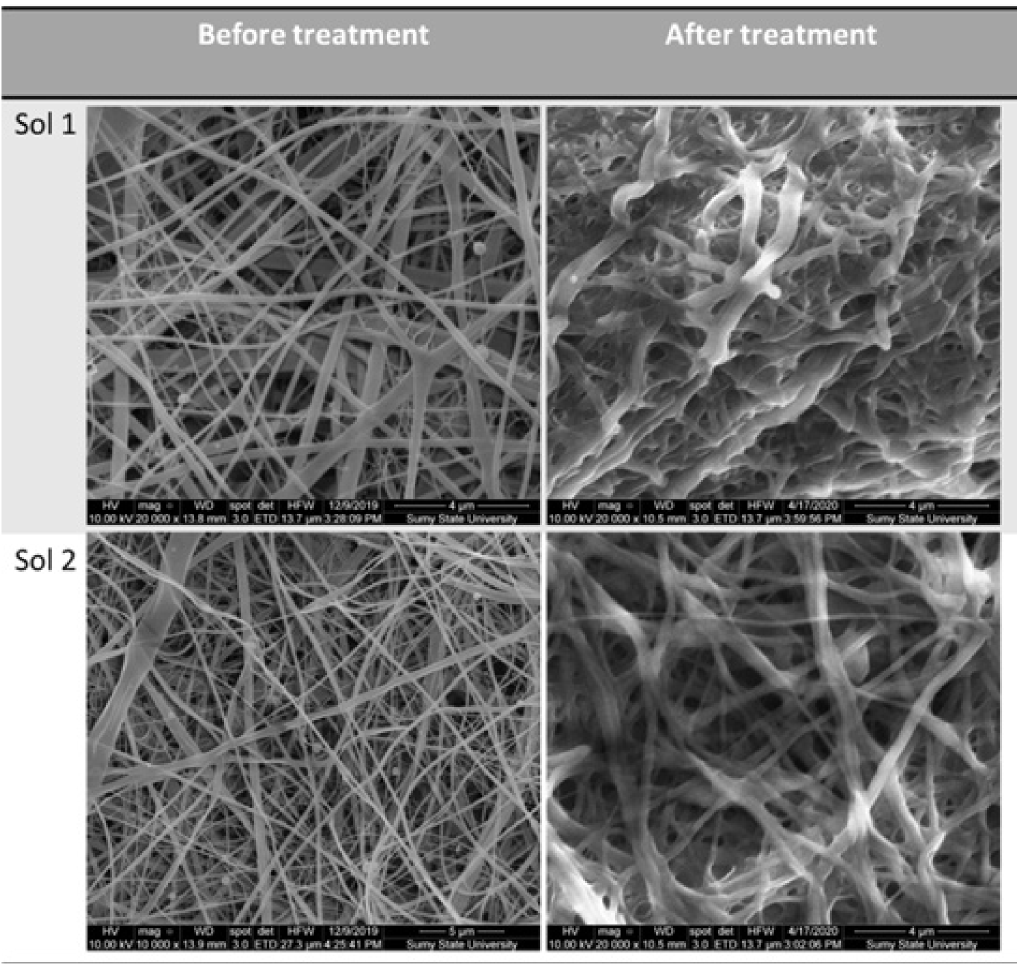

Figure 1. Scanning Electron Micrographs ofelectrospunCh-TFA/DCM nanofibrous membranes (Solution 1 and Solution 2)

Fiber size distribution was observed with increasing TFA content in the solution (Table 3) before treatment [7].

To obtain stable chitosan nanofibers membranes samples were immersed in NaOH alkali solution for 24h. The chitosan nanofiber membranes are becoming insoluble in aqueous medium under the –NH2 form. Figure 1 shows the SEM images of membranes before and after immersion in neutralizing media. SEM images analyses confirmed the stability of Solution 2 membrane structures after treatment with ethanol-water (70/30).

After treatment with NaOH chitosan membranes fabricated from Solution 1 increased in the size of thenanofibers by swelling with change of fibrous morphology.

The diameter was increased by around 13% for Solution 2 and 35% for Solution 1 compared with the initial size (Table 3) [11].

Table 3

Distribution fiber diameter before and after treatment with 1M NaOH solution (ethanol)

Samples	*Average diameter before treatment, µm*	*Average diameter after treatment, µm*
Solution 1 (TFA:DCM=7:3)	0.18±0.009	0.28±0.11
Solution 2 (TFA:DCM=9:1)	0.2±0.01	0.23±0.1

After co-cultivationof microorganisms with nanofibrous mats it should be noted that membranes could inhibit the growth of both *S. aureus* and *E. coli* (Figure 2), however, Solution 2 showed stronger antibacterial effect.

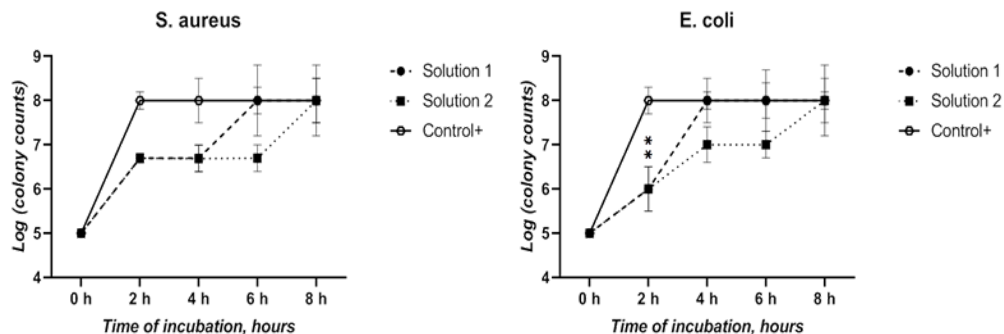

Figure 2. Antibacterial effectivenessof chitosan membranes fabricated from Solution 1 and Solution 2 against *S. aureus* and *E. coli*, CFU/mL

It is clear that formulations of Solution 2 could reduce the amount of *S. aureus* more efficiently related to Solution 1, such that the number of colonies reached the same in control samples only after 8 hours of experiment. The results indicate that Ch-TFA/DCM samples fabricated of Solution 2 preventthe microbial growth of *E. coli* after 2, 4 and 6 h incubation and only after 2 h created of Solution 1. The size of the pores in the electrospunCh-TFA/DCM membranes is smaller than the size of the microorganisms that prevent the bacterial penetration and intensity of the microbial growth rate. Another mechanism could be clarified by the fact that NH3+groups on chitosan bond with negatively charged bacteria and inhibit their penetration [12].

The formulations containing higher amount of TFA shown stronger antimicrobial effects, attributed to chitosan. With respect to both microorganisms, this

formulation reduced the microbial growth at the early time points of incubation, but was not not reduce it after 8 hours of experiment (Figure 3).

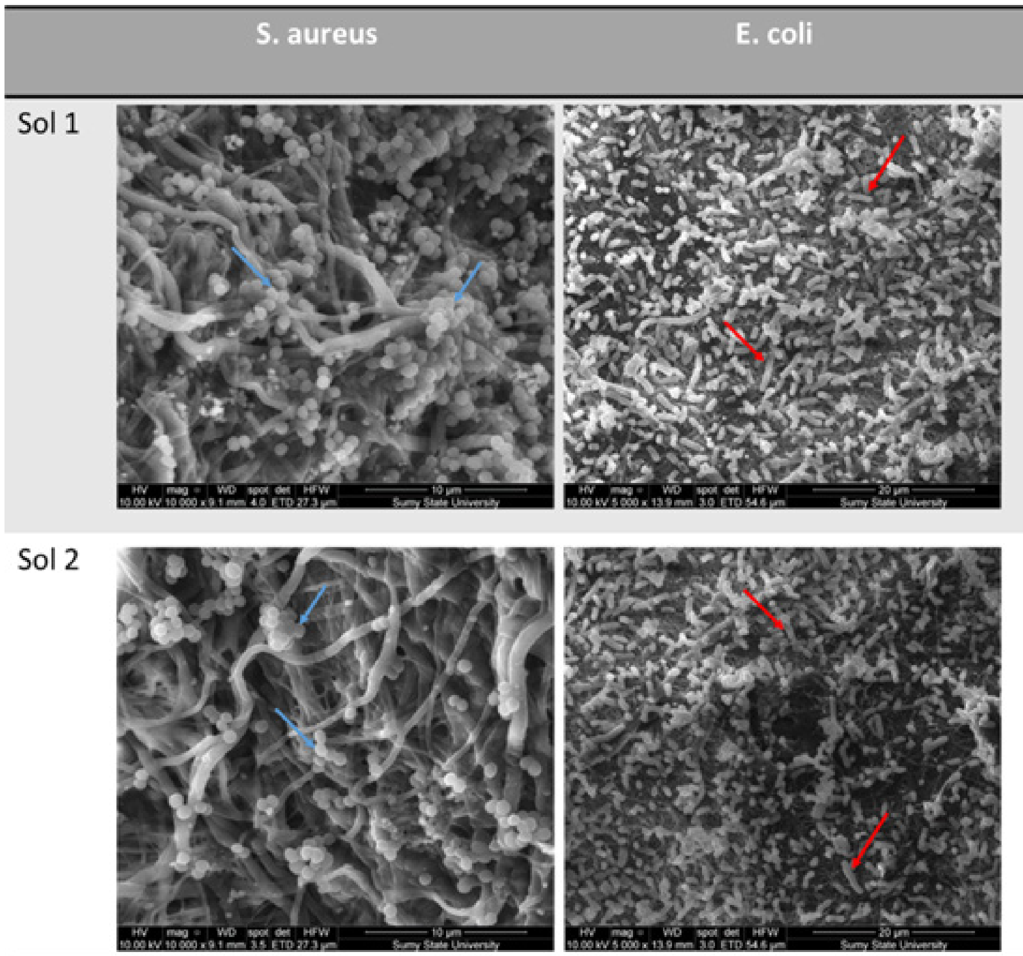

Figure 3. Electrospun Ch-TFA/DCM nanofibrous membranes (Solution 1 and Solution 2) after 8 h cultivation in bacterial suspension. Red arrows indicate *E. coli,* blue arrows – *S. aureus*

It could be explained by the time-depending rate of chitosan degradation and oligomers release from nanofibers that allows to implement the antimicrobial effect of chitosan through are shift of cell permeability, cell membranedestruction, and penetration of its oligomers into the cell leading to inhibition of transcription [13].

Conclusions

The results revealed that blending chitosan with various ratio of TFA/DCM and neutralization with 1M NaOH solution caused thickening of the electrospun-nanofibers in the range of average diameter from 0.23±0.1µm to 0.28±0.11µm. The bead formation was observed at high concentrations of TFA in solvent system TFA/DCM. NaOH alkali treatment used to achieve insoluble in aqueous medium chitosan nanofibers membranes led to increasing of the fibers diameter that can be

caused by itsswelling. The reduction rate ofbacteriawas greater for nanofibers with TFA/DCM 9:1solvent.

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UDC 579.6

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ATTACHMENT OF *LACTOBACILLUS PLANTARUM* ONU 311 TO HUMAN BUCCAL EPITHELIOCYTES

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Abstract. *Lactobacillus plantarum ONU 311 exhibited high level of attachment to human buccal epitheliocytes: mean index of attachment reached 11,25 ± 1,2, and index of adhesiveness – 12,5 ± 2,3 bacteria per epitheliocyte. The studied strain could be recommended for use in probiotics production.*

Key words: *Lactobacillus plantarum, epitheliocytes, attachment*

Introduction

Attachment to host epitheliocytes is necessary for the successful colonization of intestinal tract. Probiotic microorganisms should be tested on adherence ability before their introduction into biological preparation production. If microorganisms do not settle, attach and survive inside the host organism, they would be only the representatives of transient microbiota.

Lactobacilli are well known for their high attachment activity [Bron et al., 2012; Anderson et al., 2010].

Adhesion of lactobacilli to epitheliocytes depends on surface polysaccharides, teichoic acids and S-layer proteins [Kos, 2003; Lorca, 2002; Mobili, 2010].

Our investigations was **aimed** on the study of the attachment of lactobacilli from the strain *Lactobacillus plantarum* ONU 311, initially isolated from grape must, to human buccal epitheliocytes.

Materials and Methods

Lactobacilli were cultivated on solid MRS (deMan, Rogosa, Sharpe) medium [deMan et al., 1960] overnight, and bacterial suspension in PBS buffer with concentration 10^8 cells/ml was brought to the experiment.

Buccal epitheliocytes were gathered and prepared for the experiment according to [Sobel et al., 1981].

Investigations were carried out in four independent experiments, and buccal epitheliocytes were taken from four persons. Mean index of attachment and index of adhesiveness of bacteria per epitheliocyte were calculated [Livinska et al., 2012].

Results

Bacteria *L. plantarum* ONU 311 initially isolated from grape must were able to attach to human buccal epitheliocytes (Fig. 1).

According to previous investigations, bacteria from this strain demonstrated high attachment level to plant surfaces – roots, stems, leaves and seed shells

[Limanska et al., 2019]. This ability was predictable because of the initial habitat of *L. plantarum* ONU 311. But the ubiquitous nature of this species [Siezen et al., 2010; Aleksandrzak-Piekarczyk et al., 2019; Ołdak et al., 2019] allowed us to hypothesize that lactobacilli would also attach to animal cells. The results of our experiments proved this suggestion.

Thus, the mean index of attachment (mean amount of bacteria that attached to one epitheliocyte) and index of adhesiveness (mean amount of bacteria that attached to one epitheliocyte participating in adhesion process) for the studied bacteria were sufficient high to recommend the strain for use in probiotics production (Table 1).

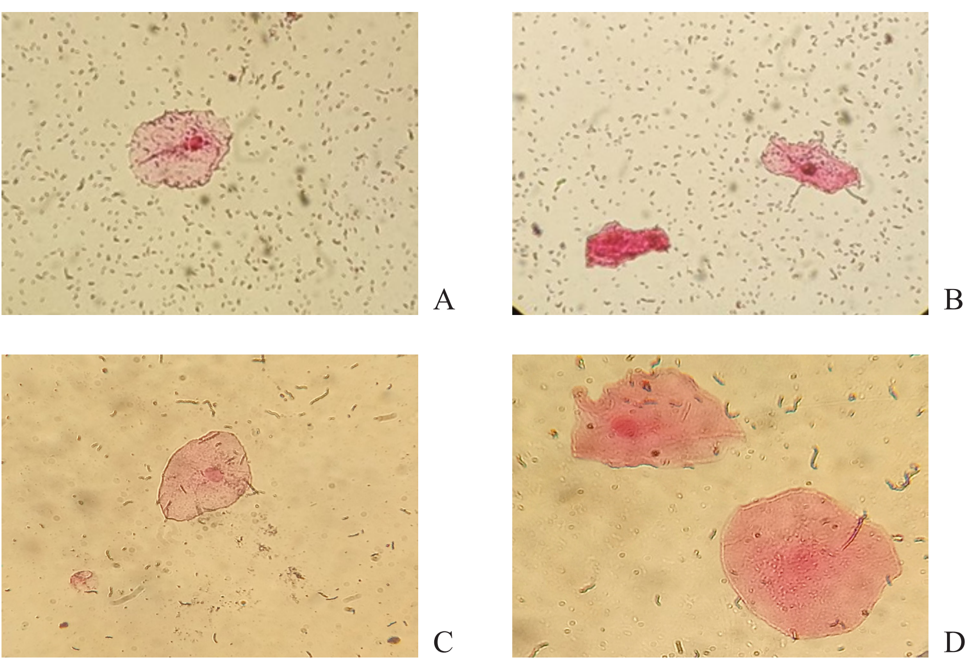

Fig. 1. Epitheliocytes with attached *L. plantarum* ONU 311 cells (A, B, C - 600x; D – 800x)

According to literature data, mean index of attachment > 10 is a characteristic of the strains with high adhesiveness activity [Livinska et al., 2012].

Table 1

Attachment of *L. plantarum* ONU 311 to buccal epitheliocytes

Mean index of attachment	Index of adhesiveness
11,25 ± 1,2	12,5 ± 2,3

We could make a **conclusion** that *L. plantarum* ONU 311 is a strain with high attachment ability, and it is perspective for probiotics production, because bacteria of this strain would actively attach to epitheliocytes and colonize host intestinal tract.

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THE CHARACTERISTICS OF PHENAZINE PIGMENTS THAT PRODUCED BY SOME *PSEUDOMONAS* STRAINS

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Abstract. *At present time phenazine compounds produced by Pseudomonas bacteria are applied in many industries. These substances are used in the biotechnology, including agricultural production. The aim of this work was to study the effect of the nutrient composition on the intensity of accumulation of phenazine compounds by pseudomonad cultures. The dependence of the intensity of accumulation of phenazine antibiotics on the composition of the nutrient medium, in particularly the presence of metal cations in it was detected. It can be assumed that the phenazine derivative synthesis is strain specific process. It was also noted that the intensity of the production of these compounds did not correspond to the rate of cell biomass accumulation by the studied Pseudomonas strains.*

Keywords: *phenazine compounds, Pseudomonas strains, microbial cell biomass, nutrient medium composition.*

Introduction

Phenazines are 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. 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. Natural and synthetic phenazines are of significant interest because of their potential impact on bacterial interactions and biotechnological processes [Beifuss, 2015]. From a biotechnological perspective, the interest in phenazines is due largely to their physicochemical properties, including their oxidation–reduction (redox) properties and their bright pigmentation and ability to change color with pH and redox state [Bloemberg, 2010].

In nature, phenazins are formed in cells whose separation has been discontinued, that is, these compounds do not play the role of energy source or reserve nutrients [Davies, 2012]. Bacteria are the only known source of natural phenazines [Beifuss, 2015]. These secondary metabolites have been studied intensively because of their broad antibiotic properties and roles in virulence. Many phenazine-producing bacteria are commonly found associated with host organisms.

The Gram-negative *Pseudomonas* bacteria was known as the first and for several years also considered as the only microbial genera to produce phenazine pigments [Schoonbeek, 2012]. But phenazines are produced by a wide variety of *Eubacteria* including both Gram-negative and Gram-positive species. Phenazine producers include *Nocardia*, *Sorangium*, *Brevibacterium*, *Burkholderia*, *Erwinia*,

Pantoea agglomerans, *Vibrio*, *Pelagiobacter* and members of the *Actinomycetes*, especially *Streptomyces*. Additionally, *Methanosarcina*, a member of the *Archaea*, was shown to contain a phenazine derivative. New phenazine producers continue to be identified, such as *Brevibacterium* sp. KMD 003 isolated from a marine purple sponge [Kobayashi, 2009].

The *Pseudomonas* genus is characterized by ability to synthesize more than 300 different antimicrobial substances. The most common antibiotics are isolated *Pseudomonas* are phenazine [Sorensen, 2008]. Phenazines isolated from *Pseudomonas* strains (e.g., *aeruginosa, aureofaciens, fluorescens* and *cepacia*) are mostly simple hydroxyl- and carboxyl-substituted structures [Parsons, 2016].

The most studied phenazine is pyocyanin (5-N-methyl-1-hydroxyphenazine), which is blue when oxidized. It is produced by *Pseudomonas aeruginosa*, a common soil inhabitant and opportunistic human pathogen. Pyocyanin was isolated originally from patient wounds and subsequently demonstrated to be associated with infections caused by *P. aeruginosa* [Maddula, 2012].

Phenazines produced by fluorescent pseudomonads also are studied extensively for their application in plant disease management.

Phenazine-1-carboxylic acid, also known as tubermycin B14 because of its antibiotic activity against *Mycobacterium tuberculosis*, and phenazine-1,6-dicarboxylic acid are believed to be metabolic precursors for other phenazines [Kobayashi, 2009]. For example, chlororaphine is a 1:1 complex of phenazine-1-carboxamide and its 5,10-dihydro-derivative; iodinin is 1,6-dihydroxyphenazine-5,10-dioxide. Other phenazines produced by pseudomonads include aeruginosins A and B, isolated from *P. aeruginosa* and *P. iodina*, and the dimeric structure, di(2-hydroxy-1-phenazinyl)methane, isolated from a pigmentation mutant of *P. chlororaphis* [Pearson, 2013].

Tubermycin B and chlororaphine efficiently inhibited growth of *Bacillus cereus* but showed only modest antibiotic activity against *Micrococcus luteus* and *Staphylococcus aureus*. Iodinin exhibited great antibiotic activity against Gram-positive bacteria, several actinomycetes and some fungi but none against Gram-negative bacteria. 1,6-Dihydroxyphenazine exhibited modest activity against Gram-positive bacteria and actinomycetes but was not active against Gram-negative strains or fungi. Pyocyanin is characterized by broad-spectrum bactericidal effects and is believed to protect the pyocyanin producing organism against competing microbes [Laursen, 2014].

It is increasingly evident that bacteria produce multiple phenazine derivatives. Bioinformatic comparisons of the phenazine biosynthetic genes among several bacteria demonstrate a high degree of conservation of five genes. These are considered the «core» genes as each is required for the synthesis of the basic three-ringed phenazine structure. Recent evidence suggests that these «core» biosynthetic genes moved among diverse bacterial genera via horizontal transmission. In most phenazine-producing bacteria, the «core» biosynthetic genes are flanked by one or more accessory genes that encode different terminal-modifying enzymes that result in the production of additional phenazine derivatives [Sorensen, 2008].

For example, *Pseudomonas chlororaphis* 30-84, a root-associated beneficial bacterium, produces three phenazines: phenazine-1-carboxylic acid, 2-hydroxy-phenazine-1-carboxylic acid and 2-hydroxy-phenazine [Turner, 2014].

This *Pseudomonas* species is unique in that it contains phzO, a gene that encodes a monooxygenase, located immediately downstream of the «core» genes. The presence of phzO converts a small amount (~ 10%) of the yellow phenazine-1-carboxylic acid into the bright orange 2-hydroxy-phenazine-1-carboxylic acid. Additionally, a third minor derivative, 2-hydroxy-phenazine, is generated spontaneously from 2-hydroxy-phenazine-1-carboxylic acid. *P. chlororaphis* PCL1391 and *P. aeruginosa* PAO1 contain phzH, a putative transamidase that converts a portion of phenazine-1-carboxylic acid into phenazine-1-carboxamide, enabling these strains to produce both phenazine-1-carboxylic acid and phenazine-1-carboxamide [Laursen, 2014].

Two additional genes in *P. aeruginosa* PAO1, phzM, a methyltransferase, and phzS, a flavin-containing monooxygenase, together are responsible for the conversion of phenazine-1-carboxylic acid to pyocyanin. PhzS alone can facilitate conversion of phenazine-1-carboxylic acid to 1-hydroxy-phenazine [Davies, 2012].

Monitoring of gene expression at the transcription level is the main mechanism for modeling the production of secondary metabolites. The work of the genes responsible for the synthesis of phenazines is regulated by a complex of general regulatory systems of secondary metabolism [Kobayashi, 2009].

The aim of this work was to study the effect of the nutrient composition on the intensity of accumulation of phenazine compounds by pseudomonad cultures.

Material and methods

In this work, strains *P. chlororaphis* (ONU 304, ONU 305, ONU 306), *P. fluorescens* ONU 303, *P. aeruginosa* (ATCC 15692, ATCC 27853, ATCC 10145) were used. Preliminary cultivation of bacteria was carried out on the MPA for 24 hrs, after which the bacterial biomass was transferred to the following nutrient media: Hiss liquid medium; MPB, enriched with 5 % glucose and King medium without adding agar-agar. The initial cell concentration was 5 10^7 CFU / ml. Microorganisms were grown for 7 days at a temperature of 25 °C and 37 °C, taking into account the physiological characteristics of the strains. The optical density of the suspension was measured every 24 hrs using a «µQuant» BioTek spectrophotometer at 540 nm wavelength. Extraction of the phenazine group antibiotics produced by the studied pseudomonad strains was carried out according to the scheme given in [Levitch, 1964].

Each experiment was conducted twice the number of examples in each of them was 4. Statistical analysis of the results was carried out using the Exel-2010 program.

Results

The study showed that over time there was a gradual increase in biomass of the cultures. The maximum growth level for most strains was noted at 5th day, after which the cell biomass amount remained unchanged. This, evidently, indicates that the studied pseudomonads reached the stationary development phase. When comparing the composition of the nutrient medium with the intensity of biomass accumulation, it was noted that the largest number of cells for almost all studied strains was observed in the liquid variant of the King medium. The optical density in the

case of *P. aeruginosa* cultures exceeded values in 10 and 5 times for *P. fluorescens* and *P. aureofaciens*, respectively.

During the experiment, it was noted that it was in the liquid variant of King medium that the most intense color change occurred. This fact was an indicator of the intensive formation and accumulation of metabolites of microorganisms in this environment. It is known that during the stationary development phase microorganisms start to produce secondary metabolites, which also include phenazine compounds [Davies, 2012]. These components are intended to help microorganisms in the struggle for resources at a high density of culture biomass and are allocated in order to reduce the competitive pressure from other representatives of the strains.

The next investigation of the concentration of phenazine compounds in the culture fluid showed that their most active producers were *P. aeruginosa* ATCC 15692 and *P. fluorescens* ONU 303. The content of the corresponding culture metabolites can be arranged as follows: *P. aeruginosa* ATCC 15692 > *P. fluorescens* ONU 303 > *P. chlororaphis* ONU 305 > *P. chlororaphis* ONU 304 > *P. aeruginosa* ATCC 10145> *P. aeruginosa* ATCC 27853> *P. chlororaphis* ONU 306.

Conclusions

Thus, as a result, the dependence of the intensity of accumulation of phenazine antibiotics on the composition of the nutrient medium, in particularly the presence of metal cations in it was detected. They play an essential role in initiating the process of microbial phenazine synthesis as well as in stabilizing the corresponding compounds. In addition, it can be assumed that the phenazine derivative synthesis is strain specific process. It was also noted that the intensity of the production of these compounds did not correspond to the rate of cell biomass accumulation by the studied *Pseudomonas* strains which indicates a lack of direct correlation between these two characteristics.

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CENTRAL HEMODYNAMICS AND WAVE STRUCTURE OF THE HEART RATE OF STROKE BLOOD VOLUME, THEIR SYNCHRONIZATION WITH THE HEART RATE IN MEN AND WOMEN UNDER DIFFERENT CONDITIONS

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Abstract. *Measurements of heart rate wave structure were measured in 41 healthy young men and 32 women at rest lying down, with orthoprobe and psychoemotional load. In 32 women, registration was performed three times - in the folliculin, ovulatory and luteal phases of the ovarian-menstrual cycle. The values of heart rate variability in men and women at rest differed significantly due to the greater overall power of the spectrum of R-R interval fluctuations in men. During the transition of the body to an upright position and psycho-emotional load, the decrease in total spectral power in women was greater than in men, and power reactivity and its distribution in the low heart rate range differed significantly in both direction and amplitude. It was found that under all conditions, except for regulated respiration of 6 cycles per minute, the maximum maximum spectral power of the oscillations of the stroke volume and the duration of the R-R interval were lower in women. Particularly large differences were recorded in orthoprobic and psychoemotional stress. In women, both at rest and at different loads, lower blood pressure, cardiac output than in men due to smaller body size, overall variability in heart rate and stroke. The level of functioning of spontaneous sensitivity to the barereflex in women to loads is lower than in men.*

Key words: *heart rate variability, stroke volume, baroreflex sensitivity, blood pressure, conditions*

Introduction. Functional state of blood circulation according to the concept of R.M. Baevsky (2003) [1] and V.M. Pokrovsky (2002-2012) [11] is considered as a universal indicator of the adaptive capacity of the organism. As informativeness of reserve possibilities of an organism use quality of an estimation of reactivity of system hemodynamics. The end result of the reaction of the cardiovascular system to various stimuli is a completely normal blood supply to the functional systems of the body, which is maintained by the optimal value of blood pressure [4].

Individual differences in the neurohumoral regulation of the heart and blood vessels affect the hemodynamic structure of blood pressure [9]. However, some authors indicate the variability of systemic hemodynamics under different conditions [2, 3].

Analysis of blood pressure variability and R-R intervals can be used to quantify changes in autonomic nervous system function and predict adverse clinical events. It is known that age and sex have a profound effect on the state of the cardiovascular system. Studies of the reactivity of systemic hemodynamics, which are in the literature in most cases, concern the elderly and senile [12]. In this case, age, sex and evolutionary processes in different periods of postnatal ontogenesis can affect hemodynamics associated with morphological and functional changes in adolescence.

Materials and methods

The study involved 41 men and 32 women aged 18-23 years. The study was conducted in compliance with the basic bioethical provisions of the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Helsinki Declaration of the World Medical Association on the ethical principles of scientific medical research with human participation (1994-2008). Each woman was tested three times: in the folliculin (I), ovulatory (II), and luteal (III) phases of the ovarian-menstrual cycle (OMC). Determination of the phases of the cycle was performed by anamnesis, measurement of basal temperature and using a set of jet tests for ovulation "Solo" (IND Diagnostic, Inc., Canada).

In the morning (from 8 to 11 o'clock) after a 15-minute rest, 5-minute recordings of the electrocardiogram and differentiated impedance rheogram from the RA-5-01 rheoanalyzer (Kyiv Research Institute of Radio Measuring Equipment) were performed. Similar recordings were performed during orthoprobe (5 minutes) and psychoemotional stress (10 minutes).

Statistical and spectral analysis of cardiointervalograms was performed in the program "CASPICO" (a/s of Ukraine №11262). The power of the R-R oscillation spectrum in the standard frequency ranges was calculated: 0-0.04 Hz (VLF); 0.04-0.15 Hz (LF); 0.15-0.4 Hz (HF); 0.003-0.4 Hz (TP); normalized power - in the range of 0.15-0.4 Hz (HFnorm) [13].

To elucidate the detailed features of the wave structure in the LF range, median spectrograms with a step of 0.01 Hz were constructed [5]. Psycho-emotional load was modeled in the conditions of 10-minute neurodynamic testing in the feedback mode according to the method of M.V. Makarenko.

Due to the fact that most of the indicators were distributed abnormally, the values of the median, the boundaries of the upper and lower quartiles were calculated. The probability of differences was determined by the Wilcoxon pairwise comparison criterion.

Results

Blood pressure is the leading physiological constant that provides stable blood flow and reflects the pumping function of the heart. Gender differences in some indicators of central hemodynamics under different conditions were noted. The following was found: in men under different conditions, the level of systolic blood pressure (SBP) and diastolic blood pressure (DBP) significantly exceeds the corresponding values in women. The value of the average arterial pressure (AAP) at rest was 76.6 ± 0.7 mm Hg (compared with men - 91.7 ± 0.6 mm Hg), and with

psycho-emotional load 76.1 ± 0 , 6 mm Hg (compared with men 96.3 ± 0.8 mm Hg). Gender differences were also observed in the values of (OV) at rest while lying down and under psycho-emotional stress. Thus, in women at rest, the value of blood supply to the thoracic cavity at rest was 22.74 ± 0.50 shock units, and in men 24.05 ± 0.52 shock units Under conditions of psycho-emotional load 20.5 ± 0.5 shock units. and 22.4 ± 0.4 shock units. This indicates a lower level of blood supply to the thoracic organs.

The mean values of the minute volume of blood (MVB) in men under all conditions were higher than in women. However, no differences were observed in cardiac index (CI) at rest and in regulated breathing and psycho-emotional load. In the orthoprobe in women, this figure was lower than in men (respectively 2019 ± 45.1 ml · $min^{-1}m^{-2}$ and 2302 ± 270.9 ml ($min^{-1}m^{-2}$, $p < 0.05$).

Thus, studies have shown gender differences in the levels of central hemodynamics, which is manifested in lower levels of blood pressure in women and cardiac output mainly due to differences in body size in all conditions, lower blood supply to the thoracic cavity.

The variability of stroke volume is a characteristic of central hemodynamics, which may indicate its functional state [6].

Table 1 presents the levels of the wave structure of the variability of the stroke volume of blood in men and women at rest lying down and in orthoprobe.

Table 1

Characteristics of the wave structure of the variability of the stroke volume of blood in men and women at rest lying down and during orthoprobe

Indicators	**Terms**			
	Calm lying down		**Orthoprobe**	
	men	**women**	**men**	**women**
VLF^{sv}, ml^2	5 [3;10,2]	6,6 [3,4;13,3]	4,2 [2,1;8,1]	2,5 ** [1,5;5,1]
LF^{sv}, ml^2	7 [4,5;12,5]	5,5 * [2,8;7,3]	4,6 [2,8;8,1]	3,0 * [1,8;4,9]
HF^{sv}, ml^2	27,7 [19,3;46,3]	21,1 * [12,2;36,9]	12,2 [7,2;19,2]	8,2 ** [4,5;12,9]
aLF^{sv}, ml^2/Hr	0,9 [0,5;1,7]	1,5 * [0,7;3,3]	0,6 [0,3;1,19]	1,3 ** [0,6;2,3]
$HFnorm^{sv}$, %	79,4 [70;88,5]	79,9 [70,6;88,9]	72,4 [63,3;80]	73,5 [63,2;81,4]
TP^{sv}, mc^2	45,3 [29,9;69,2]	35,4 * [24,3;53,7]	21,8 [15,5;34,1]	14,8 *** [9,9;22,6]

Notes: * - $p<0,05$; ** - $p<0,01$; *** - $p<0,001$ comparable women and men

Thus, the total power of the oscillations of the stroke volume of blood in all states was higher in men.

The power of the oscillations of the stroke volume of blood in all ranges was lower in women.

However, the normalized oscillation power of the stroke volume in the range in these two groups did not differ.

To analyze the spontaneous baroreflex sensitivity in men and women, the amplitude of the maximum peak cross-spectral power of the oscillations of the shock volume of blood and t-R-R under different conditions was determined (Table 2).

Table 2

Maximum cross-spectral power of the oscillations of the shock volume of blood and the duration of the interval R-R (ms · ml) in the range of 0.04-0.15 Hz in men (n = 82) and women (n = 96) under different conditions

groups	Terms			
	Lying down	**6 cycles · min^{-1}**	**Orthoprobe**	**Psycho-emotional**
men	6,13 [3,43; 10,47]	186,16 [31,40; 317,08]	7,78 [3,87; 13,75]	8,53 [4,38; 15,53]
women	4,33* [2,70; 6,80]	132,00 [58,49; 318,39]	5,86** [3,08; 8,31]	4,38*** [2,52; 7,82]

Notes: * - p<0,05; ** - p<0,01; *** - p<0,001 comparable women and men

Thus, in all conditions except for regulated breathing 6 cycles per minute, this figure was lower in women.

Particularly large differences were recorded in orthoprobe and psychoemotional stress.

Discussion

A number of reviews [7, 8, 10, 13] provide data on age and gender changes in some indicators of HRV. In studies by Ketel I.J. et al [17], conducted in randomized samples on 149 middle-aged men and 137 women, found that HRV levels were inversely related to age and heart rate (HR) in both sexes. LF levels in men are probably higher than in women. The same gender and age features of the heart rhythm wave structure are confirmed in measurements on 302 men and 312 women performed by Bai X. et al [15], on 653 persons performed by Aubert A.E. et al [14] and on 276 individuals conducted by C.J. Barrett et al [16].

There are significant differences in the reactivity of the power of oscillations of the duration of the R-R interval and blood pressure in men and women to physical, mental, cold loads. Thus, in the studies of O.V Peshakova [10] showed that for women under these conditions is characterized by greater centralization of the mechanisms of regulation of the cardiovascular system, and for men - an increase in the activity of the sympathetic part of the autonomic nervous system.

Conclusions

1. It was found that under all conditions except for regulated respiration 6 cycles per minute, the maximum cross-spectral power of the oscillations of the stroke volume of blood and the duration of the interval R-R was lower in women. Particularly large differences were recorded in orthoprobe and psychoemotional stress.

2. In women, both at rest and at different loads, lower blood pressure levels, cardiac output than in men due to smaller body size, general variability in heart rate and stroke volume.

3. The level of functioning of spontaneous baroreflex sensitivity in women at loads is lower than in men.

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FUNCTIONAL ENRICHMENT ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN HUMAN PLACENTA OVER THE COURSE OF NORMOTENSIVE PREGNANCY

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Abstract. *The dynamics of gene expression in human placenta in the course of physiological pregnancy is rarely systematically analyzed by genome-wide approaches though it is important for understanding the exact process and for comparison with any ongoing pathological processes affecting placenta. The aim of our study was to integrate the available in open access disparate data on gene expression obtained by microarray technology and analyze the changes of differentially expressed genes common for 1 vs 11 and 11 vs 111 trimesters, that is 1025 genes. A large group of immune response and chemotaxis genes increased expression from I to II trimester and decreased from II to III. The expression of genes responsible for multicellular organisms development, cell differentiation, cell cycle and cell division decreased from I to III trimester. Obtained differentially expressed genes' lists along with fold-change values can serve as control expression dynamics for pathological cases affecting placenta, such as preeclampsia.*

Keywords: *placenta, microarray, integrative analysis, pregnancy*

Introduction

Human placenta undergoes rapid development throughout the pregnancy. Hence, its function in both physiological and pathological cases must be studied in dynamics of placental development. In particular, in comparative studies it is not simply enough to match a case with respective control by gestational age, since the course of pregnancy can be accelerated or halted by a pathological condition. In order to properly address gestational age contribution in differential expression between physiological and pathological cases, the dynamics of the physiological case should be studied and used for reference.

Methods

Gene expression data (6 datasets, 16676 common genes among datasets) from 1st (7.4±2 weeks), 2nd trimester (16.9±1.1 weeks), (38.4±1.2 weeks) and term **bulk** placental **tissue** were obtained from GEO and ArrayExpress databases and complemented with standardized metadata from our IGEA database [1] (available igea.sysbio.org.ua). Raw microarray data was integrated into one dataset, using the Empirical Bayes method for cross-normalization and batch-effect removal [2]. Differentially expressed genes (DEGs) were identified by generalized linear models [3]. Analysis was done in R programming language. For the assembly of

gene coexpression and protein interaction networks, clustering and functional enrichment analysis we used String web tool [4] that employs general (not organ-specific) data from public databases such as KEGG, Uniprot and Gene Ontology.

Results

Using generalized linear models we selected 1025 genes that are differentially expressed simultaneously between I and II trimesters and between II and III trimesters and provides the opportunity to interrogate the quantitative changes in gene expression over the course of physiological pregnancy though in a restricted number of genes.

Differentially expressed genes were arranged into four groups according to expression changes between trimesters - UpUp, where the gene expression increases from I to II and from II to III trimester, UpDown, DownDown and DownUp, respectively. Thei protein interaction networks were created for each group of genes using String web-tool. The groups were ranked by the number of DEGs and the interaction networks' average node power (av.n.p) reflecting the rate of interrelation between genes: UpDown (466 genes, 3.95, av.n.p.), DownDown (273, 5.61 av.n.p.), UpUp (150 genes, 0.413 av.n.p) and DownUp (136 genes, 0.235 av.n.p).

The UpDown group includes the majority of genes involved in the immune system processes, particularly, chemotaxis (34 genes), neutrophil degranulation (28) and vehicle mediated transport and endocytosis (23). Changes in gene expression in the middle of the second trimester signify the existence of a turning point in expression of these genes, which roughly coincides with the period when preeclampsia manifests itself by the onset of clinical symptoms (> 20 weeks).

The second largest DownDown group possesses the highest average node power. The majority of genes are involved in multicellular organisms development (101), cell differentiation (76) and cell cycle (48).

The UpUp and UpDown groups comprise more promiscuous genes according to their functions e.g. G-protein coupled receptor signaling (7 genes), GTP binding (4) for UpUp and regulation of cell motility (4 genes), glucose metabolic process (4), regulation of hormone secretion (3) for DownUp.

Discussion

The quantitative characteristics of placental gene expression over the course of gestation revealed the steady up- and down-regulated processes and UpDown and DownUp processes which reveal II trimester as a turning point in expression of the corresponding genes. The changes in trend of gene expression coincide in time with an appearance of clinical symptoms of preeclampsia.

Conclusions

On the basis of integrated openly available microarray data we quantitatively characterised DEGs and the most pronounced biological processes in the course of physiological pregnancy. The newly found characteristics are the baseline for subsequent gene expression analysis in human placenta.

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UDC 579.6

Razghonova Ye., Zinchenko O.

ANTIMYCOBACTERIAL ACTIVITY OF *GRYLLOTALPA ORIENTALIS* AND *GALLERIA MELLONELLA* EXTRACTS

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Abstract. *The study of biological activity of extracts obtained from animal raw materials, namely Gryllotalpa orientalis tissues and Galleria mellonella wastes towards Mycobacterium smegmatis MC² 155 has been conducted. Antimycobacterial activity was evaluated by detection of the ability of studied extracts to inhibit the growth of mycobateria on a solid nutrient medium, 40% ethanol was used as extractant. The highest inhibitory effect was achieved at the proportion of mole cricket in the extract of 10% and wax moth – of 30%. The maximum value of M. smegmatis growth inhibition zone diameter as a result of mole cricket and wax mole extract action reached 30±1,9 and 30±0,9 mm, respectively, this effect was observed on the fifth day of infusion.*

Key words: *Mycobacterium smegmatis, wax moth, mole cricket*

Introduction

Tuberculosis remains a major public health problem despite intense national and international efforts to control the disease, and *Mycobacterium tuberculosis* is one of the most dangerous pathogens, taking millions of lives worldwide each year.

In 2018, the number of deaths caused by tuberculosis decreased: 1,5 million people died compared to 1,6 million in 2017. The number of new cases in recent years has also been steadily declining. However, one of the main obstacles in the fight against the epidemic is the growing resistance to drugs that previously allowed the successful treatment of tuberculosis [WHO report, 2018; WHO report, 2019].

In recent years, intensive research has been conducted to improve the effectiveness of TB therapy through the introduction of new environmentally safe, affordable and effective tools [WHO report, 2019].

Insects attract the attention of researchers as a source of biologically active substances, because a large number of antimicrobial peptides which are equated to antibiotics and, accordingly, can be used to treat bacterial and fungal infections have been isolated from their tissues [Пурыгин и др., 2011].

The objective of this study was to evaluate antibacterial activity of folk remedies – mole cricket and wax moth extracts – toward model organism *Mycobacterium smegmatis* MC² 155.

It is known that selected objects have antituberculosis, immunostimulatory and antioxidant effects [Zimmer, 2005].

Raw materials of plant or animal origin are used mainly in the form of infusions and decoctions as adjunctive therapy of tuberculosis.

Materials and methods

Raw materials from dried insects *G. orientalis* were obtained from an online store (country of origin China), wax moth products *G. mellonella* were purchased in an apiary in Odesa region.

The strain *M. smegmatis* MC^2 155 was used as a test organism.

Alcohol extracts were obtained from the studied raw materials of animal origin. The raw material was ground with a mortar and pestle.

The extraction was carried out without heating and removing the extractant, by maceration.

The extractant was ethanol at a concentration of 40%. The infusion lasted 8 days in a dark glass bottle at a temperature of 18-20 °C [Гроссман, 2014]. An aliquot of the extract of each species was taken daily and antimicrobial activity against mycobacteria was evaluated. The share of mole cricket and waste of wax moth in the extracts was 10% and 30%, respectively.

Antimycobacterial activity was determined by the method of wells in nutrient agar based on active compound diffusion through the solid medium [Methods…, 1998]. Obtained extracts of animal raw materials were diluted with ethanol to obtain working solutions, which were used in the experiment. For mole cricket's extract, the activity of solutions with a concentration of 10, 5 and 1,5% was investigated, for waste of wax moths - 30, 15 and 5% extracts.

An overnight culture of *Mycobacterium smegmatis* MC^2 155 grown under sterile conditions in meat-peptone broth at 37 °C for 24 hours. Antimicrobial activity was determined by the diameter of the growth inhibition zone of test organism. 40% ethanol was added to the wells in control dishes to exclude the presence of an inhibitory effect of the extractant. Each extract concentration and control were studied in triplicates.

Statistical data processing was performed using standard statistical methods and Microsoft Excel software.

Results and discussion

Evaluation of 10% mole cricket extract activity showed a positive correlation (r = 0.72) between the increase of inhibition zone diameters and the duration of infusion, with a maximum value of 30±1,9 mm on the fifth day (fig. 1). 5% extract showed a weak statistical correlation (r = 0.56), 1,5% extract, on the contrary, – a strong negative (r = -0.92) correlation, but this picture was observed only for first four days, extracts obtained on 5th-8th day of infusion demonstrated no inhibitory effect.

The data on the activity of the extracts of wax moth larvae excrements shows a similar relationship, with a gradual increase of the inhibitory effect with prolongation of the extraction period and subsequent decline. The peak of activity for the three samples occurred on the 5th day, the maximum size of the growth inhibition zone was 30±0,9 mm (Fig. 2). Correlation coefficients for extracts with a concentration of 30%, 15% and 1,5% were 0,79; 0,30 and 0,34, respectively.

If the correlation coefficient is close to - 1, it indicates a strong negative correlation between the variables - days of infusion and the strength of inhibition, and, if coefficient is close to + 1, it shows positive connection.

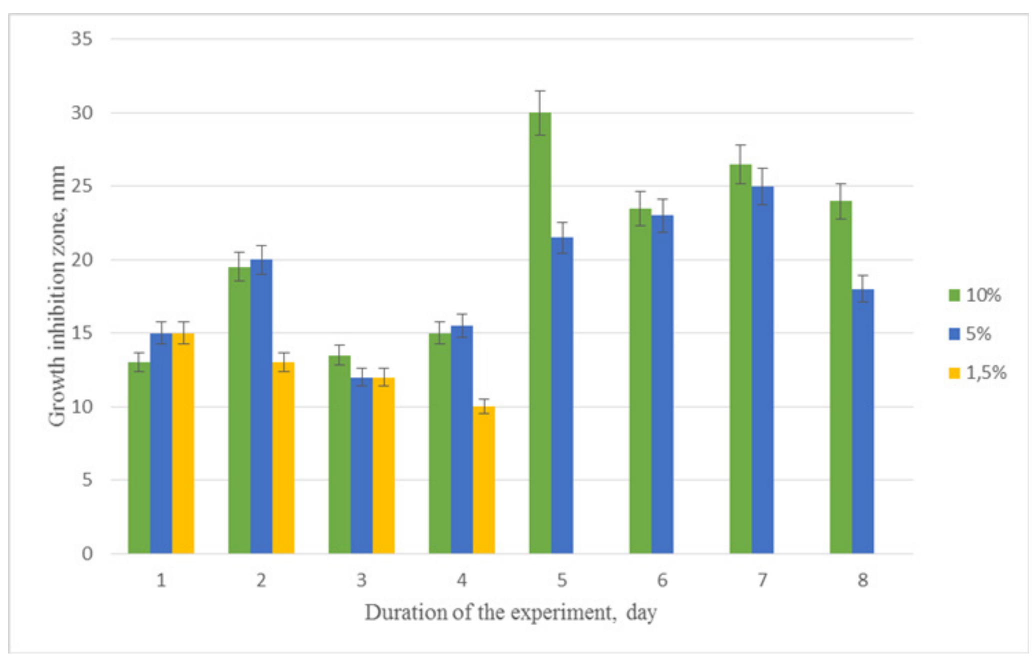

Fig. 1. Dynamics of inhibitory effect of mole crickets extracts towards *Mycobacterium smegmatis* MC² 155

Note: 10, 5, 1,5% - concertration of mole cricket extract

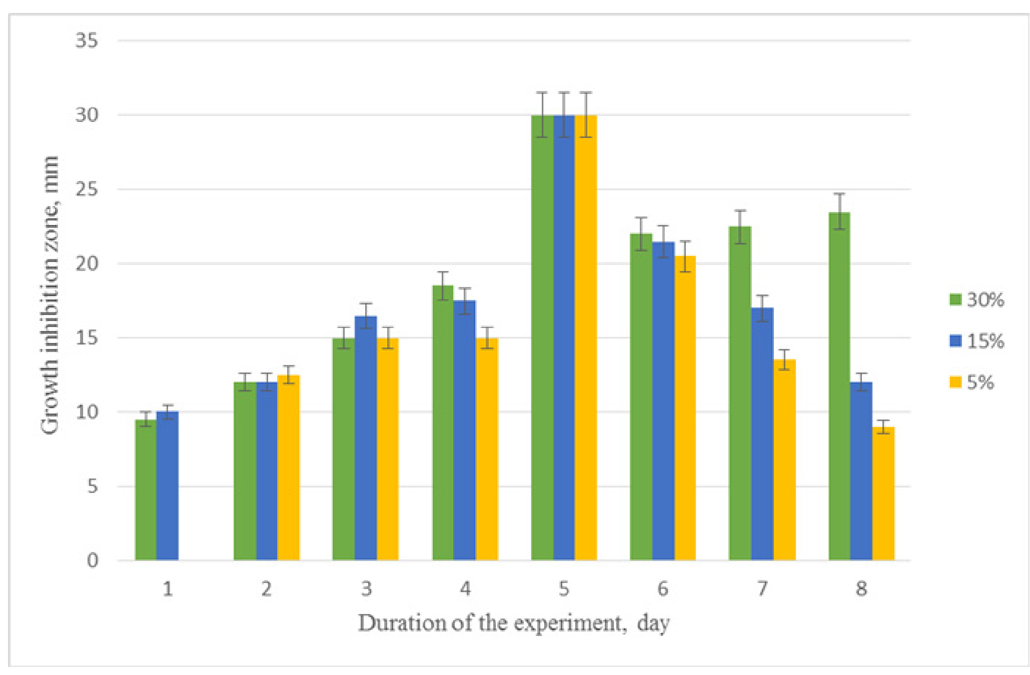

Fig. 2. Dynamics of inhibitory effect of wax moth wastes extracts towards *Mycobacterium smegmatis* MC² 155

Note: 30, 15, 5% - concentration of wax moth wastes extract

For the most part, there was a positive correlation, which means that prolongation of infusion time leads to an increase of the inhibitory effect of extract.

Thus, extracted biologically active compounds of wax moth wastes and mole cricket tissues showed significant inhibitory activity towards mycobacterial test strain, which gradually increased with prolongation of infusion and reached its maximum on the 5th-6th day. The highest inhibitory effect was achieved at the proportion of mole cricket in the extract of 10% and wax moth – of 30%.

Comparing the maximum diameters of growth inhibition zones of *M. smegmatis* MC^2 155 due to the action of the studied extracts, we can conclude that the wax moth waste product was more effective than mole cricket axtract, causing the growth inhibition zone of 30 ± 0,9 mm in all used concentrations.

In general, investigated insects have been used in Chinese medicine as remedies that have a multifactorial effect on human body for a long time.

Researchers [Ma et al., 2018] were found that extracts of mole crickets have inhibitory effects to *S. aureus* and *M. tuberculosis*. Studies [Мельник, 2012] showed that the wax moth extract, which has minimal antimycobacterial activity *in vitro* ranging from 15 µg/ml to 31 mg/ml, what is noteworthy for scientists and general practitioners.

Conclusion

Despite the rapid development of synthetic drugs, the huge potential of herbal and animal drugs for the prevention and treatment of diseases is not exhausted. Moreover, the resources of insects in our country are extremely rich, and the number is very large. Our results indicate that insect extracts have antimycobacterial activity, which stimulate to study mechanism of this compounds action. The molecular mechanisms of *in vitro* growth inhibition of *M. smegmatis* by biologically active compounds of insect tissues are not yet understood and provide a rich source for scientific search for new antituberculosis drugs.

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THE CHARACTERISTICS OF MICROBIAL INTERACTION BETWEEN SOME REPRESENTATIVES OF HUMAN MICROBIOTA AND PROBIOTIC STRAINS

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Abstacts. *The work was dedicated to the study of the interaction of strains isolated from probiotics, with some opportunistic microorganisms. It has been established that between L. acidophilus, L. sporogenes, B. clausii and the opportunistic strains (Candida albicans ATCC 18804, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923) antagonistic, neutral and mutual interactions develop. Over time, the form of interaction may change. The most effective, stable antimicrobial effect caused by the biologically active exometabolite production was determined for L. acidophilus to all investigated test microorganisms.*

Key words: *human microbiota, antagonistic activity, probiotics, Lactobacillus sp.*

Introduction

Since the early observations of Ilya Mechnikov, the beneficial effects of *lactobacilli* in human and animal health have been investigated. He believed that when consumed, the fermenting bacillus (*Lactobacillus*) positively influenced the gut microbiota, decreasing «putrefaction» and toxic microbial activities there. It was established that bacteria are not necessarily detrimental to man but may, on the contrary, play an important role in our well-being (Bibel, 2008). It is, however, much later the word «probiotic» was proposed by Parker for "organisms and substances which contribute to intestinal microbial balance" (Fijan, 2016).

According to the Food and Agriculture Organisation of the United Nations and the World Health Organization, probiotics are defined as live microorganisms, which when administered in adequate amounts confer a health benefit on the host. The most common probiotic bacteria are certain strains from the genus *Lactobacillus* (i.e., *L. rhamnosus, L. acidophilus, L. plantarum, L. casei, L. delbrueckii* subsp. *bulgaricus,* etc.) and *Bifidobacterium* (i.e., *B. infantis, B. animalis* subsp. *lactis, B. longum*, etc.). Other probiotic bacteria include *Pediococcus acidilactici, Lactococcus lactis* subsp. *lactis, Leuconostoc mesenteroides, Bacillus subtilis, Enterococcus faecium, Streptococcus thermophilus, Escherichia coli*. Certain yeasts such as *Saccharomyces boulardii* are also probiotics (Koning, 2010).

The existing clinical attitude always favored the use of antibiotics for killing and eradication of microbial pathogens. Probiotic therapy is considered as a natural

way of suppressing the growth of pathogens in a non-invasive way, is free from undesirable side effects and is preventive in nature in comparison with antibiotic therapy. It can be an alternative as well as an addition to antibiotic therapy (Millette, 2007).

A broad antimicrobial/antagonistic ability is especially important for probiotics as one of their functional beneficial requirements as well as strong antagonism against pathogenic bacteria. The antagonistic activity of one microorganism against another can be caused by competitive exclusion, immune modulation, stimulation of host defense systems and production of organic acids or hydrogen peroxide that lower pH and antimicrobials such as bacteriocins, antioxidants, signalling molecules (Ratsep, 2014).

The application of probiotics as a biotherapeutic agent has already started and in the coming future it is conceivable that they will become more effective tools in the treatment of different diseases (Arshad, 2018).

Objectives

The aim of the work was to characterized antimicrobial properties of some *Lactobacillus* probiotic strains towards microorganisms that belong to the human microbiota (*Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*).

Materials and methods

The work was carried out at Biotechnological Research and Educational Center and Department of Microbiology, Virology and Biotechnology.

In the work strains of *Lactobacillus acidophilus* and *Lactic Acid Bacillus (Bacillus coagulans (= Lactobacillus sporogenes))* that were isolated from probiotic preparations «Gynoflor» (Medinova AG, Switzerland), «Lactovit Forte» (Mepro Pharmaceuticals Private Limited, United Kingdom) and «Enterozhermina» (Sanofi Aventis, Italy) were used. Pre-cultivation was carried on the De Man, Rogosa and Sharpe Agar (MRS) medium at 37 °C for 24 hrs.

Antagonistic activity of the studied strains due to *Candida albicans* ATCC 18804, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 was detected using the «agar well» and «culture droplet» methods (Chernivets Yu., 2006; Glushanova N., 2004).

Results

The probiotic characteristics are determined by a set of biologic properties of the microbial strains. Obtaining data characterizing the diverse biological potential and the validity of their probiotic activity is one of the fundamental approaches to understanding the probiotic action mechanisms [Nowroozi, 2004].

The interactions between microorganisms in the common ecological niche, in particular agar nutrient medium, were determined in the work. By means of «culture droplet» method modified by Glushanova, the ability of the studied microorganisms to compete on a common habitat, as well as the dependence of antagonistic activity on the rate of growth, was characterized.

The growth retardation of one of the studied culture or the growth of one strains on the surface of the other was evaluated as an antagonism phenomenon;

the merger of the colonies of the studied cultures in the zone of their co-cultivation was assessed as the antagonism absence; the intensification of the microorganism growth in the dripping area was assessed as a mutualism.

Simultaneous cultivation of microorganisms on the surface of a agar medium was characterized by various forms of their interaction: antagonistic, neutral, and mutualistic.

In some cases, there were some noticeable changes in this process over time. Thus, for *E. coli* ATCC 18804 and *S. aureus* ATCC 25922, the neutral form of interaction with *B. clausii* at the beginning of cultivation changed on the microbial growth increase in the droplet zone. This situation is a result of the mutualism interaction development. Also, during the experiment there was a change in the antagonistic effect of *L. sporogenes* on *S. aureus* ATCC 25922 on a neutral interaction.

However, for *L. acidophilus* and used test microorganisms, there were no differences in growth in the «common» part of the droplet when comparing different time intervals.

The mechanism of probiotic strain positive influence, in particular lactobacilli, is multifactorial and is due to the production of lactic and fatty acids, bacteriocin and hydrogen peroxide, immune response modification (induction of IgA and anti-inflammatory cytokine synthesis), synthesis of biosurfactants and collagen-binding proteins and specific molecules that are able of pathogen virulence reducing and a number of other factors [Coman, 2004; Lin, 2016].

In contrast to the previous study, the determination of the microorganism interactions by the «agar well» method allows to study in more detail the role of biologically active substances in this process. First of all, microorganisms are cultivated in the most favorable conditions for their nutritional needs. Secondly, the cultivation of the studied strains in the liquid medium promotes more intensive accumulation of exometabolites, in particular of lactic and other organic acids, and their further diffusion into the medium thick [Nowroozi, 2004].

Determination of the microbial form interaction by the «agar well» method was carried out every 24 hrs during the week: however, the largest changes in the growth of the test strains were noted on the 7th day compared to the 2nd one.

For the *L. acidophilus*, the antimicrobial activity level against the test microorganisms used over time increases: the average growth retardation zone diameter around the probiotic culture well increases by 2-4 mm for the corresponding period.

Regarding the *L. sporogenes* culture against *E. coli* ATCC 18804 and *C. albicans* ATCC 25923, the suppressive activity gradually became, on the contrary, less notable: the test microorganism growth retardation zone decreased by 25 – 35%, respectively. In the case of the interaction of *L. sporogenes* with *S. aureus* ATCC 25922, the degree of antagonism was constant: the inhibition zone remained unchanged throughout the week.

During the experiment, the smallest antimicrobial activity was noted for *B. clausii*. At the same time during the 2nd – 7th days an intensive decrease of the inhibition zone diameter of all test cultures occurred. In the case of the *E. coli* ATCC 18804 it complete disappeared. This trend indicates a lack of bactericidal action by *B. clausii* on *E. coli* cells.

Thus, *Lactobacillus* sp. probiotics are characterized by the most prevailing

antimicrobial activity. So, it may indicate the bacteriostatic effect of lactobacillus exometabolites.

The absence of effective inhibition test organisms by *B. clausii* strain may be due to the fact that, generally, bacilli inhibit the microorganism growth by means of the production of polypeptide antibiotic substances that have a sufficiently high molecular weight (for example, M of gramicidin C is 1146) and are characterized by a lower ability to diffuse into a nutrient medium that was used in the experiment. Also, in most cases the synthesis of antibiotic compounds in this microorganism group is associated with the spore formation process, which was also absent in the relevant conditions of the study [Prabhurajeshwar C., 2012].

Conclusions

During investigation of the *Lactobacillus* strain antagonistic activity following results were obtained:

• the most antimicrobial effect was on the *E. coli* ATCC 25922 growth;

• the most resistant microorganism to *Lactobacillus* influence was *Candida albicans* ATCC 18804 ;

• the direct influence of *Lactobacillus* strains was not detected – the microbial growth rate was exactly the same in all cases (by means of «culture droplet» method);

• the indirect microbial influence caused by biologically active substances produced by the microorganisms was the most expressed in the case of *Lactobacillus acidophilus* and *Escherichia coli* ATCC 25922 (by means of «agar well» method).

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Semenets A., Kaleva A., Prishchenko I., Galkin M.

BIOSURFACTANTS SYNTHESIS BY *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM MUSSEL SURFACES OF BLACK SEA

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Abstract. *Biosurfactants are a structurally diverse group of surfactant molecules synthesized by microorganisms. These compounds reduce the surface tension and interface at both aqueous solutions and mixtures of hydrocarbons, making them potential candidates for increasing oil yield and emulsion destruction processes.During the research such marine Pseudomonas spp strains were used: P. aeruginosa M1, P. aeruginosa M4 and wild- type strain P. aeruginosa PA01 in suspension and biofilm formation systems (LB and Giss media). P. aeruginosa strains M1 and M4 isolated from Black Sea mussel's surfaces synthesize 25% and 66% more surfactants than the reference strain PA01 and in comparison with P. aeruginosa PA01 synthesize in 4-7 times more biosurfactants. In biofilm cultures the same surfactant synthesis dependence on the composition of the nutrient medium is observed as in suspension cultures. According to the intensity of rhamnolipid production in biofilm cultures, the studied strains can be arranged in the following row: P. aeruginosa M4> P. aeruginosa M1 >> P. aeruginosa PA01.*

Key words: *Pseudomonas aeruginosa, biosurfactants, rhamnolipids, biofilms, marine bacteria*

Introduction

Marine microorganisms have unique properties because of their adaptation ability to marine conditions such as high or low temperature, alkaline or acidic environment, high pressure and substrate limitation in deep water [Amani et al., 2013]. These distinctive characteristics have attracted many researchers to a detailed study of the possibility of using marine microorganisms in industry.

Among various biologically active compounds, biosurfactants (bioemulsifiers) are of great interest due to their structural and functional diversity [Fracchia, 2012]. The diverse marine environment of microorganisms makes it possible to discover new compounds such as antibiotics, enzymes, vitamins, drugs, biosurfactants, bioemulsifiers and other valuable compounds of commercial importance, because due to the vastness of the marine biosphere, much of the marine microbial world remains unexplored [Dang et al. 2016].

Biosurfactants are functional molecules that are of interest to many researchers due to their biocompatibility, versatility and applications in several biotechnological fields. Five classes of biosurfactants are known now: glycolipids, lipopolysaccharides and polysaccharide-lipid complexes, lipopeptides, fatty acids and neutral lipids [Banat, 2014]. The physiological role of microorganism's biosurfac-

tants is adhesion to the substrate and nutrients emulsification, desorption from the surface, antibacterial and antifungal activity and receptor for bacteriophages.

Biosurfactants have a number of advantages over chemical surfactants, including low toxicity, high biodegradability, better environmental compatibility, high foaming, selectivity, specific activity to elevated temperatures, pH and salts, and the ability to synthesize from renewable materials [Darvishi et al., 2011]. Compared to chemical surfactants, they are needed in small quantities, are effective in a wide range of oils and are environmentally friendly.

Bacterial biosurfactants are not inferior to synthetic surfactants in terms of emulsification efficiency. However, unlike synthetic surfactants, they have such advantages as biodegradability and lack of toxicity, which makes them promising in the development of new environmentally friendly technologies.

Factors influencing on the biosurfactants synthesis are pH, temperature, aeration, mixing intensity, etc.

Pseudomonas is a genus that belongs to the type of *Proteobacteria* that includes several known plant pathogens, such as *P. syringae*, opportunistic human pathogens *P. aeruginosa*, soil bacterium *P. putida*, and some species that cause spoilage of unpasteurized milk and other dairy products. *Pseudomonas aeruginosa* biosurfactants are a rhamnolipids mixture with different structure, among which the main part consists of di- and monoramnolipids, each containing two fatty acid residues and, above all, β-hydroxydecanoyl-β-hydroxydecanoate (C10–C10) [Thavasi et al., 2011].

Rhamnolipids synthesized by *Pseudomonas aeruginosa* have a wide range of biological activity, in particular, have antimicrobial and antitumor activity [Chakraborty et al., 2016].

Biosurfactants can be used in the peat dehydration, paper, coal, textile and mining industries. The only obstacle to the practical use of biosurfactants is the high cost of their production, which is 3-10 times higher than their chemical counterparts [Banat, 2014].

Materials and methods

Cultivation conditions

During the research were used such marine *Pseudomonas spp* strains: *P. aeruginosa* M1, *P. aeruginosa* M4 and wild-type strain *P. aeruginosa* PA01.

Pseudomonas aeruginosa strains cultivation was performed at 37 °C for 120 and 168 hours in flasks under shaking conditions (suspension cultures) and in stationary conditions for biofilm cultivation.

Two nutrient media were used:

• LB (1% peptone, 1% yeast extract, 0.5% NaCl);

• Giss medium (1% peptone, 1% glucose, 0.5% NaCl).

After cultivation, the cell density was determined spectrophotometrically at 600 nm and the suspensions were diluted with nutrient medium to a concentration of 10^9 cells in 1 ml. To obtain cell-free supernatants, the samples were centrifuged for 20 min at 3000 rpm. The supernatants samples were collected in tubes and used to biosurfactants detection.

Biosurfactants detection

- Drop test

200 μl of the supernatant was added to 5 μl 1% methylene blue solution and 20 μl was applied to the center of the square (1×1 cm) on the parafilm surface. The control was a sample that contained distilled water instead of the supernatant [Jain, 1991]. After the drops dried, the parafilm strips were scanned and the spot diameters were determined using the Altami Studio computer program.

- Rhamnolipids isolation and identification

The quantitative content of rhamnolipids was evaluated by the color reaction of rhamnose with orcin [Koch et. al, 1991]. After samples centrifugation, 2 ml of ethyl acetate was added to 2 ml of culture. The upper phase of ethyl acetate with dissolved rhamnolipids was taken in vials and left to completely solvent evaporation.

Then 0.5 ml of methanol was added to the vials and 0.1 ml was transferred into glass tubes. To the tubes was added 1 ml of a solution of 10 g of orcin in 25 ml of 56% sulfuric acid. The tubes were heated for 20 minutes at 80-100 ^{o}C until the appearance of yellow-brown color. In the control sample, 100 μl of methanol was added to the orcin reagent. After cooling the samples to room temperature, the optical density of the test samples was measured against the control sample on a spectrophotometer at 420 nm.

Biofilm biomass determination

The method is based on the ability of the crystalline violet dye to bind to cells and the biofilm matrix [Christensen et al., 1985].

After the plankton culture was removed from the wells, the biofilm in the plates was washed from the unattached cells with saline and fixed with 96% ethanol for 10 minutes. After fixation, the samples were dried and stained with an aqueous solution of crystalline violet for 5 minutes. The biofilm in the plates after 24 hours of drying at room temperature was destroyed with 0.1 M NaOH + 1% SDS and incubated at room temperature for 1.5 hours. The results were recorded on a BioTek μQuant spectrophotometer at a wavelength corresponding to the maximum absorption of the dye 592 nm. The presence of biological activity of the compounds was analyzed by the presence of the difference in optical density between the experimental and control samples.

All experiments were performed in three independent experiments with 3-4 replicates in each.

Results

Analysis of drop test data (Fig. 1) shows that the *P. aeruginosa* ability to synthesize biosurfactants strains can be arranged in the following row: *P. aeruginosa* M4(7.20 ± 0.41 mm) > *P. aeruginosa* M1 (5.44 ± 0.25 mm)> *P. aeruginosa* PA01 (4.35 ± 0.18 mm). Thus, in comparison with the collection strain, marine isolates were synthesized by 25% (strain M1) and 66% (strain M4) more surfactants.

The study of rhamnolipids amount in suspension cultures (Fig. 2) showed that the amount of rhamnolipids on LB medium significantly exceeds the content of these compounds on Giss medium for all studied strains. Thus *P. aeruginosa* PA01

rhamnolipids content during growth on Giss medium was 1.1 mg per 10^9 CFU on the 5th day and 1.6 mg on 10^9 CFU on the 7th day, then on LB medium it was 8-10 times higher - 8.9 and 17.8 mg 10^9 CFU, respectively. Amount of rhamnolipids on the 5th day of incubation in the *P. aeruginosa* M1 supernatant cultures on Giss medium was 1.8 mg per 10^9 CFU, and on the 7th day of incubation - 2.6 mg per 10^9 CFU. At the same time, the content of rhamnolipids on LB medium was almost 20 times higher and was 41.5 mg on the 5th and 48 mg on the 7th day of incubation.

The highest level of rhamnolipid biosynthesis was observed for strain *P. aeruginosa* M4. On Giss medium on the 5th and 7th day of incubation the rhamnolipids amount was 1.9 and 3.2 mg per 10^9 CFU, on LB medium - 63.7 and 70.6 mg per 10^9 CFU, respectively.

Given that the strains *P. aeruginosa* M1 and *P. aeruginosa* M4 were isolated from biofilms that were on the mussel shells surface, cultivation was also carried out in flat-bottomed Nuclon plates, which ensured the biofilms formation on their bottom. The rhamnolipid amount was calculated in mg per biofilm mass unit. There are no significant differences in the ability to form biofilms between strains. Also, this process did not depend on the nutrient medium (Table 1).

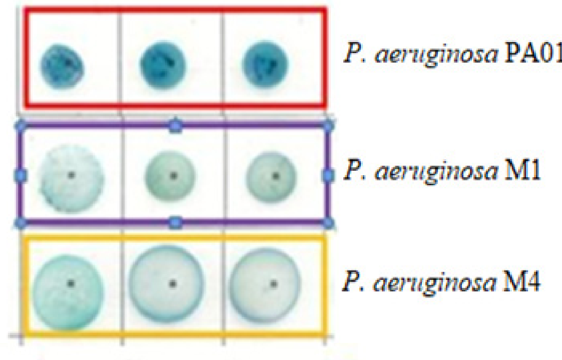

Fig. 1. The results of the ability of *Pseudomonas* to synthesize biosurfactants in the drop test

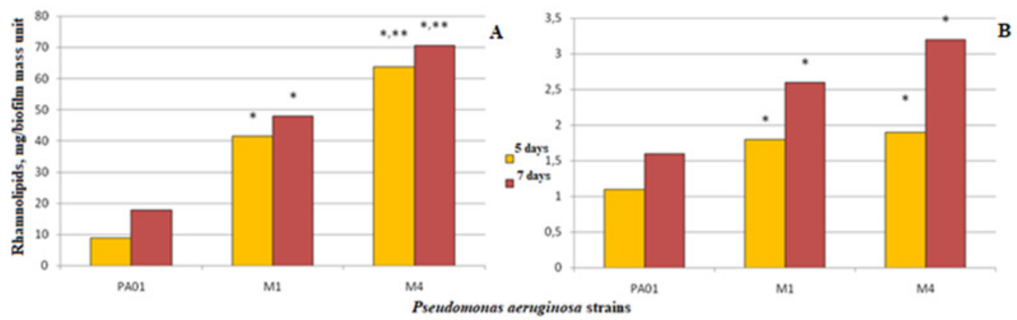

Fig. 2. The amount of rhamnolipids during the suspension cultivation in LB (A) and Giss (B) media (* - the difference is significant in comparison with *P. aeruginosa* PA01; ** - the difference is significant in comparison with *P. aeruginosa* M1)

Table 1

P. aeruginosa biofilms masses (OD 592 nm)

Medium	Day	*P. aeruginosa* PA01	*P. aeruginosa* M1	*P. aeruginosa* M4
LB	5	3,243 ± 0,264	3,581 ± 0,286	2,283 ± 0,205
	7	2,834 ± 0,230	3,154 ± 0,261	2,106 ± 0,153
Giss	5	3,027 ± 0,184	3,096 ± 0,193	2,185 ± 0,152
	7	2,873 ± 0,174	2,965 ± 0,180	1,937 ± 0,169

The study of rhamnolipid synthesis in biofilm cultures (Fig. 3) showed in general the same picture as in the case of suspension culture, except that the rhamnolipids accumulation, it was more intense than in suspension cultures for all strains on the 7th day of incubation.

As in the previous variant, the greatest rhamnolipids production ability was showed by *P. aeruginosa* M4 strain. On the 5th day of incubation, the rhamnolipids amount cultured on Giss medium was 1.9 mg, which was equal to the content of these compounds in suspension cultivation under the same conditions, and on LB medium was almost in 58 times more - 111 mg. On the 7th day, these values reached 4.3 mg on Giss medium, and 130 mg on LB medium.

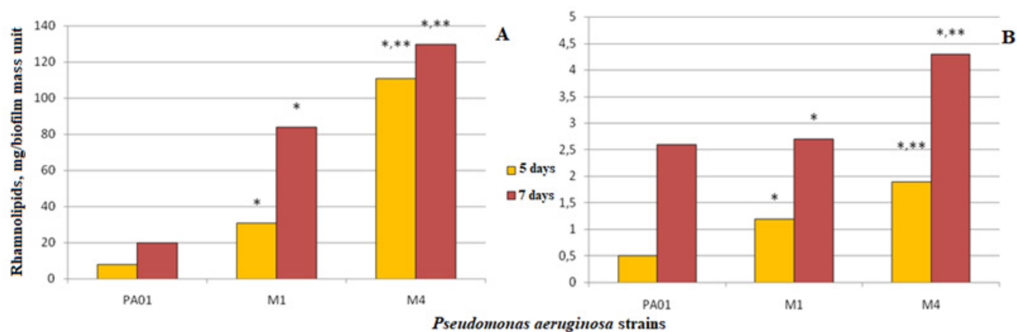

Fig. 3. The amount of biofilm rhamnolipids during the cultivation in LB (A) and Giss (B) media (* - the difference is significant in comparison with _P. aeruginosa_ PA01; ** - the difference is significant in comparison with _P. aeruginosa_ M1)

Biofilm cultures supernatants of *P. aeruginosa* M1 and PA01 strains contained significantly less rhamnolipids, which on the 7th day of incubation was equal to 2.6 and 2.7 mg in the case of Giss medium and 20 and 84.1 mg for LB medium, respectively.

Due to the fact that *P. aeruginosa* is an obligate proteolytic bacteria and practically does not utilize sugars other than glucose, it can be concluded that LB medium is much more optimal for growth and cell development of populations of this microorganism, which should also affect on the various exoproducts synthesis.

Conclusions

Obtained results showed that *P. aeruginosa* strains isolated from the Black

Sea are more efficient rhamnolipids producers than the reference *P. aeruginosa* PA01strain. The intensity of surfactants biosynthesis significantly depends on the nutrient medium composition. In the future to establish the role of other cultivation conditions: pH, temperature. For marine strains it will be interesting to determine the seawater effects and synthetic marine environment.

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BIOTECHNOLOGICAL APPROACHES TO THE STUDY OF THE MOLECULAR MECHANISMS OF TUMOR GROWTH AND TREATMENT

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Abstract. *We have studied the expression of genes encoding proteins PRMT2 and PVR/CD155 in the U87 glioma cell with a deficiency of ERN1: full deficiency, introduced by dnERN1 (cells without both protein kinase and endoribonuclease activities), and with a deficiency in ERN1 endoribonuclease, introduced by dnrERN1 (cells without ERN1endoribonuclease activity only). It was demonstrated that inhibition of ERN1 endoribonuclease and protein kinase leads to up-regulation of PRMT2 gene expression and down-regulation of PVR gene as compared to control glioma cells, transfected with the empty vector. Besides, we showed that the expression of PRMT2 genes is not significantly changed in glioma cells with without ERN1endoribonucleaseactivity. Nevertheless, such deficiency in ERN1 endoribonucleasecauses appreciable down-regulation of PVR gene expression compared to control glioma cells.The expression of PRMT2 and PVR mRNAs is regulated byERN1 signaling of endoplasmic reticulum stress throughdifferent mechanisms: PRMT2 – via protein kinase and PVR – via endoribonuclease.*

Key words: *ERN1 knockdown, mRNA expression, PRMT2,PVR, U87 glioma cells*

Introduction

The study of the molecular mechanisms of malignant tumor growth, in particular at the level of gene expression, is a highly relevant direction of modern scientific research in the field of biology and medicine. Besides, using modern biotechnological approaches is an essential component of such researches. The most aggressive types of cancer are brain tumors, peculiarly glioblastomas, which are characterized by a lack of efficient treatment. Consequently, the studies of the molecular mechanisms, which underlie the pathogenesis of gliomas, are essential to fight tumorigenesis, improve existing approaches to cancer prevention and treatment as well as development new ones.

Malignant cells, including gliomas, require metabolism reprogramming that provides specific biosynthetic needs, rapid proliferation, adaptation to challenging microenvironmental conditions. The important factors for tumor growth are endoplasmic reticulum stress and hypoxia [1, 2]. They provide resistance to apoptosis and act as key inducers of angiogenesis promoting the proliferation and survival of tumor cells even in the unfavorable microenvironment. Furthermore, endoplasmic reticulum stress is a necessary factor for the intense proliferation of malignant tumors, their resistance to apoptosis and to the negative effects of hypoxia [3, 4].

The endoplasmic reticulum stress is mediated by the three main signaling pathways: PERK (PKR-like endoplasmic reticulum kinase), ATF6 (activating transcription factor 6), and ERN1 (endoplasmic reticulum to nucleus signaling 1), which is the most important for genome reprogramming in malignant tumors.

Oligomerization and transautophosphorylation of PERK lead to inhibition of the protein translation through phosphorylation of eukaryotic translation initiation factor 2 (eIF2a), while activation of the ERN1 signaling pathway causes changes in the expression of a large number of genes controlling various metabolic processes, proliferation, apoptosis, and cell survival. The cytosolic portion of ERN1 contains two functional domains: Ser/Thr-kinase and endoribonuclease.Thus, ERN1 endoribonuclease controls the expression of a big number of genes by providing alternative splicing of the XBP1 (X-box binding protein 1) transcription factor. The XBP1 plays a key role in triggering the unfolded proteins response and restoring cellular homeostasis by inducing the expression of genes encoding chaperones, foldases, components of the endoplasmic reticulum stress-dependent pathways aimed at protein degradation and lipid synthesis [4, 8-11].

Thereby, we have obtained dominant-negative genetic constructs using biotechnological approaches to suppress endoplasmic reticulum stress, in particular the ERN1 signaling pathway or only ERN1 endoribonuclease activity [5-7]. We showed a linkage between tumor cell proliferation and long-term activation of endoplasmic reticulum stress, in as much suppression of the ERN1 signaling pathway resulted in a sharp reduction of glioma cell proliferation. Moreover, inhibition of only endoribonuclease activity affects to a greater extend. However, the molecular mechanisms of the control of tumor cell proliferation processes by the ERN1 signaling pathway are complex yet and warrant further study.

The central organelle in many metabolic and signaling pathways that undergo remodeling in tumors and are critical for cell survival or death is mitochondria. Its dysfunction is closely related to oncotransformation. The functional activity of mitochondria is controlled by numerous nucleus-encoded proteins. Most of these factors and enzymes are polyfunctional and play an important role in controlling proliferation and apoptosis both inside and outside the mitochondria. Furthermore, numerous mitochondrial genes are targets of the ERN1-dependent transcriptional reprogramming that indicates the possible role of mitochondria in response to endoplasmic reticulum stress.

The aim of this work was to study the expression of genes encoding functionally different proteins PRMT2 and PVR / CD155 in the U87 glioma cell line with inhibition of full ERN1 activity (protein kinase and endoribonuclease) or only its endoribonuclease to identify the possible role of these genes in inhibiting the glioma proliferation through the ERN1-dependent pathway of endoplasmic reticulum stress.

Materials and methods

Cell lines and culture conditions. The glioma cell line U87 was obtained from ATCC (USA) and grown in high glucose (4.5 g/l) Dulbecco's modified Eagle's minimum essential medium (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with glutamine (2 mM), 10% fetal bovine serum (Equitech-Bio, Inc.,

USA), penicillin (100 units/ml; Gibco) and streptomycin (0.1 mg/ml; Gibco) at 37 °C in a 5% CO2 incubator. We used three sublines of cell line U87[5-7]. One subline was obtained by selection of stable transfected clones with overexpression of vector pcDNA3.1, which was used for creation of dnERN1 and dnrERN1. Second subline was obtained by selection of stable transfected clones with overexpression of ERN1 dominant/negative construct (dnERN1), having suppression of both the protein kinase and endoribonuclease activities of this signaling enzyme [6].The third sub-line was obtained by the selection of stable transfected clones with the overexpression of dominant-negative ERN1 endoribonuclease mutant (dnrERN1), which was obtained by truncation of the carboxy-terminal 78 amino acids of ERN1 [7]. All used in this study sublines of glioma cells are grown in the presence of geneticin (G418) while these cells carrying empty vector pcDNA3.1, dnERN1 or dnrERN1 constructs.

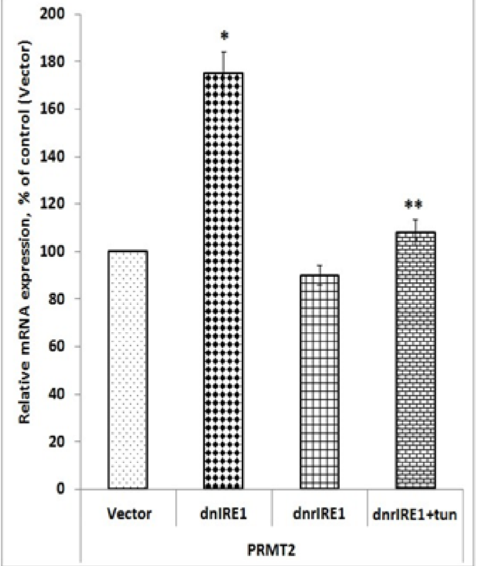
RNA isolation. Total RNA was extracted from glioma cells using the Trizol reagent according to manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). The RNA pellets were washed with 75% ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95% ethanol and re-dissolved again in nuclease-free water. RNA concentration and spectral characteristics were measured using NanoDrop Spectrophotometer ND1000 (PEQLAB, Biotechnologie GmbH).

Reverse transcription and quantitative PCR analysis. The level of expression of *PRMT2* and *PVR* genes was measured by quantitative polymerase chain reaction using SYBRGreen Mix (ABgene, Thermo Fisher Scientific, Epsom, Surrey, UK) and qPCR „RotorGene RG-3000" (Corbett Research, Germany) and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, USA). Thermo Scientific Verso cDNA Synthesis Kit (Germany) was used for reverse. Polymerase chain reaction was performed in triplicate. The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The pair of primers specific for each studied gene was received from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Quantitative PCR analysis was performed using a special computer program "Differential expression calculator" and statistical analysis using Excel program and OriginPro 7.5 software. Comparison of two means was performed by the use of two-tailed Student's t-test. A p-value <0.05 was considered significant in all cases. The values of gene expression were normalized to the expression of beta-actin mRNA and represent as percent of control (100%). All values are expressed as mean ± SEM from triplicate measurements performed in 4 independent experiments. The amplified DNA fragments were also analyzed on a 2% agarose gel and that visualized by SYBR* Safe DNA Gel Stain (Life Technologies, Carlsbad, CA, USA).

Results

We studied the expression of PRMT2 (proteinargininemethyltransferase 2) mRNA in U87 glioma cells in relation to inhibition of boththe protein kinase and endoribonuclease activities of ERN1 signaling enzyme. As shown in Figure 1, the expression of PRMT2 mRNA in U87 glioma cells, transfected by dnERN1, is strongly up-regulated (+75 %; p< 0.01) in comparison with cells, transfected by empty vector.



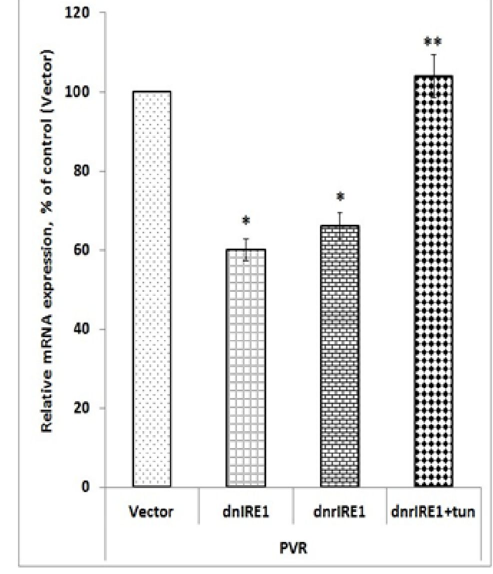


Fig. 1. PRMT2 (Protein Arginine Methyltransferase 2) mRNA expression level in U87 glioma cells: effect of inhibition of both enzymatic activities of ERN1 sensor and signaling protein (dnERN1) or its endoribonuclease activity only (dnrERN1) as well as induction of endoplasmic reticulum stressby tunicamycin (0.01 mg/ml – 2 hours) in cells without endoribonuclease (dnrERN1+tun). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n = 4; * - P < 0,01 as compared to control (Vector); **- P < 0,01 as compared todnrERN1

Fig. 2. PVR (PVR cell adhesion molecule; CD155) mRNA expression level in U87 glioma cells: effect of inhibition of both enzymatic activities of ERN1 sensor and signaling protein (dnERN1) or its endoribonuclease activity only (dnrERN1) as well as induction of endoplasmic reticulum stress by tunicamycin (0.01mg/ml – 2 hours) in cells without endoribonuclease (dnrERN1+tun). The values of this mRNA expression were normalized to the expression of beta-actin mRNA, expressed as mean ± SEM and represented as a percent of control (100%); n = 4; * - P < 0,01 as compared to control (Vector); **- P < 0,01 as compared todnrERN1

At the same time, full ERN1 deficiency causes down-regulation (-60 %; p< 0.01) of *PVR*(PVR cell adhesion molecule; CD155) gene expression as compared to control glioma cells (Figure 2).

Besides, inhibition of ERN1 endoribonuclease did not show significant changes in the expression level of *PRMT2* gene in comparison with control glioma cells (Figure 1).

Nevertheless, as shown in Figure 3, the deficiency in ERN1 endoribonuclease leads to down-regulation of *PVR* gene expression almost to the same extent as in U87 glioma cells without both enzymatic activities.

Thus, these results revealed different mechanisms of endoplasmic reticulum stress-dependent regulation of gene expression. Namely, the *PRMT2* gene expression is preferentially depends on protein kinase activity of ERN1 signaling enzyme,

because the inhibition of endoribonuclease of this signaling protein did not significantly affect the expression of *PRMT2*. At the same time, the expression of *PVR* gene is regulated by the ERN1 endoribonuclease since inhibition of both enzymatic activities of the ERN1 as well as inhibition of only endoribonuclease activity has a similar effect. Moreover, it was shown that the induction of endoplasmic reticulum stress by tunicamycin (10 μg / ml - 2 hours) in glioma cells without endoribonuclease activity leads to the increase inthe expression level of both studied genes (+20% for *PRMT2*; $p < 0.05$, and +55% for *PMR*; $p < 0.01$), indicating the involvement of other stress signaling systems in the regulation of expression of both the *PRMT2* and *PVR* genes (Fig. 1 and 2).

Consequently, showed increase in the expression of the *PRMT2* gene in relation to inhibition of both kinase and endoribonuclease activities of the ERN1 signaling enzyme,accompanyingbyreduction of glioma cell proliferation [5], conforms to the anti-proliferative and pro-apoptotic role of PRMT2 [16]. Furthermore, shown decrease of *PVR* gene expression in glioma cells with full ERN1 deficiency is consistent with the data that there is an increased expression level of this gene in various malignant tumors [17].

The results obtained in this work are fundamentally new and not only show the dependence of *PRMT2* and *PVR* gene expressions on the endoplasmic reticulum stress, mediated by the ERN1 signaling enzyme, but also reveal the molecular mechanisms of these changes, indicate the important role of endoribonuclease and protein kinase activities of ERN1 in the regulation of gene expression during tumor growth.

Conclusions

1. It was shown that the level of expression of PRMT2 and PVR mRNAs depends on endoplasmic reticulum stress, particularly on the ERN1 signaling pathway, which is tightly linked to angiogenesis and cell proliferation process as well as tumor growth.

2. Inhibition of the ERN1 endoribonuclease does not significantly affect the expression of *PRMT2* gene, while full ERN1 deficiency leads to up-regulation of this gene expression that reveals the essential role of protein kinase activity of ERN1 in the regulation of *PRMT2* gene expression.

3. It was also shown that the expression of *PVR* gene is regulated by ERN1 endoribonuclease since inhibition of both enzymatic activities of ERN1 as well as inhibition of only endoribonuclease activity have a similar effect on expression of *PVR* gene.

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STUDY OF THE INTEGRITY CYTOPLASMIC MEMBRANES OF WHITE BLOOD CELLS IN PULMONARY TUBERCULOSIS PATIENTS

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Abstract. *Tuberculosis (TB) – is a leader among infection diseases in morbidity and mortality rates. The state of white blood cells (WBC) and the integrity of it's cytoplasmic membranes, have influence to the functional activity of WBC, thus are important elements of anti-TB immune response. Aim of work was to study the integrity of cytoplasmic membranes of WBC in patients with pulmonary TB undergoing anti-tuberculosis treatment. The study was performed on 30 patients with diagnosis of pulmonary TB and 12 healthy donors. The assessment of the integrity of cytoplasmic membranes of WBC was performed by adding the markers CD45 +, 7AAD by flow cytometry. Statistical processing of the obtained results was carried out by analyzing Microsoft Excel 2016 and StatsoftStatistica 8.0. Significant differences being considered to be at p <0.05. The proportion of injured cells that reacted with 7AAD was significantly (p<0.05) higher in group of TB patients, then in control group (14.2 ± 2.8 % versus 4.3 ± 0.3 %). Our results suggest that pulmonary TB is accompanied with deep violation of the integrity of cytoplasmic membranes of CD-45+ WBC, that can be caused by progression of disease and the usage of anti-TB drugs, which requires further study.*

Key words: *tuberculosis, 7 AAD, flow cytometry, apoptosis, necrosis*

Tuberculosis (TB) – is a chronic infection disease, that is caused by the bacillus *Mycobacterium tuberculosis*. Among infection diseases TB has the leading positions in the main epidemiological indicators – morbidity, mortality, prevalence. According to the last report of World Health Organization, 10 million of people fell ill with TB in 2018 and 1.5 million deaths were estimated, globally [1].

Anti-tuberculosis immunity very differs from immunity to other infections. Immune response depends from activity of phagocytosis by blood cells, formation of specific antibodies and delayed-type hypersensitivity, all this processes are performed or depends from white blood cells (WBC) [2]. So the state of WBC and the integrity of it's cytoplasmic membranes, have influence to the functional activity of WBC, thus are important elements of anti-tuberculosis immune response.

7-aminoactinomycin D (7AAD) – a vital DNA-dye that penetrates the cell in violation of cell membrane integrity [3, 4]. 7-AAD does not readily pass through intact cell membranes; if it is to be used as a stain for imaging DNA fluorescence, the cell membrane must be permeabilized or disrupted. 7-AAD is also used as a cell viability stain. Cells with compromised membranes will stain with 7-AAD, while

live cells with intact cell membranes will remain dark. 7 AAD – biomarker of irreversible prosesses, like late stage of apoptosis or necrosis [3, 4].

It is known that apoptosis / necrosis of immunocompetent cells gives ability to organism to eliminate *Mycobacterium tuberculosis* that located inside cell and activates native and adapted immune response of «host-organism», on the other hand, apoptosis / necrosis has destructive effect and allow to spread MTB and increase MTB-population [5]. But these processes in TB patients studied insufficiently and the control values still are not determined.

Aim: to study the integrity of cytoplasmic membranes of white blood cells in patients with pulmonary tuberculosis undergoing anti-tuberculosis treatment.

Materials and Methods

The study was performed on 30 patients with diagnosis of pulmonary TB, who were treated at the Regional Tuberculosis Hospital №1 (Kharkiv, Ukraine). All diagnostic and treatment were prescribed according to the order of the Ministry of Health of Ukraine No. 620 of 14. 09. 2014. The control group consisted of 12 healthy donors.

Characteristic of groups: average age in group of TB patients was 39.3 years, in the control group – 39.9 years. In group of TB patients firstly diagnosed TB cases were registered in 80.5 % and recurred cases – 19.5 %, all cases were proved by positive cultural method, provided on the liquid medias on MGIT BACTEC-960 and / or on the Löwenstein-Jensen solid medias; according to the results of drug susceptibility testing mono- or poly resistance of MTB to the anti-TB drugs was established in 44.7 %; according to radiological methods of patient examination the destruction of pulmonary tissue had 85.8 % of patients, 87.3 % of patients had infiltrative clinical form of TB, others – disseminated TB.

The biomaterial for the study was blood sample collected from the peripheral vein in the morning in a volume of 10 ml and placed in an EDTA tube. Samples were collected at the end of 2 months of standard anti-tuberculosis treatment. The study the integrity of cytoplasmic membranes of WBC was performed by flow cytometry using a FACS Calibur flow cytometer. The assessment of the integrity of cytoplasmic membranes of WBC was performed by adding the markers CD45 +, 7AAD (BectonDickinson, USA) [6].

Statistical processing of the obtained results was carried out by analyzing Microsoft Excel 2016 (license № 00201-10554-16848-AA351) and StatsoftStatistica 8.0. (licenseSTA862D175437Q). Significant differences being considered to be at $p < 0.05$.

The work was performed according to the requirements for researches with the participation of people: Statute of the Ukrainian Association for Bioethics and the GCP norms (1992), requirements and norms of ICH GLP (2002), typical ethics provisions of the Ministry of Public Health of Ukraine 66 dated February 13, 2006.

Results and Discussion

Analysis of the integrity of cytoplasmic membranes of WBC in the blood of pulmonary TB patients showed that the proportion of intact cells was lower by 10,2 % from the results of control group ($p<0.05$). The proportion of injured cells

that reacted with 7AAD was significantly ($p<0.05$) higher in group of TB patients, then in control group (14.2 ± 2.8 % versus 4.3 ± 0.3 %). Results are presented in the Figure 1.

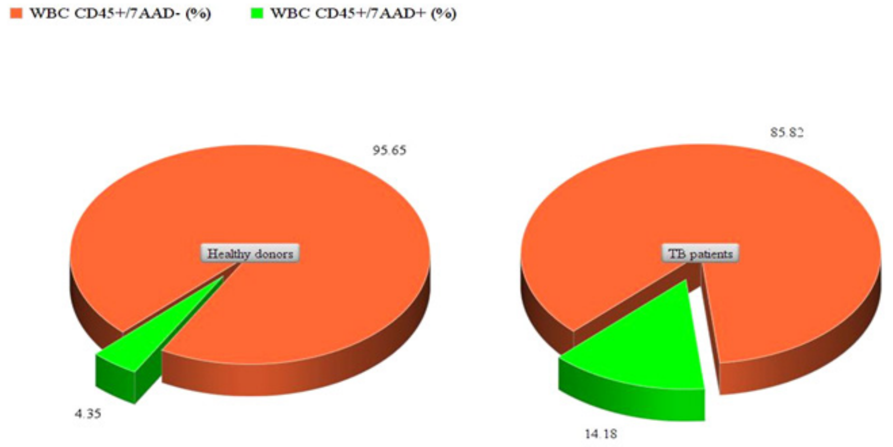

Figure 1. Proportion of intact (WBC CD45+/7AAD-) and damaged (WBC CD45+/7AAD+) white blood cells in groops of TB patients and healthy donors

The quantity of injured WBC in the blood of TB patients was in 2,7 times increased, comparing with control group. This results indicate a decrease in the active WBC's pool of patients with active pulmonary TB. While the study was conducted in the period when patients were undergoing anti-tuberculosis treatment, we can suggest that deficiency of alive and functionally active WBC can be caused not only by progression of disease, but also by the usage of anti-tuberculosis drugs, which requires further study.

Conclusions

Our results suggest that pulmonary tuberculosis is accompanied with deep violation of the integrity of cytoplasmic membranes of CD-45+ white blood cells, because significant ($p<0.05$) difference between proportion of white blood cells that were involved in reaction with 7AAD in groups of TB patients and healthy donors was found.

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NEW BIOLOGICALLY ACTIVE COMPOUND OF *BACILLUS* GENUS

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Abstract. *The known ability of representatives of the genus Bacillus to synthesize a variety of biologically active secondary metabolites provides the basis for raising the question about taxonomic constraints for metabolites of various classes. Via the chromatography-mass spectrometric assay of culture extracts of the three antagonistic strains of the genus Bacillus, 20 compounds were detected, which were previously found only as products of metabolism of other prokaryotes.*

Keywords: *Bacillus, antibiotics, secondary metabolism, polyketids*

Introduction

The genus *Bacillus* includes bacteria with diverse ecological and physiological characteristics. The ability to form resistant endospores, combined with the flexibility of both primary and secondary metabolism, allowed them to settle a significant part of the biosphere [3].

From the applied point of view, one of the most interesting aspects of *Bacillus* biology are biologically active secondary metabolism products, in particular antibiotics and cytostatics. Such classes of secondary metabolites of bacilli, such as lipopeptides, polyketides (which, although, are represented by only three types of compounds), isocoumarine derivatives (in particular amicoumacins), and a certain number of compounds that cannot be classified at least in a set of metabolites if bacillary origin, e.g. aminosugars neotregalosodiamine and canosamine, aminoglycosides butyrosines, lysophospholipidbacillolysin and ieodomycins - modified fatty acids [2, 6, 7, 8]. We can note the relatively low degree of intersection of the groups of these compounds, characteristic of *Bacillus*, with compounds produced by other microorganisms. In particular, amicoumacins, except for bacilli, are characteristic only for actinobacteria of the genus *Nokardia*. In general, the question of the relationship between taxonomic positions of the producer strain and the spectrum of its secondary metabolites is poorly investigated [4].

The aim of this work was to reveal and identify in the culture fluid of antagonistically active strains of bacilli some compounds, new to the representatives of this genus, but present in other prokaryotes.

Materials and methods

As materials of the study, were used 100 strains of endosporeforming facultative anaerobic bacteria isolated from deep-sea bottom sediments of the Black Sea. Sampling was carried out in 2011 during an expedition to the Meteor ship

of the Bremen University. After determining the antagonistic activity against the test strains, the most active isolates of *Bacillus subtilis* 4, *Bacillus megaterium* 14, *Bacillus subtilis* 52 were selected. To determine the spectrum of exometabolites, they were grown on tryptone-soybean broth, and exometabolites were extracted with ethyl acetate and butanol. Organic extracts for each strain were analyzed using liquid chromato-mass spectrometry to identify the spectra of metabolites. Each received fraction was tested for antimicrobial activity. The active fractions were also subjected to liquid chromato-mass spectrometry. The system automatically identified the detected compounds and provided background information on their chemical and biological properties.

From the list of identified metabolites for analysis in this work, were selected compounds of prokaryotic origin, except those which were known for representatives of the genus *Bacillus*.

Results and discussion

In order to simplify the understanding, we will later name the compounds found in bacilli, but previously not known for this group, as "new compounds" (Table 1-3).

A rather interesting fact is the relatively uniform distribution of new compounds by strains, regardless of the total number of metabolites. Thus, in the culture fluid of the strain *Bacillus subtilis* 4 compounds were detected 14 from 90 (15%), *Bacillusmegaterium*14 - 3 of 33 (9%) and *Bacillus subtilis* 52 - 3 of 43 metabolites (6.7%).

All new compounds of strain Bacillus subtilis 52 are attributed to *Streptomyces*, for the *Bacillus megaterium* 14 strain - two to actinobacteria and one for archaea (Table 2), and for the *Bacillus subtilis* 4 strain only compounds known for actinobacteria, cyanobacteria and pseudomonads were available. Microorganisms of all these groups are known as active producers of various secondary metabolites.

By the type of physiological activity, the revealed compounds are very diverse. There are present antimicrobial, antifungal compounds, cytostatics and one compound with herbicidal activity (Table 3).

Among the compounds detected are dominated by non-ribosomal peptides: insulapetolides and micropeptins. Two polypeptides were found - butyrolactol A and azalomycin F3, macrolactoneoctalactinA and one aminoglycoside antibiotic - casugamycin. Quite unexpected was the discovery of a quorum sensing system mediator of *Pseudomonas aeruginosa* - 3-aminodihydro-2 (3H) -furanone- (S) -N-undecanoyl, and a nocardial antibiotic Y 03559J-A with epoxide and nitrile groups in the molecule. One of the cyanobacterial new compounds was not identified (peak with RT 6.33 - *Tolypothrixbyssoide*, Table 1).

The revealed compounds also differ in degree of characterization. Among them there are well-studied substances with known molecular structure and biosynthesis way (e.g., butyrolactol A, casugamycin, etc.), compounds for which only the molecular structure is known (antibiotic Y 03559J-A) and compounds for which, in addition to biological activity only the molecular weight and the class of chemical compounds to which they belong are known (antibiotic MT 10 for which it is known to be a depsipeptide) [1, 5].

Table 1

Secondary metabolites of the strain *Bacillus subtilis* 4

RT	M+H	M	Name	Accurate Mass	Error (ppm)	Biological Source		Use / Importance
4,11	270,206	269,198854	3-Aminodihydro-2(3H)-furanone; (S)-form, N-Undecanoyl	269,199094	-0,89	Pseudomonas aeruginosa	Bacteria	
4,42	326,218	325,210974	Sperabillin C	325,21139	-1,28	Prod. by Pseudomonas fluorescens	Bacteria	Active against Gram-positive and -negative bacteria
5,34	1035,54	1034,533584	Micropeptin 88Y	1034,532452	1,09	cyanobacteria Microcystisaeruginosa	CyanB	Chymotrypsin inhibitor Depsipeptide antibiotic
1,99	328,122	327,115394	KimbeamideA	327,115668	-0,84	Isol. from a consortium of marine cyanobacteria,	CyanB	Moderate sodium channel blocker
5,08	1021,52	1020,517414	Insulapeptolide E	1020,516802	0,60	Nostocinsulare	CyanB	Potent inhibitor of human leukocyte elastase
6,03	1063,56	1062,562484	Micropeptin 88E	1062,563752	-1,19	Prod. by Microcystisaeruginosa NIES-88	CyanB	Chymotrypsin inhibitor
6,33	1491,83	1490,829964	MIXED PEACK	1490,831944	-1,33	from Tolypothrixbyssoide	CyanB	Antifungal agent
7,02	398,227	397,220524	Antibiotic MT 10	397,221287	-1,92	Prod. by Streptomyces indicus	Strpmc	Depsipeptide antibiotic. Struct. Unknown Active against Gram-positive bacteria
7,52	357,228	356,221614	Octalactin A.	356,21989	4,84	by a marine Streptomyces sp.	Strpmc	Cytotoxic
7,63	640,399	639,392244	Antibiotic M 2847	639,398249	-9,39	Streptomyces platensis	Strpmc	Active against Gram-positive organisms
4,77	380,165	379,158284	Kasugamycin	379,159082	-2,10	Prod. by Streptomyces kasugaensis, also from Streptomyces rutgersensis, Streptomyces albulus and Nocardiasp	Strpmc	Inhibitor of the fungus responsible for rice blast
4,61	312,217	311,210004	Antibiotic Y 03559J-A YM-47515	311,209659	1,11	Micromonospora sp. Y-03559	Bacteria	Active against Gram-positive bacteria incl. MRSA

Table 2

Secondary metabolites of the strain *Bacillus megaterium* 14

RT	M+H	M	Name	Accurate Mass	Error (ppm)	Biological Source		Use / Importance
5,82	194,15432	193,147044		193,146664	1,97	from an actinomyceteAmycolatopsis sp.	Actmct	Herbicide
3,27	370,234590	369,227314	Pyrrolysine Derivative: Nα-tert-Butyloxycarbonyl, Me este	369,226372	2,55	methanogenic bacteria Methanosarcinabarkeri	Bacteria	
8,51	1068,719380	1067,712104	Azalomycin F3	1067,650502	57,70	by Streptomyces hygroscopicus var. zalomyceticus ATCC 13810 and a mangrove-derived Streptomyces sp. 21172	Strpmc	Active against Gram-positive bacteria, mycobacteria, yeasts and fungi

Table 3

Secondary metabolites of the strain *Bacillus subtilis* 52

RT	M+H	M	Name	Accurate Mass	Error (ppm)	Biological Source		Use / Importance
4,03	527,323490	526,316214	Butyrolactol A	526,314185	3,86	Prod. by Streptomyces rochei	Strpmc	Active against fungi and yeasts
3,76	420,298480	419,291204	Prodigiosin R1	419,293662	-5,86	by Streptomyces griseoviridis 2464-S5	Strpmc	
	434,310990	433,303714		433,298079	13,00	by a marine-derived Streptomyces sp. NSU893	Strpmc	

Conclusions

The obtained results give grounds for re-estimating the concepts about the connection of certain classes of secondary metabolites with the taxonomic position of its producers. From a theoretical point of view, such a result is not unexpected because of the fact that the genetic determinants of secondary metabolites belong to an unstable peripheral part of the genome. In general, the findings will establish a number of new questions for the genetics and biochemistry bacilli, for which should be devoted further work in this direction.

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UDC 531/534/: [57+61]

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BIOLOGICAL PROPERTIES OF BACTERIA *BACILLUS SUBTILIS* ONU551 AND *AEROMONAS ICHTHIOSMIA* ONU552 – PHENOL DESTRUCTORS

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Abstract. *The biological properties of bacteria of Bacillus subtilis ONU551 and Aeromonas ichthiosmia ONU552 are studied - destructions of phenol. The stamm of Bacillus of subtilis of ONU551 is presented by gram-positive sticks that form субтермінально ендоспори is located. A stamm of Aeromonas ichthiomia ONU552 is gram-negative direct sticks. The features of fat-acid composition of stamms of Aeromonas ichthiomia ONU552 ONU552, B. subtilis ONU551 of destructions - to the phenol, that distinguish them from other bacteria - in cellular lipids each of stamms presence of fat acids : 16: 1 w7c alcohol, 17: 0 iso, 17: 0 anteiso.*

Keywords: *phenol, purification of water, bacteria of destructors, Bacillus subtilis ONU551, Aeromonas ichthiosmia ONU552*

Introduction

The risk of phenolic compounds entering the sewage into the environment is due to their toxicity to biological objects and resistance to decomposition.

Microbiological detoxification is a promising method of purifying the environment, during which the cleavage of the aromatic ring occurs and the formation of non-toxic compounds - carbon dioxide and water [1, 2].

In this regard, the current issue is the development of New Environmentally Safe Biotechnologies for the Purification of Sewage from Phenol [3].

The **aim** of the work was to study the biological properties of bacteria *Bacillus subtilis* ONU551 and *Aeromonas ichthiomia* ONU552 – phenol destructors promising for usein biotechnology wastewater treatment.

Materials and methods

The objects of the study were strains *Bacillus subtilis* ONU551 and *Aeromonas ichthiosmia* ONU552, isolated from wastewater produced by pharmaceutical preparations.

Morphological properties of strains were investigated using classical bacteriological methods.

The analysis of fatty acid profile of strains was carried out by gas chromatography using the system of identification of microorganisms MIDI Sherlock (MIDI, USA). the cultivation of microorganisms was carried out Tryptic soy agar, at 24 ° C for 24 hours.

Lyses and lipids were washed with 50% CH3OH and 3.7 M NaOHat 95-100 °C. for 30 minutes, methylation with acidic methanol solution, 80 ° C., 10 min, neutralization, 0.3 M NaOHsolution. Chromatographic separation was carried out at 170-270 ° C with a gradientof 5 ° C / min.

For the identification of microgranisms, a system for the identification of microorganisms MIDI Sherlock, a library of fatty acid profiles of aerobic microorganisms RSTBA6 Version 6.2 was used.

Results

As a result of the research, it was found that the strain *Bacillus subtilis* ONU551 is represented by mobile, large gram-positive sticks measuring 2.2 × 5.5.0 μm for mingoval endospores that are subterminally placed.

Fatty acid composition of the total bacterial lipids of Bacillus subtilis ONU551 representations in Table 1 and Fig. 1.

Table 1

Fatty acid composition of common lipid bacteria *Bacillus subtilis* ONU551

Fatty acid	% of the total peak areas	Fatty acid	% of the total peak areas
12:0	0.36	16:0 iso	1.85
14:0 iso	0.52	16:1 w11c	1.21
14:0	0.28	16:0	1.30
15:0 iso	34.72	17:1 iso w10c	3.18
15:0 anteiso	33.72	17:0 iso	7.11
15:1 w5c	1.85	17:0 anteiso	10.24
16:1 w7calcohol	1.08	17:1iso I/ anteiso B	2.57

From Table 1 of Fig. 1 shows that in the total bacterial lipids, 14 fatty acids with a predominant content of long chain fatty acids of branched structure 15: 0 (13-methyltetradecanoic acidand 12-methyltetradecanoic acid) and 17: 0 (15-methyl hexadecanoicacidand 14-methylhexodecanine acid) in the form of iso and anteiso.

The methyltetradecanoic acid iso was in the minor amount, and the anteiso is absent.

The mole fraction of other fatty acids in bacteria *Bacillus subtilis* ONU551 is 3% and lower. *Bacillus subtilis* ONU551 hydroxy acids are absent.

On the basis of morphological properties and fatty acid composition of common lipids, the species belonging the ONU551 strain to *Bacillus subtilis* was confirmed.

The strain *Aeromonas ichthiomia* ONU552 is a gram-negative straight stick with rounded ends, measuring 0.5 x 2.5 microns. In smears, they are located individually. The bacteria *Aeromonas ichthiomia* ONU552 grow at 20-30o C, pH 7.0 a simple nutrient media - MAA.

The spectrum of fatty acids of strain *Aeromonas ichthiomia* ONU552 is presented in Table 2 and Fig. 2. It is evident from the data in Table 2 that in the total of the lipid bacteria *Aeromonas ichthiosmia* ONU552 23 fatty acids were found with

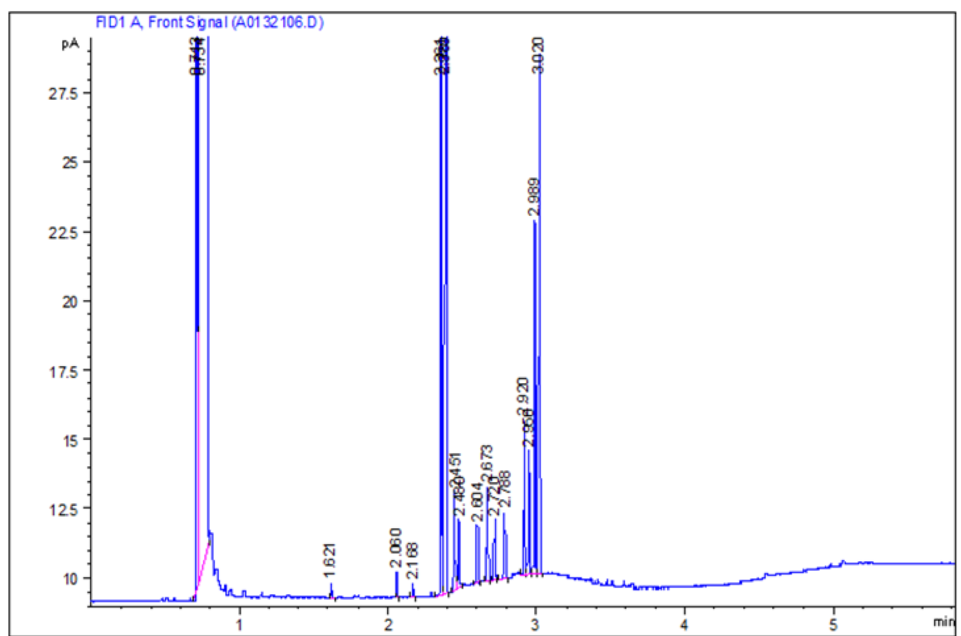

Fig. 1 Fatty acid spectrum of *Bacillus subtilis* ONU551 strain

16: 0 (hexodecanoicacid), the sum of (9-hexodecenic acidand 10-hexodecenic acid) hexodecenicacidsand 11- octadecenicacid.

Table 2

Fatty acid composition of common lipid bacteria *Aeromonas ichthiosmia* ONU552

Fatty acid	% of the total peak areas	Fatty acid	% of the total peak areas
10:0	0.17	∑16:1 w7c/16:1 w6c	36.89
12:0	6.94	16:1 w5c	0.12
12:0 3OH	0.23	16:0	21.84
13:0 iso	0.20	17:1 iso w9c	1.35
13:0	0.15	17:0 iso	1.49
14:0	3.77	17:0 anteiso	0.27
∑14:0 3OH/16:1 iso I	6.59	17:1 w8c	0.60
15:0 iso	0.97	17:1 w6c	0.26
15:0 iso 3OH	3.85	17:0	0.33
15:0 3OH	0.35	18:1 w7c	8.53
16:1 w7c alcohol	3.45	18:0	0.30
16:0 N alcohol	1.34		

The content of other acids wasat a level of 7% or less.

Aeromonas ichthiosmia ONU552 Hydroxy Acids Present and they are biomarkers for the differentiation of this strain at the generic level.

Conclusion

1. The morphological properties of two strains iso lated from sewage – *Bacillus subtilis* ONU551 strain represented bygram-positivesticks, which form subterminally located endospores, strain *Aeromonas ichthiomia* ONU552 - gram-negative straight sticks.

2. Specific feature sof the fatty acid composition of strains *A. ichthyosis* ONU552, *B. subtilis* ONU551 destructors- phenol that distinguish them from other bacteria - in cellular lipids of each strainthe presence of fatty acids: 16: 1 w7c alcohol, 17: 0 ISO, 17: 0 anteiso.

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THREE DIMENSIONAL RECONSTRUCTION MODEL OF HUMAN NECK MORPHOGENETIC STAGES AT EMBRYONIC AND PREFETAL PERIODS OF PRENATAL DEVELOPMENT

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Abstract. *This work is devoted to practical use of three-dimensional reconstructions methodin anatomical micro-structures investigations in human embryos (specifically neck region). Method of morphological 3d reconstruction is based on series of consequent specimens (histological, macroscopic or computer tomograms) which are further transformed into digital image that can be studied or measured. As a result, this method allows to receive a virtual copy of the real anatomical structure or reconstruction can be used for modeling, variant anatomy, age changes, organ movements, pathological process, etc.*

Key words: *prenatal visualization, three-dimensional reconstruction,human embryology.*

Introduction

Morphological studies on human prenatal development are aimed to study out features of human formation on each of the developmental stages of organogenesis and chronical consistency. Nowadays morphologists use new methods of investigation that complement classical one for a proper visualization of macro- and microstructures, especially when talking about human prenatal development [7]. Three dimensional reconstruction is one of them, as it gives opportunity to study a real model of anatomical structures, obtained previously by the means of series of histological slides [2, 3]. Our study shows possibilities of this method while investigating prenatal morphogenesis of the head and neck region in human embryos.

Materials and methods

We have used 6 specimens of human embryos and prefetuses (10,0-80,0 mm of parieto-coccygeal length (PCL) to investigate early developmental changes in the head and neck region. Material was obtained from Chernivtsy Regional Pathologists Office, concerted by bilateral agreement on scientific cooperation with the Department of Histology, Cytology and Embryology of BSMU. The research was approved by Ethics Committee of BSMU as the one that follows the provisions of Helsinki Declaration (2000) on researches, that involve human materials. Obtained material was fixed, dehydrated and concluded in paraffin blocks. Histological sections from the head and neck region were prepared on microtome with sagittal cuts

at a thickness of 10 microns with simultaneous photographing of the paraffin block surface by the means of histological equipment [1, 4]. We have made 50-70 cuts from each specimen.

Results and discussion

Obtained digital images were proceeded in corresponding sequence [1]. We have conducted segmentation (creation of anatomical contours on each of the suitable slide) within computer program [5, 6]; calibration and finally creation of volumetric three-dimensional models. As a result, each piece of material that was enclosed in paraffin block has been recreated in a digital model.

The privileges of using this method is first of all possibility to visualize microscopic structures in human embryo at their length. For example, we can trace vagus nerve or carotid arteries formation and topographic variations at during different periods of organogenesis (during 2-3 months in this case). The problematic question of tracking on the earliest stages of development (10,0-40,0 mm PCL) appears first of all with obtaining necessary material (which means an embryo at specific stage of development) and secondly the condition and position at which material was fixed and embedded in paraffin (damaged neck area makes it impossible to create a proper digital model). Correct obtaining procedure and preservation of material will lead to creation a full and precise virtual digital model. This point is crucial when reconstructing neck, because at 10,0-80,0 mm PCL human embryos this region is quite low at often turned to one of the shoulders.

Secondly, created models give opportunityto conduct morphometric calculations in a program. That is why we can measure size of important anatomical structures that are the prenatal ultrasound diagnostic key points for possible congenitalmalformations occurrence. Investigations of our 6 digital models has shown variations of sternocleidomastoid muscle length and width after splitting from the common precursor muscle mass with the trapezoid muscle; gradual movement and differentiation of the infrahyoid muscles (sternohyoid, omohyoid, thyrohyoid); evaluation of longitudinal indexes of the neck, comparing to transverse ones gradually in late embryonic period (14,0-20,0 mm PCL); topographo-histological changes within hyoid bone and its disposition from earlier to later periods (predominantly 54,0-80,0 mm PCL).

Conclusions

Our research shows that three-dimensional reconstruction is an informative and valid method for early human development processes investigations. Focused on the head and neck region, our studyis an example on how classical morphological methods (morphometry, histological slides preparation, macroscopy) are complemented by creation of digital models for more profound understanding and visualization. Digital models may be used for muscles (superficial and deep neck), blood vessels (arteries and veins), bones and cartilages visualization. Method requires appropriative technical support, fixation of the material and skills on histological slides preparation and comparison, and contorting of anatomical structures.

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CARBOHYDRATE-SPECIFIC ACTIVITY OF LACTOBACILLI AND LACTIC ACID COCCI

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Introduction

Novel antibacterial agents with the capacity to inhibit bacterial biofilms are important for the treatment of bacterial infections. A promising class of antimicrobial molecules is the family of lectins,since lectin molecules have unique hydrocarbon-recognizing capacities, and pathogens are often decorated with sugars that affect their survival and infectivity [Francois et al., 2012]. Lectins are defined as carbohydrate binding proteins without catalytic activity that are prevalent in all organisms. They often have important functions in cell signaling and cellular interactions [Dias et al., 2015].

An additional bonus from the presence of lectins is the increase due to their action of probiotics. Positive effect from probiotics indirectly due to direct or indirect endogenous flora and the immune system modulation. In this case, the direct contact of the probiotic culture with active epithelial cells terminal is the necessary condition of macroorganism niche. Studies have shown that lactic acid bacteria are in contact with epithelial cells and induces the eRNA of some genes. A clear correlation had shown increased expression between eRNA and extracellular secretion of mucin [Barnett, 2018; Yadav, 2015].

Similar to plants and animals, bacteria can also express lectins on their surface, but reports on their characterization, and especially antipathogenic potential, are very scarce[Dias et al., 2015].

The research aim was to investigate carbohydrate-specific activity of lactobacilli and lactic acid cocci that were isolated from different objects.

Materials and methods

Adhesive activity was studied by the method of determining hemagglutinum activity (HAA) of culture fluid (CF) of all bacterial strains examined.

Lactobacillus and *Lactococcus* strains which were isolated from sea sponges of mussels and sturgeon intestineswere used for the study. The studies were conducted using trypsinized erythrocytes of ram, chicken and sturgeon, fixed by glutaraldehyde. The presence of hemagglutinum or lectin activity was determined by the reaction of hemagglutination (RHAA) by double serial dilutions in sterile 96-well polystyrene microplates with U-shaped wells at room temperature [Луцик и др., 1980]. In all cases, in order to prevent errors associated with autoagglutination of erythrocytes, wasput control of 2% of erythrocyte solution in the physiological solution.

A set of 13 carbohydrates and polyhydric alcohols was used to determine a potential lectin oflactobacilli and lactic acid cocci strains. The degree of inhibition

of GAA by carbohydrates was expressed as the minimum dose of carbon necessary for the complete suppression of RGA with sheep, rabbit and chicken erythrocytes.

The 2% suspension of erythrocytes is added at 0.1 cm^3 each into the U-shaped wells of 96-well polystyrene microplates. Then, the culture fluid with the test compounds is added in the same amounts, and the suspension is gently mixed. The microplatesis covered with a lid and placed in a thermostat at a temperature of 37 ° C for one hour.

After the tablet is incubated in a thermostat, it is removed and preliminary results are taken into account. In the presence of adhesive properties in the studied compounds, red blood cells are distributed evenly over the entire surface of the bottom.

With a negative result, all red blood cells are collected in the form of a point in the middle of the well of the tablet. The final results of the study are taken into account 18 hours after incubation of the tablet at room temperature.

Results

As we see from table 1, the carbohydrate-specific activity of lactobacilli, which is possibly due to the presence of free lectins isolated from sea sponges, has a fairly high variability in terms of the ability to bind to carbohydrates and polyhydric alcohols. According to our data, potential lectins of the strains of *Lactobacillu parabuchneri* ONU10.1, *Lactobacillus vaccinostercus* ONU22 and *Lactobacillus bifermentans* ONU68 are not associated with xylose, and *Lactobacillus parabuchnerus* ONU10. 2a, *Lactobacillus parabuchneri* ONU19.2b and *Lactobacillu parabuchneri* ONU52.1. That is, the only strain that is not able to bind to pentoses is the *Lactobacillu parabuchneri* ONU10.1 strain.

By the level of lectin binding activity, we can separate both the most inactive strain (*Lactobacillus vaccinostercus* ONU2) and the most active strains (*Lactobacillus parabuchneri* ONU39, *Lactobacillus bifermentans* ONU68 and *Lactobacillus bifermentans* ONU53).

Strains of lactic acid bacteria, isolated from sturgeon intestines, showed greater ability to bind carbohydrates and polyhydric alcohols (Table 2) and less variability of this trait.

We also noticed that, in contrast to the data given for strains isolated from sponges, the potentially lectins of strains of lactobacilli isolated from sturgeon are actively associated with sucrose, to which almost no lectins of strains isolated from sponges were bound. The lectins of these strains were least active in relation to fructose and xylose. According to the final results, it was shown that the minimum activity with respect to sugar and polyhydric alcohols demonstrates the strain of *Lactobacillus sp*. ONU2.8 (Tab. 2). The maximum activity is demonstrated by strains of *Lactobacillus sp*. ONU3.6, *Lactobacillus sp*. ONU2.7 and *Lactobacillus sp*. ONU2.2 (Tab. 2). When analyzing the results of the hydrocarbon-specific activity of lectins to hydrocarbons and polyhydric alcohols of strains that were isolated from mussels, we noticed that they are worst associated with fructose, mannose, lactose and glucose. Conversely, they are actively associated with galactose, xylose, maltose, sucrose and mannitol (Tab. 3).

Table 1

Carbohydrate-specific activity of strains of lactobacilli isolated from sea sponges in relation to carbohydrates and polyols

	Rhamnose	Xylose	Dulcite	Galactose	Maltose	Sorbitol	Glucose	Fructose	Mannose	Sucrose	Mannitol	Lactose	Arabinose
Lactobacillus vaccinostercus ONU2	–	+	–	–	+	–	+	–	+	–	+	–	+
Lactobacillu parabuchneri ONU10.1	–	–	–	+	+	+	+	+	+	+	–	–	–
Lactobacillus bifermentans ONU10.2	+	+	–	+	+	+	+	+	+	–	–	–	–
Lactobacillus bifermentans ONU19.2a	+	+	–	+	+	–	+	+	+	+	–	–	–
Lactobacillus parabuchneri ONU19.2b	–	+	–	+	+	+	–	+	+	–	–	–	+
Lactobacillus vaccinostercus ONU22	+	+	+	–	+	–	–	+	+	+	+	–	–
Lactobacillu parabuchneri ONU52.1	+	+	+	+	+	–	+	+	+	–	–	–	–
Lactobacillus bifermentans ONU53	–	–	+	+	+	+	+	–	+	+	+	–	+
Lactobacillus bifermentans ONU68	+	–	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus parabuchneri ONU39	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2

Carbohydrate-specific activity of strains of lactic acid bacteria isolated from sturgeon in relation to carbohydrates and polyols

	Mannose	Dulcite	Arabinoza	Rhamnose	Galactose	Maltose	Glucose	Lactose	Xylose	Sucrose	Sorbitol	Mannitol	Fructose
Lactobacillus sp. ONU1.1	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU1.2	+	+	+	+	+	+	+	+	+	++	+	+	±
Lactobacillus sp. ONU1.3	+	+	+	±	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU1.4	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU2.1	+	±	+	+	+	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.2	++	+	+	+	+	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.3	+	+	+	+	+	++	+	+	+	+	+	+	±
Lactobacillus sp. ONU2.4	+	+	+	+	+	+	±	+	+	+	+	+	±
Lactobacillus sp. ONU2.5	+	+	+	++	+	+	+	+	+	+	+	+	++
Lactobacillus sp. ONU2.6	+	+	+	+	±	+	+	+	+	++	+	+	+
Lactobacillus sp. ONU2.7	+	+	++	+	+	++	+	+	+	+	+	+	±
Lactobacillus sp. ONU2.8	+	+	+	+	+	+	++	±	+	++	±	+	+
Lactobacillus sp. ONU3.1	+	+	+	++	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.2	+	+	+	±	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.3	+	+	+	±	+	+	+	+	+	+	++	+	+
Lactobacillus sp. ONU3.4	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus sp. ONU3.6	+	+	+	+	++	+	+	+	+	++	+	+	+

Table 3

Carbohydrate-specific activity of strains of lactic cocci isolated from mussels in relation to carbohydrates and polyhydric alcohols

	Mannose	Dulcite	Arabinoza	Rhamnose	Galactose	Maltose	Glucose	Lactose	Xylose	Sucrose	Sorbitol	Mannitol	Fructose
*Lactococcus sp.*LM7	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactococcus sp. LM8	+	+	+	+	+	+	+	+	+	±	+	+	++
Lactococcus sp. LM10	+	+	+	+	+	+	+	±	+	+	+	±	+
*Lactococcus sp.*LM25	+	+	+	++	++	+	+	+	+	+	+	+	+
Lactococcus sp. LM27	±	+	+	–	++	++	++	++	+	++	++	+	±
Lactococcus sp. LM30	±	+	++	+	++	++	±	++	++	++	+	++	±
Lactococcus sp. LM31	±	+	+	++	++	++	±	±	++	++	+	++	±
*Lactococcus sp.*LM32	±	+	++	++	++	++	±	±	++	++	+	++	±
*Lactococcus sp.*LM33	±	+	+	+	+	++	++	±	++	++	+	++	±

Lectins of the strain *Lactococcus sp*. LM27 were the only ones that did not bind to rhamnose. Lectins of strains of *Lactococcus sp*. LM30, *Lactococcus sp*. LM33, *Lactococcus sp*. LM31 and *Lactococcus sp*. LM32 polyhydric alcohols (Tab. 3).

Conclusion

A study of lactobacilli and lactic acid cocci isolated from sea sponges, mussels and sturgeon intestines showed a significant difference between these strains in their ability to form lectins and their ability to bind to various carbohydrates and polyhydric alcohols.

Thus, we have shown that the hydrocarbon-specific activity of lactic acid bacteria is a fairly variable factor, which depends on the source of isolation and the activity of the strain in the sense of adhesive activity.

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THE CHARACTERISTICS OF CELLULAR DIMORPHISM OF SOME *CANDIDA ALBICANS* STRAINS

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Abstract. *The study revealed clear morphological differences for both colonies of C. albicans and at the cellular level. The data obtained indicate that strains of C. albicans, which are characterized by the ability to form hyphae, form on the surface of Spider agar colonies with wrinkles on the surface. Smooth colonies of C. albicans, in most cases with a matte surface, consist of yeast-like cells.*

Key words: *cellular dimorphism, Candida albicans, colony morphology, cell microscopy*

Until recently, opportunistic pathogens *Candida albicans* in the human body were considered mainly as representatives of normobiota. However, in recent years there has been an increase in the number of visceral forms of candidiasis, a feature of which is the polymorphism of their clinical features, as well as many sites of localization of *C. albicans* in one macroorganism. Thus, in many patients fungi were detected from mucous membranes, skin, nail rollers, urine and feces. These pathogenic strains of *C. albicans* fungi differed in a number of biochemical and physiological properties from fungi of the same species isolated from virtually healthy individuals [6].

One of the main virulence factors that determine the pathogenicity of the fungus *C. albicans* is the ability of fungal cells to dimorphism, which determines the course of the invasion, which leads to the development of candidiasis [8].

Cellular dimorphism is accompanied by a change in two forms of growth in the life of the fungus depending on the conditions of cultivation. *In vivo C. albicans* is a polymorphic microorganism that can exist either as a true yeast-like fungus with an oval shape of cells (up to several microns in diameter) or as undivided hyphae (about 2 nm in diameter), as well as elongated ellipsoidal cells with constrictions, so-called. pseudohyphae (2.8 nm in diameter) [3].

It is known that *C. albicans* cells undergo a reverse morphological transition from yeast to hyphae in response to various environmental signals. The range of environmental factors that contribute to the filamentation of *C. albicans* is quite wide: temperature 37-40 °C, pH about 7.0 and others. For example, at low pH (<6) *C. albicans* cells predominantly grow in yeast form, whereas at high pH (> 7) hyphae growth is induced. In addition, certain chemicals are required, such as N-acetyl-D-glucosamine, amino acids, biotin, sulfhydryl compounds, heme group, zinc, serum, etc. However, so far no "universal" factor has been identified that could be used to artificially switch the morphology of *C. albicans* [1].

Today, *C. albicans* cells have been shown to be able to regulate their own morphology in at least two circumstances: a slow response to adverse nutrient media and a rapid response to certain physicochemical conditions. In the latter case, filamentous forms develop under conditions of temperature and pH, which are found in the tissues of the host in vivo [3].

As a rule, fungi of *C. albicans* are components of microbial associations in which mutual influences of participants of associations on each other and on a macroorganism are closely intertwined. These influences can be carried out in the following main directions:

1) associates can change the biological properties, stimulate or inhibit the reproduction and development of the main pathogen;

2) in the conditions of new mutual relations between microbes their influence on a macroorganism can change both at the expense of strengthening of virulence of the activator, and at the expense of formation of the new factors aggravating a disease course;

3) additional human sensitization by microbes included in the association [9].

In this regard, an important addition to establishing the pathogenicity of the strain is to study the relationship of the main virulence factors of different strains of *C. albicans* and study their changes in vitro when cultured in liquid and dense nutrient media, which are most common in studies of these microorganisms , in order to clarify their role in various manifestations of candidiasis and the response to antifungal drugs [9].

Materials and methods

Strains of *C. albicans* UKM U-2501T (ONU 415), UKM U-1918 (ONU 423), UKM U-1518 (ONU 425), ATCC 18804, which were obtained from the collection of cultures of microorganisms of the Department of Microbiology, Virology and biotechnology of ONU named after I.I. Mechnikov.

Preliminary cultivation of the studied microorganisms was carried out for 24 hours on beveled meat-peptone agar at 37 ^{O}C.

The study of the peculiarities of the process of "switching" the development of used strains of *C. albicans*, ie the ability to dimorphism, was carried out using nutrient media of the following composition:

• Spider agar (CA, g/l: meat-peptone broth - 20.0; mannitol - 20; K_2HPO_4 - 4.4; final pH value 6.5 ± 0.2);

• Sabouraud agar (AS, g/l: peptone - 10.00; casein hydrolyzate - 5.00; peptic digestion of animal tissue - 5.00; glucose - 40.00; chloramphenicol - 0.05; agar-agar - 15, 00, the final pH value of 5.6 ± 0.2);

• Mueller's agar (AM, g/l: meat infusion - 300.00; casein acid hydrolyzate - 17.50; starch - 1.00; glucose - 20.00; agar-agar - 17.00; final pH value 7.3 ± 0.1);

• corn agar (KA, g/l: corn flour - 50.00; agar-agar - 15.00; final pH value 6.0 ± 0.2) [4].

Sterilization of nutrient media was carried out by autoclaving at 1 atm, if it did not contain carbohydrates, and at 0.5 atm in the presence of them in the composition. The medium was then poured into Petri dishes for solidification and drying.

Sowing of daily cultures of microorganisms on appropriate media was per-

formed by the classical method in aseptic conditions using a bacterial loop. The biomass of individual strains was distributed by sectors of the nutrient medium, which was poured into one Petri dish. This allowed to create the most similar conditions for the development of different strains of the studied microorganisms. Incubation was carried out at 37 ^{O}C in a thermostat for 3 days.

Observations of the peculiarities of the development of the studied microorganisms were performed every 24 h: the morphological characteristics of the colonies were recorded (photo using Xiaomi Mi A3 camera), as well as their cell structure (crystal violet smear microscopy) using a Zeiss Primo Star light microscope (Germany). magnification x1000, photography was performed using a Canon EOS 750D camera (Japan) and Zen 2 software (ZEISS Efficient Navigation)).

Results and discussion

The study of the process of development of *C. albicans* strains ONU 415, ONU 423, ONU 425 and ATCC 18804 under the conditions of cultivation on dense nutrient media of different composition and morphological changes that occur.

The nutrient media used in the experiment, according to the literature, are used in microbiological studies of fungi of this genus. Thus, Sabouraud agar (AS) is most often used for isolation and cultivation of both saprophytic and pathogenic fungi, in particular *C. albicans* [7]. To determine the invasive potential of cellular forms of *C. albicans* in clinical studies used nutrient medium Spider (SA): mannitol, which is part of it, stimulates the formation of specific cellular forms, including hyphal [1]. Mueller agar (AM) is a modified version of the source Mueller-Hinton medium, which is used to determine the sensitivity of fungi to antifungal compounds according to CLSI standards [7]. Corn agar (CA) was also used, which is one of the main variants of media for the cultivation of fungi *C. albicans* and obtaining chlamydospores during their development [3].

The obtained data on the morphological characteristics of *C. albicans* colonies, which were grown on the surface of dense media, are shown that colonies of different strains have quite different morphological properties when cultured on the surface of dense media of different composition. First of all, all the studied environments contributed to the rather intensive development of microorganisms, with the exception of the spacecraft. On its surface for the entire period of cultivation (for 3 days) only strains of *C. albicans* ATCC 18804 and ONU 425 (UKM U-1518) formed colonies.

However, the most intensive growth of the studied microorganisms was recorded when using Spider agar and Mueller agar. From the first day of incubation, all strains formed colonies, which differed significantly from each other. The most noticeable differences were observed for the studied strains on the surface of CA. Thus, *C. albicans* ONU 425 was characterized by shrunken colonies with a folded surface. With regard to *C. albicans* ATCC 18804, the morphology of the colonies was defined as rounded convex formations with a smooth surface. Strains of *C. albicans* ONU 415 and ONU 423 were characterized by less intensive growth, forming more "fragile" colonies with a glossy sheen.

In the case of the AM environment, the morphology of the studied strains turned out to be somewhat different. For example, for *C. albicans* ONU 425 more

wrinkles were also recorded, but they were less pronounced and were located on the entire surface of the colonies. A similar wrinkled structure was found for *C. albicans* ONU 423. In the case of *C. albicans* ATCC 18804 matte formations with an uneven "wavy" edge were determined. Strain *C. albicans* ONU 415 on this medium accumulated biomass more slowly, forming separate, with a glossy sheen and uneven surface of the colony, which over time did not merge into a continuous association.

When using AS as a nutrient medium for the cultivation of microorganisms, the obtained colonies of the studied strains of *C. albicans* had almost no differences. Their characteristics corresponded to dense matte associations with a uniform smooth surface. Only the strain of *C. albicans* ONU 415, as in the previous case, formed a smaller, with a glossy luster colony.

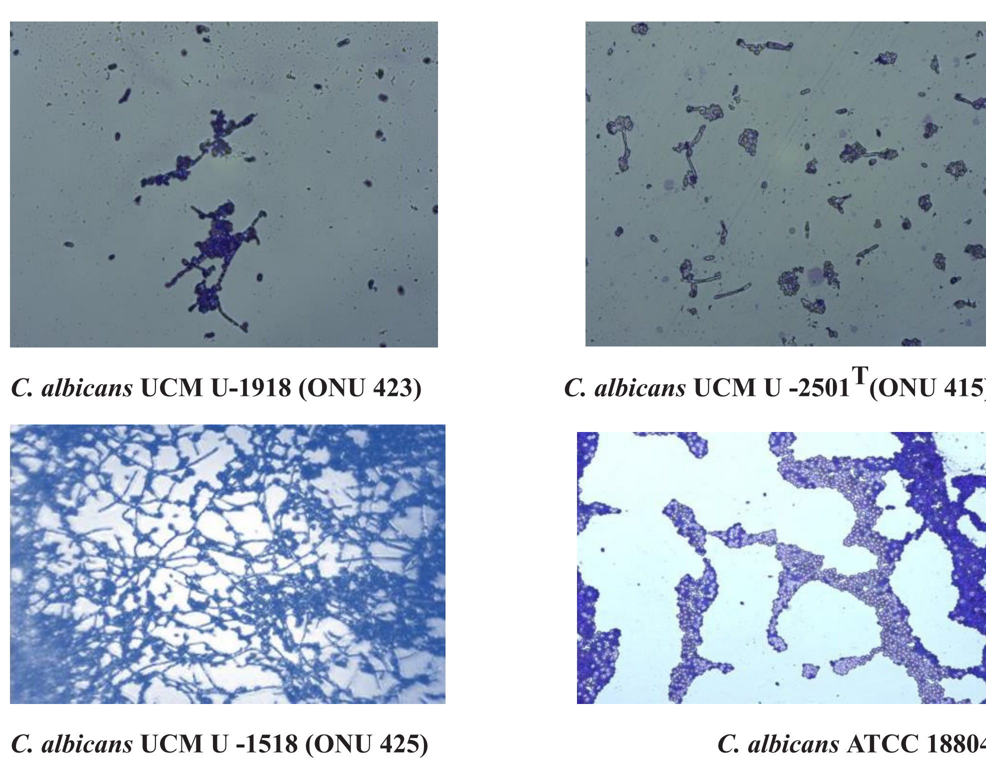
The phenomenon of fungal dimorphism inherent in some species is the ability to form two forms of growth - mycelial, consisting of long, usually branched filaments with or without septa, and yeast - rounded budding cells [1]. These forms under certain changes in conditions, in particular cultivation on different nutrient media, can spontaneously undergo reversible inter-conversions. The competence of a cell of dimorphic fungi, including *C. albicans*, to an alternative growth path allows us to consider it as a bi-stable element and a convenient model for studying phase transitions in morphogenesis, as well as the realization of pathogenic potential. Implementation of the choice of colony shape as one of the possible states of dynamic equilibrium, in turn, leads to the subsequent organization of the microorganism, in general, leading to the formation of different structural structures, differences for which arise not only at the visual morphological level of colonies but also in cellular organization, as well as the genetic and biochemical level [8, 10].

The next stage of the work was a microscopic study of the morphology of *C. albicans* cells that formed colonies on the surface of the studied nutrient media (Fig.).

Due to the fact that visually the greatest differences in the morphology of the colonies were observed during cultivation using Spider agar, the microscopy of cells in the colonies was performed primarily for this variant of the experiment. The presented results on the cell morphology of the studied strains indicate the presence of quite significant differences between microorganisms that were cultured on the surface of CA. Thus, the colonies of *C. albicans* ONU 425 consisted entirely of elongated cells - pseudohyphs, which formed a fairly dense structure. Chlamydospores - rounded "swellings" with a double-contoured shell - were found in some parts of these structures.

For *C. albicans* strains ONU 423 and ONU 415, elongated cells were also recorded, but "normal" oval-shaped yeasts were also present. The ratio of these forms in microscopic examination indicated the following trend: for the first of the cultures, the number of pseudohyphal elements was higher than yeast. However, for *C. albicans* ONU 415 the opposite picture was observed, ie most of the cells in the smears had a rounded shape.

As for *C. albicans* ATCC 18804, the vast majority of cells in the colonies were characterized by a rounded "classic" form for the yeast-like variant of existence.



C. albicans **UCM U-1918 (ONU 423)** *C. albicans* **UCM U -2501T(ONU 415)**

C. albicans **UCM U -1518 (ONU 425)** *C. albicans* **ATCC 18804**

Fig. Morphology of cells of the studied strains of *C. albicans* when cultured on Spider agar medium: photo - original (camera Canon EOS 750D (Japan)); magnification x1000, crystal purple color

Thus, the study revealed clear morphological differences for both colonies of *C. albicans* and at the cellular level. These characteristics were most pronounced when using Spider agar as a nutrient medium. It is known from the literature that not only morphological changes occur during the change of the form of existence of *C. albicans*, but also there is a restructuring of some biochemical processes, in particular the synthesis of pathogenic factors. Therefore, the search for the most suitable and "universal" nutrient medium for assessing the ability of *C. albicans* to switch morphogenesis is a very important area of study of microorganisms of this species.

Conclusions

Thus, the data obtained indicate that strains of *C. albicans*, which are characterized by the ability to form hyphae, form on the surface of Spider agar colonies with wrinkles on the surface. Smooth colonies of *C. albicans*, in most cases with a matte surface, consist of yeast-like cells.

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SYNTHESIS CHARACTERISTICS OF SIDEROPHORES OF DIFFERENT TYPES FORMED BY PSEUDOMONADS UNDER DIFFERENT CULTIVATION CONDITIONS

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Abstract. *The aim of this work was to develop a method of obtaining siderophores and to study the influence of different cultivation conditions, in particular the composition of nutrient media, on the spectrum and level of their synthesis by some members of the Pseudomonas genus. Strains of Pseudomonas chlororaphis ONU 306, Pseudomonas fluorescens ATCC 13325 and Pseudomonas aeruginosa ATCC 10145 produced more intensely chelating compounds during cultivation in King B liquid medium. The corresponding values were higher than 9% for P. aeruginosa ATCC 10145) to 47% (for P. chlororaphis ONU 306). The spectrum of compounds of the siderophore series formed by the studied strains of pseudomonads mainly consisted of substances of hydroxamate and catechol type.*

Key words: *siderophores, pseudomonads, CAS-reagent, nutrient medium*

Introduction

Most aerobic and facultative anaerobic microorganisms synthesize at least one type of siderophores [1]. Today, according to the peculiarities of the structure, there are 3 main groups of siderophores: one of them contains phenolates and catecholates (enterobactin), the other is hydroxamate ligands (ferrichrome) [2]. There is also a third group - with mixed ligands (pyoverdine). In the molecules of catechol siderophores short peptides are acylated with 2,3-dihydroxybenzoic acid, in carboxylate along with amino acids there are also residues of hydroxycarboxylic acids [11].

In terms of chemical structure, many siderophores are modified peptides in which certain groups participate in the formation of the iron-binding center. Hydroxamate siderophores contain residues of ornithine or lysine, to the terminal amino groups of which are attached various substituents [10]. Based on the side chain of the functional group, hydroxamate siderophores are divided into three categories: ferrioxamines, ferricchrome and aerobactin. They are found in *Escherichia coli*, *Pseudomonas* spp., *Klebsiella pneumoniae* [1].

Due to the fact that all siderophores differ significantly in their structure, there is no single system for their isolation [2].

Of the bacterial iron chelators, the most studied today are siderophores of gram-negative bacteria, especially members of the genus *Pseudomonas* [3].

The aim of this 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 species of pseudomonads.

Materials and methods

The study was done on the basis of the Biotechnological Research and Training Center of ONU named after I.I. Mechnikov. *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 a nutrient medium King B, which contained (g/l): peptone - 20; $MgCl_2$ - 1.5; K_2SO_4 - 1.8; yeast extract - 2; glycerol - 10. The pH of the medium was 7.4 ± 0.2. The minimum medium MM9 of the following composition (g/l) was also used in the work: K_2HPO_4 - 0.5; NH_4Cl - 1; $MgSO_4 \times H_2O$ - 0.2; NaCl - 0.5; glucose - 10.0; gluconic acid - 2.5; malic acid - 2.5; casamic acids - 0.5. The pH of the medium was 7.0 ± 0.1. 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) [7].

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 [9]. 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 [5], and the hydroxamate type method - the Atkin method [3].

To detect siderophores by the first method, 1.0 ml of the test sample was sequentially mixed with 1.0 ml of 0.5M HCl, 1.0 ml of nitrite-molybdenum reagent and 1.0 ml of 1M NaOH. The sample was incubated at room temperature for 5 min to develop color. For blank, 1.0 ml of distilled water was used instead of the test sample of culture fluid. The nitrite-molybdenum reagent was prepared by dissolv-

ing 10 g of $NaNO_2$ and 10 g of Na_2MoO_4 in 100 ml of distilled water. If the microorganisms produced catechole-type siderophores, the resulting mixture developed an orange-pink color that was stable for 1 h [4].

Determination of hydroxamate-type siderophores by the Atkin method to 0.05 ml of the test sample was added a 5 mm solution of $Fe(ClO_4)_3 \times nH_2O$ in 0.1 M $HClO_4$. In the presence of siderophores of hydroxamate type, a yellow color was formed.

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 (Table 1).

Table 1

Type of siderophores produced by the studied strains of pseudomonads

A variant of the nutrient medium	Microorganism-producer	Type of siderophores	
		hydroxamate	catechol
MM9	*P. chlororaphis* ONU 306	+	-
	P. fluorescens ATCC 13325	+	-
	P. aeroginosa ATCC 10145	+	+
Кінг В	*P. chlororaphis* ONU 306	++	++
	P. fluorescens ATCC 13325	++	++
	P. aeroginosa ATCC 10145	++	++

Note: ++ - qualitative reaction when determining the type of siderophores was manifested immediately after adding the appropriate components to the nutrient medium; + - signs of the reaction developed at the end of the incubation period; - - the reaction did not take place, ie the production of the corresponding group of siderophores was not carried out.

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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THE ANTIBIOTIC INFLUENCE ON ANTAGONISTIC ACTIVITY OF MICROORGANISMS THAT ARE THE BASE OF SOME PROBIOTICS

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The work was devoted to the study of the strain interactions that isolated from probiotics with some opportunistic pathogens. In the co-cultivation of L. sporogenes and B. clausii probiotic strains, obtained from "Lactovit Forte" and "Enterogermina" preparations and strains of E. coli ATCC 25922 and S. aureus ATCC 25923, an antagonistic interaction was determined. The addition of 0.0313 mg/ml of ciprofloxacin enhanced the antagonistic interaction between probiotic strains, in particular B. clausii and cultures of E. coli ATCC 25922 and S. aureus ATCC 25923.

Key words: *human microbiota, antagonistic activity, probiotic drugs, antibiotics*

Probiotic drugs are microorganisms and substances of microbial and non-microbial origin, which have a natural effect of positive effect on physiological, biochemical and immune responses of the host organism by optimizing the functions of its microbiome status. Most bacteria with biotic properties are members of the genera *Lactobacillus* and *Bifidobacterium*, as well as members of the genera *Propionibacterium, Enterococcus, Escherichia, Leuconostoc, Pediococcus* and yeasts of the genus *Saccharomyces*.

According to the type and number of strains used, probiotic drugs are divided into the following generations:

I - classic monocomponent drugs, which usually contain one strain of bacteria of the genus *Lactobacillus* or *Bifidobacterium*.

II - drugs based on antagonistic microorganisms that have the ability to eliminate themselves: members of the genus *Bacillus*, mainly *Bacillus subtilis*, *Bacillus licheniformis*.

III - combined drugs consisting of several types of bacterial strains (multicomponent) or those containing additives that enhance their action (eg, Acipol, Linex, Bifi-forms);

IV - live bacteria immobilized on the sorbent, representatives of normobiota.

Today, there are certain schemes for the use of probiotics in the treatment of most infectious diseases. Prescribing an antibiotic and then a probiotic is a classic treatment option. The antibiotic is used to destroy pathogenic microorganisms. But during this process there is a high probability of similar activity against represen-

tatives of the normative biota of the macroorganism. Therefore, after a course of antibiotics, a probiotic is prescribed, which restores the host's normobiota. Also, if a person shows signs of dysbacteriosis, without prior antibiotic therapy or other diseases associated with a violation of the microbiota of the body, it is possible to use probiotics. They are also used to stimulate immunity or prevent acute respiratory diseases during epidemics.

But today, the simultaneous use of antibiotics and probiotics is increasingly common. The purpose of this form of therapy is either to enhance the effect of the antimicrobial drug on the background of probiotics, or "mitigation" of severe side effects of antibiotic therapy, for example, with a significant weakening of the macroorganism and the inability to stop the antibiotic. In this case, the antibiotic substance exerts its effect not only directly on the pathogenic microorganism, but also on the basis of the probiotic drug, which can lead to changes in their abilities, namely antagonistic activity.

The aim of the study was to determine the effect of some antibiotics on the form of exposure of strains that are the basis of probiotic drugs "Lactovit Forte" and "Enterogermina" on *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Materials and methods

The microorganisms *Lactobacillus sporogenes* and *Bacillus clausii* isolated from the probiotic Lactovit Forte (Mepro Pharmaceuticals Private Limited, Great Britain) and Enterogermin (Sanofi S. P. A., Italy) were used, as well as the strains obtained from the collection of microorganisms of the Department of Microbiology, Virology and Biotechnology of ONU named after I.I. Mechnikov: *Escherichia coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923.

The studied antibiotic Ciprofloxacin is a synthetic broad-spectrum antibacterial drug and belongs to the group of fluoroquinolones. The minimum inhibitory concentration of this drug against test microorganisms was previously determined using the serial dilution method. Cultures were grown in liquid His medium at 37 °C for 24 hrs with constant shaking. The concentration range of the studied antibiotic was 0.0165 - 2.0 mg/ml, which was prepared by twice diluting the stock solution. The results were recorded in accordance with the recommendations of the European Committee for the Determination of Susceptibility to Antibacterial Drugs (EUCAST). The minimum inhibitory concentration was the lowest amount of antibiotic that was able to inhibit the growth of test microorganisms, i.e. the corresponding sample of the nutrient medium remained transparent (no growth of cultures was observed).

In determining the form of interaction of strains of probiotic drugs and opportunistic pathogens, the following experiment was performed. From 24-day cultures of *L. sporogenes* and *B. clausii* grown on dense MRS medium, cell suspensions were prepared under sterile conditions by washing with sterile saline. The number of cells was standardized by optical density (OD) at 540 nm (immunological spectrophotometer "uQuant" (BioTek, USA)). After that, the obtained cultures were incubated in liquid MRS medium in the presence of an antibiotic, the concentration of which corresponded to the established value of MIC.

In parallel, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were cultured on MPA for 24 hours at 37 °C and a suspension of cells of these microorganisms in physiological saline was prepared.

The main co-growth of microorganisms took place with the simultaneous addition of suspensions of cells of probiotic strains, pre-incubated with (or in the absence of) antibiotic, and opportunistic pathogens for 24 hrs at a temperature of 37 °C. To compare the results and determine the form of interaction of microorganisms, the following cultivation options were used: I - self-cultivation of *L. sporogenes* or *B. clausii*; II - separate cultivation of *E. coli* ATCC 25922 or *S. aureus* ATCC 25923; III - co-cultivation of probiotic strains with opportunistic pathogens. Two series of studies were carried out in parallel: in the first case, the previous cultivation of *L. sporogenes* or *B. clausii* took place without the addition of the antibiotic to the medium, and in the second case, in the presence of the antibiotic.

The results were recorded by determining the optical density of the obtained suspensions at 540 nm (immunological spectrophotometer "µQuant" (BioTek, USA)). The form of influence of probiotic microorganisms on the development of opportunistic cultures was determined according to [3]. According to the obtained values, it was possible to determine the number of cells after co-cultivation, as well as, comparing with the values of monocultures, to determine the form of interaction. If an additive effect was observed, the indicators of optical density after co-cultivation did not differ from the sum of the indicators of each strain: $OD_{mix} = (OD_{probiotic} + OD_{E.coli \text{ or } S.\ aureus})$, i.e. the neutral nature of inter-microbial interaction was registered. With the predominance of OD after co-cultivation ($OD_{mix} > (OD_{probiotic} + OD_{E.coli \text{ or } S.\ aureus})$ - a synergism of microorganisms was observed.

Each experiment was performed in 3 replicates, the number of replicates in each was equal to 10.

Research results and their discussion

The effect of the antibiotic ciprofloxacin on the antimicrobial activity of *L. sporogenes* or *B. clausii* strains, which were obtained from probiotic drugs "Lactovit Forte" and "Enterogermina", respectively, was determined.

The previously established value of MIC for the studied antimicrobial drug was 0.5 mg/ml. Therefore, in subsequent studies, the effect of ciprofloxacin was determined at this concentration on the antimicrobial activity of microorganisms isolated from probiotics. The results obtained in the experiment are presented in the table.

Types of action of antibiotics on the antagonistic activity of probiotics:

suppression - a classic effect, as well as on other microorganisms. The antibiotic kills m / o, or stops their reproduction.

stimulation - when the interaction of antibiotic and probiotic (in a protective shell of alginate), together they acquire new properties.

As can be seen from the presented results, ciprofloxacin did not have a significant effect on strains derived from probiotic drugs. The differences in OD in the cases of monocultures of *L. sporogenes* and *B. clausii* did not exceed 15%, which did not correspond to a significant difference between the respective values. At joint cultivation influence of microorganisms is observed. The addition of the antibiotic determined some changes in it. Thus, in almost all cases there was a decrease

in the OD of suspensions of mixed cultures, under conditions of pre-incubation of strains with ciprofloxacin.

Table

Characteristics of growth of microorganisms in determining their interaction under the influence of ciprofloxacin

Research variant	Type of microorganisms	Experiment in the absence of antibiotics	Experiment with the addition of 0.5 mg / ml of antibiotic
I	*L. sporogenes*	0.405±0.011	0.397±0.004
	B. clausii	0.533±0.005	0.504±0.008
II	*E. coli* ATCC 25922	0.493±0.009	
	S. aureus ATCC 25923	0.434±0.006	
III	*L. sporogenes* + *E. coli*	1.016±0.021	0.823±0.017
	L. sporogenes + *S. aureus*	0.377±0.005	0.546±0.010
	B. clausii + *E. coli*	0.820±0.015	0.631±0.008
	B. clausii + *S. aureus*	0.621±0.009	0.543±±0.016

Note: I - separate cultivation of probiotic strains; II - individual cultivation of test microorganisms; III - co-cultivation of probiotic strains with opportunistic pathogens.

That is, the antibiotic under investigation apparently altered the activity of cultures derived from Lactovit Forte and Enterogermina against *Escherichia coli* and *Staphylococcus aureus* cells. The exception was the co-cultivation of *L. sporogenes* and *S. aureus*: the recorded changes were less pronounced and indicated, in general, the absence of mutual influence of microorganisms. The results of determining the form of interaction, which was conducted in the study, are presented in the figure.

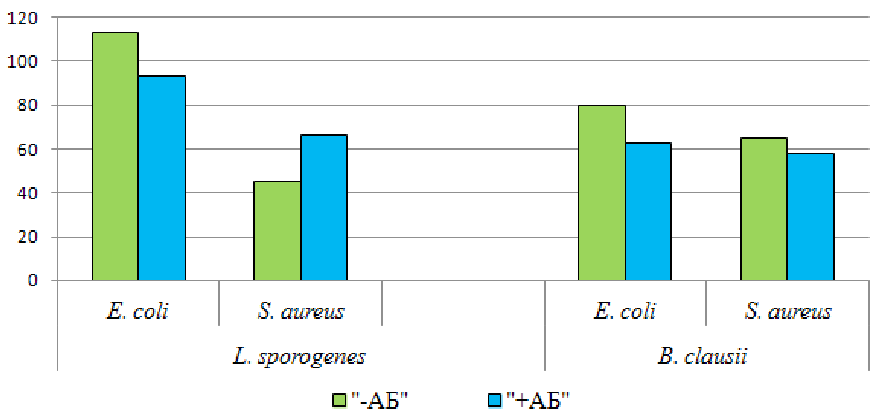

Fig. Characteristics of growth of the studied microorganisms and the form of their interaction during co-cultivation:

y-axis -% increase in biomass with simultaneous co-cultivation compared with the amounts obtained for individual test strains; "-AB" - probiotic strains have not previously been affected by ciprofloxacin; "+AB" - pre-probiotic strains were cultured in the presence of 0.5 mg/ml antibiotic

The obtained results indicate that in all cases of the study developed an antagonistic form of interaction between strains during co-cultivation. This trend was most pronounced for *L. sporogenes* or *B. clausii* and *S. aureus*. The level of OD reduction in co-cultivation was almost 2 times compared with monocotyledonous cultures.

The addition of ciprofloxacin in the pre-cultivation of probiotic strains caused a 7-20% increase in antagonistic activity. The most significant decrease in OD was recorded during co-cultivation of *B. clausii* and *E. coli*.

In general, the observed changes were more pronounced for the probiotic strain derived from Enterogermin.

Thus, the form of interaction that develops in the co-cultivation of probiotic strains of *L. sporogenes* ("Laktovit Forte"), *B. clausii* ("Enterogermina") with opportunistic strains of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923, corresponds to antagonism. The addition of the antibiotic ciprofloxacin during pre-cultivation of probiotic strains enhances their antagonistic effect, most significantly in the case of *B. clausii*.

Conclusions

1. In co-cultivation of probiotic strains of *Lactobacillus sporogenes* and *Bacillus clausii*, obtained from probiotic preparations "Lactovit Forte" and "Enterogermina", respectively, and strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, an antagonist effect was determined.

2. The greatest manifestation of antagonistic interaction is observed in the co-cultivation of *Lactobacillus sporogenes* and *Staphylococcus aureus* ATCC 25923: inhibition of biomass growth was more than 2 times compared with individual cultures.

3. The most pronounced antimicrobial effect of the antibiotic ciprofloxacin had on the development of cultures of *Escherichia coli* ATCC 25922 and *Lactobacillus sporogenes*, which corresponded to the MIC value of 0.0625 mg/ml.

4. The addition of 0.0313 mg / ml ciprofloxacin enhanced the antagonistic interaction between probiotic strains, in particular *Bacillus clausii* and cultures of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

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