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The materials reflect the content of the conference reports, which outline the results and prospects of research in various fields of microbiology and biotechnology, in which young scientists, PhD students, and students. The authors of the abstracts are responsible for the accuracy of the materials and text.

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FORMATION OF THE MULTISPECIES BIOFILM OF PHENOL BACTERIA-DESTRUCTORS ON NATURAL AND SYNTHETIC CARRIERS IN A BIOFILTER

Abstract
Fluorescent microscopy using acridine orange dye confirmed that bacteria phenol-destructors used for water purification formed biofilm on the biofilter media of different nature – zeolite, flaps mussels, synthetic carrier type VII, charcoal, peat, ceramic tubes, sand.

Keywords: phenol, purification of water, bacteria -destructors, biofilms, biofilter.

Introduction

Today priority pollutants of aquatic ecosystems are phenol and its derivatives as by-products of petrochemical enterprises, coal industry, chemical industry, pharmaceutical production, due to their toxicity, ability to accumulate in the environment and sustainability [1].

Sources of phenols in natural waters are drains of petrochemical enterprises, coal industry, mechanical engineering, chemical industry, household drains and drains of pharmaceuticals, dyes, pesticides, phenol-formaldehyde resins and non-ionic surfactants [2].

To prevent negative effects and protect the environment from pollution with phenolic compounds, a biotechnological method is applied using phenol destructors attached to different carriers [3, 4].

The aim of the study was to determine the presence of the bacteria destructors biofilm on carriers of different origin in the biofilter using a fluorescent dye.

Materials and methods

To study the formation of biofilm by museum strains of bacteria:

- *Aeromonas ichthiosmia* ONU552
- *Bacillus subtilis* ONU551
- *Pseudomonas maltophilia* ONU329
- *Pseudomonas fluorescens* ONU328
- *Pseudomonas cepacia* ONU327

on carriers of natural origin (zeolite, ceramic tubes, mussel doors, peat, coal, sand) and synthetic fibers (VII) fluorescent dye acridine orange was used [5].

All carriers were removed from the flow filter after 10 days of operation and treated with 96% ethanol for 15 minutes, after the carriers were stained by immersion in 1% acridine orange solution for 4 minutes. Then all the carriers were washed with water and dried on slides.

The samples were analyzed under a Carl Zeiss fluorescence microscope and a Carl Ceiss, Primo Star light microscope with photo-fixation.

Sterile carriers and fixed smears of the above strains treated with 1% acridine orange served as controls.

Results

The study of the formation of biofilm by strains destructors on carriers showed that on each carrier a biofilm is formed in different ways and in different volumes.

A visual comparative analysis showed (Fig. 1) that mussels, peat, zeolite, peep and ceramic tubes have the formation of a clearly visible biofilm.

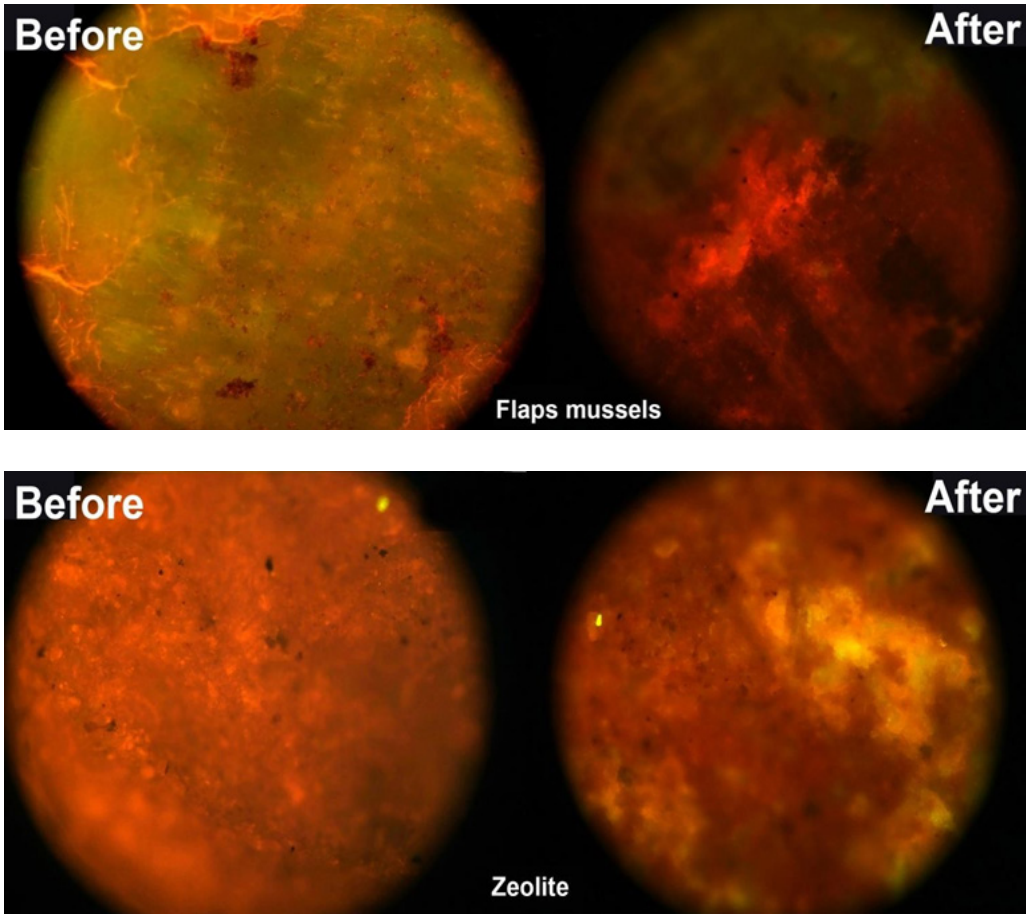

Fig. 1. Photographs of the microbial strains association biofilms on mussel valves and zeolite obtained by fluorescence microscopy after staining with acridine orange

During visual analysis of activated carbon surface under the light and fluorescent microscope the formation of biofilms was not observed. Only single cells in cracks and pores are observed when analyzed on a fluorescence microscope. On synthetic VII carriers no changes were detected after treatment with a fluorescent dye.

References

1. Часова Э. В. Эколого-химические характеристики и методы защиты окружающей среды от фенола / Э. В. Часова, В. В. Ивчук // Вестник Криворожского национального университета. – 2013. - №34(1). – С. 209–213
2. Gudzenko Tatyana, Wolodymyr Iwanycja, Olga Woljuwacz, Boris Galkin, Olga Zuk, Elena Gorszkowa. Biodegradacja fenoli i nnych cyklicznych związków aromatycznych. - Publisher: GlobeEdit is a trademark of International Book Market Service Ltd., member of OmniScriptum Publishing Group, 17 Meldrum Street, Beau Bassin 71504, Mauritius. (ISBN: 978-613-8-25347-1). – 85 p.
3. Nor Suhaila Yaacob, Rosfarizan Mohamad, Siti Aqlima Ahmad . The influence of different modes of bioreactor operation on the efficiency of phenol degradation by Rhodococcus UKMP-5M // Rendiconti Lincei. – 2016. – 27(4). – P. 749-760.
4. Songwen Tan, Xuncai Chen, Chunzhi Cui, Yang Hou, Weiguo Li, Hong You. Biodegradation of saline phenolic wastewater in a biological contact oxidation reactor with immobilized cells of *Oceanimonas* sp.// Biotechnology Letters – 2017. – Vol. 39, Issue 1, P. 91–96
5. Биопленки: основные методы исследования: учебно-методическое пособие / Марданова А.М. с соавт. – Казань: К(П)ФУ, 2016, – 42 с.

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ADHESIVE ACTIVITY OF LACTOBACILLI AND LACTIC ACID COCCI

Introduction

Lactic acid bacteria belong to human useful microbiota and has a great practical significance of its life. Lots of probiotics are based on the use of lactic acid bacteria or lactic acid cocci [4, 6]. Positive effect from probiotics indirectly due to direct or indirectendogenous floraand the immune system modulation. In this case, the direct contact of the probiotic culturewith active epithelial cells terminalis the necessary condition of macroorganismniche. 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 [3, 7].This fact explains the probability of inhibition, with different probiotic strains (such as *Lactobacillus*) epithelial adhesion to pathogenic bacteria, namely enteroinvasive ones *E. coli* [5]. Colonization of the terminal niche or contact with its wall can be achieved by various mechanisms such as adhesion, aggregation, excessive growth or constant use of probiotics [1].

In connection with the identity of glycopherin erythrocytes and glycocalyx epithelial cells, red blood cells are widely used in the study of the adhesive properties of bacteria.

The research aim was to investigate adhesive 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 strains which were isolated from sea sponges of mussels and cattle of sturgeon were 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 [2].

In all cases, in order to prevent errors associated with autoagglutination of erythrocytes, wasput control of 2% of erythrocyte solution in the physiological solution.

Results

Strains of lactobacilli, isolated from sea sponges, showed a rather high level of hemagglutinum activity. The highest rates were 1024HAA was registered for strain *L. bifermentans* ONU16 when using erythrocytes of sheep. For strains *L. parabuchneri* ONU26, *L. vaccinostercus* ONU27 i *L. parabuchneri* ONU55, the hemagglutinating activity rates were lower, – 512 HAA (fig. 7). For all other strains, the hemagglutinum activation rates for erythrocytes of ram 256 HAA (fig. 1).

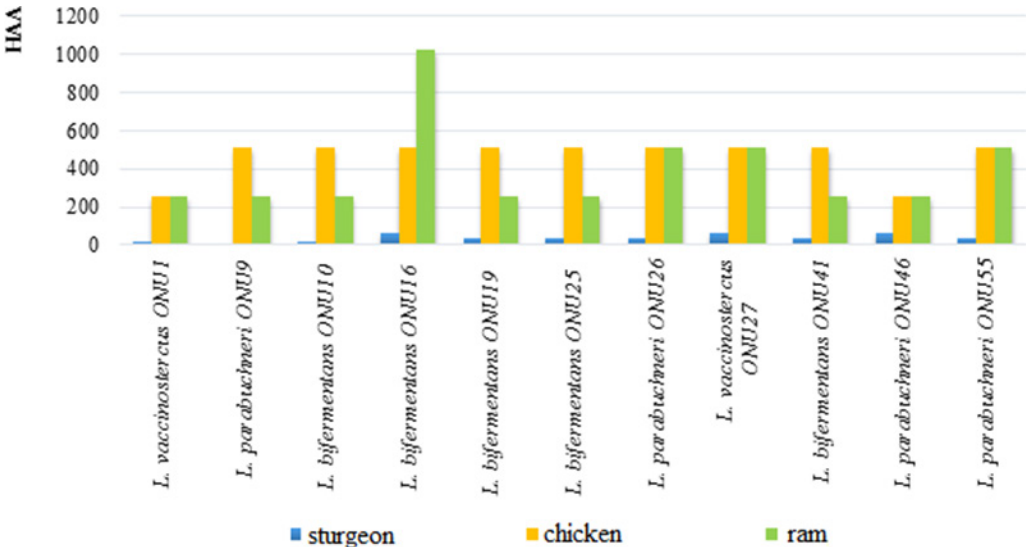

Fig. 1. Adhesive activity of strains of lactobacilli, isolated from sponges

The lactic acid cocci strains isolated from mussels exhibited low adhesive activity to all types of red blood cells (fig. 2).

Strain *Lactococcus sp.* LM33 was the most active: haemagglutinating activity for sheep red blood cells was at 64 HAA, and for sturgeon and chicken red blood cells at 16 HAA levels (fig. 2).

Generally, the adhesive activity of strains of lactic acid cocci isolated from mussels was lower than those of lactobacilli strains isolated from sponges.

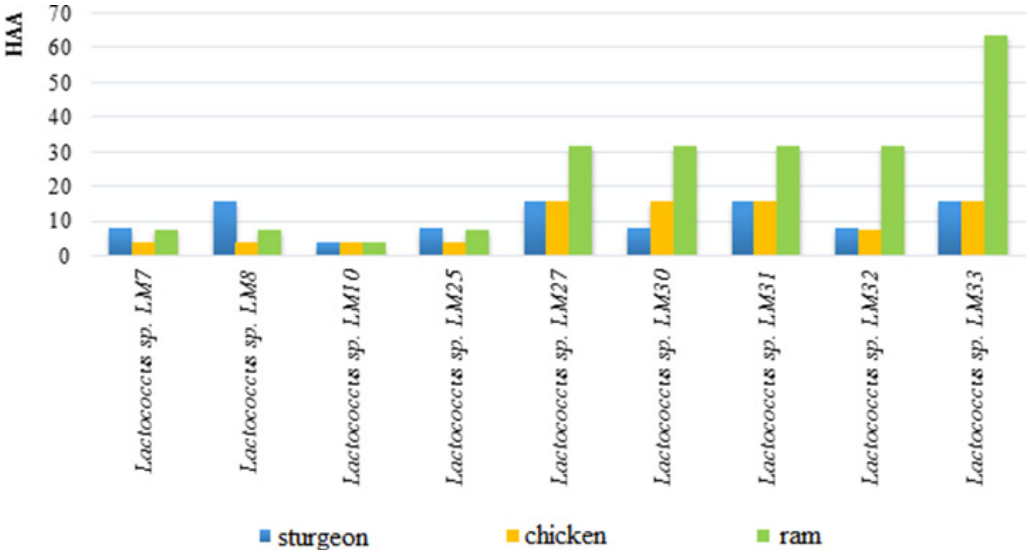

Fig. 2. Adhesive activity of strains of lactic acid cocci isolated from mussels

Also, a fair amount of adhesive activity in relation to all types of erythrocytes was shown when testing strains of lactobacilli isolated from sturgeon (fig. 3).

As in previous cases, the maximum hemagglutinum activity was recorded when sheep red blood cells were used. Indicators at the level of 64 HAA were recorded for strains of *Lactobacillus sp.* ONU 3.2, *Lactobacillus sp.* ONU3.1 and *Lactobacillus sp.* ONU 2.8 (fig. 3).

Strains of *Lactobacillus sp.* ONU 1.3, *Lactobacillus sp.* ONU 2.4 and *Lactobacillus sp.* ONU 3.4 showed the lowest adhesive activity – 16 HAA (fig. 3) relative to sheep red blood cells.

Indicators of antagonistic activity in relation to chicken erythrocytes were quite varied, unlike previous studies. Indicators of hemagglutinating activity ranged from 4HAA to 16 HAA (fig. 3).

When using sturgeon red blood cells, *Lactobacillus sp.* ONU 2.2 (32 HAA) showed the highest adhesive activity.

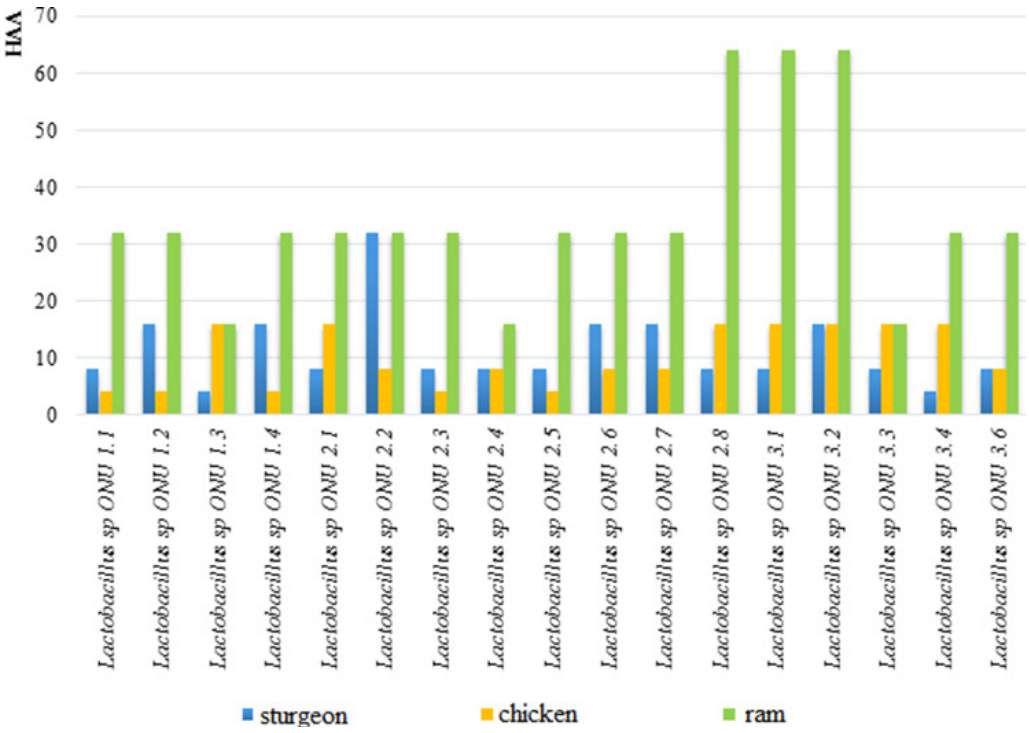

Fig. 3. Adhesive activity of strains of lactobacilli isolated from sturgeon

Thus, it has been shown that as a result of screening among larvae, mussels and sturgeon isolated from larvae, mussels and sturgeon of lactobacillus and lactic acid cocci, based on the indicators of adhesive activity, the probiotic strains of sturgeon are strains of *Lactobacillus sp.* ONU 2.2, *L. bifermentans* ONU16, *L. vaccinostercus* ONU27 and *L. parabuchneri* ONU46.

References

1. Далин М. В., Фиш Н. Г. Адгезины микроорганизмов. – Итоги науки и техники, ВИНИТИ: сер. Микробиология. – М – 1985. –Т.16 – 107с.
2. Луцик М. Д. Методы поиска лектинов (фитогемагглютининов) и определение их иммунохимической специфичности (методические рекомендации для биохимиков и иммунологов). / М. Д. Луцик, Е. И. Панасюк, В. А. Антонюк – Львов: МЗ УССР; Львов. гос. мед. ин-т, 1980. – 20 с.
3. Barnett A. M. Metabolism of caprine milk carbohydrates by probiotic bacteria and Caco-2:HT29–MTX epithelial co-cultures and their impact on intestinal barrier integrity // Nutrient. – 2018. – Vol. 10, No. 7. – P. 949 – 970.
4. Bengmark S., Gianotti L. Nutritional support to prevent and treat multiple organ failur. /World J Surg. – 1996. – Vol. 20. – P. 474-481.
5. Mack D. R., Michail S., Wei S., McDougall L., Hollingsworth M. A. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. //Am.J Physiol. – 1999. – Vol. 276. – P. 941–950.
6. Patterson J. A., Burkholder K.M. Application of prebiotics and probiotics in poultry production. //Poult Sci. – 2003. – Vol. 82. – P. 627–631.
7. Yadav A. K. Adhesion of indigenous Lactobacillus plantarum to gut extracellular matrix and its physicochemical characterization // Arch Microbiol. – 2015. – Vol. 197, No. 2. – P. 155–164.

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ELUCIDATION OF SOME MECHANISMS OF *LACTOBACILLUS PLANTARUM* ATTACHMENT TO PLANT SURFACE

Abstract

Mechanisms of attachment of Lactobacillus plantarum to plants have been studied: surface polysaccharides were suggested to be involved mostly in microcolony formation and teichoic acids – in biofilm maturation. If S-layer proteins were inactivated, no biofilms of L. plantarum on plant surfaces were formed.

Key words: attachment, adhesins, Lactobacillis plantarum, plants.

Introduction

Lactobacilli contribute to protection of mucous membranes in animals colonizing specific econiches and competing for nutrients [Sengupta et al., 2013]. The same happens in plants: being the representatives of normal microbiota, lactobacilli protect plant surfaces from pathogen invasion [Limanska et al., 2019].

Attachment to substrates is a crucial step in bacterial colonization [Velez et al., 2007]. There is scarce information about the adhesion of lactobacilli on plant surfaces [Limanska et al., 2019]. The main groups of adhesins in lactic acid bacteria are polysaccharides, teichoic acids, S-layer proteins [Lorca et al., 2002; Kos et al., 2003; Mobili et al., 2010].

The aim of investigation was to study the involvement of main adhesins in attachment of *Lactobacillus plantarum* to plant surfaces.

Materials and Methods

The study of *L. plantarum* adhesion mechanisms was carried out by the selective inactivation of the major groups of adhesins. Surface polysaccharides were inactivated with sodium metaperiodate oxidation [Lorca et al., 2002]. For this experiment, cell suspensions (10^8 CFU/ml) were incubated in 100 mM sodium metaperiodate solution in 0.1 M CH_3COONa, pH 5.5, for 18 h at 4 °C. After, bacterial cells were washed three times with saline buffer and incubated 1 h in 100 mM $NaBH_4$ at 4 °C. Cells were washed as described previously and re-suspended in MRS.

Teichoic acids were eliminated from bacterial cell walls by incubation of the bacterial cells in 30 % trichloroacetic acid at 37 °C for 1 h [Zárate et al., 2002].

S-layer proteins were eliminated by incubation of bacterial cell suspensions in 5 M LiCl in MRS on ice for 15 min [Kos et al., 2003]. After incubation, cells were washed as described previously and re-suspended in MRS.

The treated cells were brought to the experiment with biofilm formation on *Lepidium sativum* roots as described above. The viability of cells after adhesin inactivation was confirmed by inoculation of the treated bacteria on MRS plates and incubation overnight at 37 °C.

Results and Discussion

The strain *L. plantarum* ONU 355 has been selected for the study of the adhesion mechanisms due to its better ability to form biofilms on garden cress roots as compared with the other strains. Since the attachment of *Lactobacillus* is well studied on epithelial cells [Kos et al. 2003], elucidation of the adhesion mechanisms on plant surface was performed by inactivation of the major groups of the known adhesins – polysaccharides, teichoic acids and S-layer proteins.

Inactivation of the surface polysaccharides with sodium metaperiodate (Figure 1) showed no effect on bacterial cell adhesion but prevented formation of microcolonies.

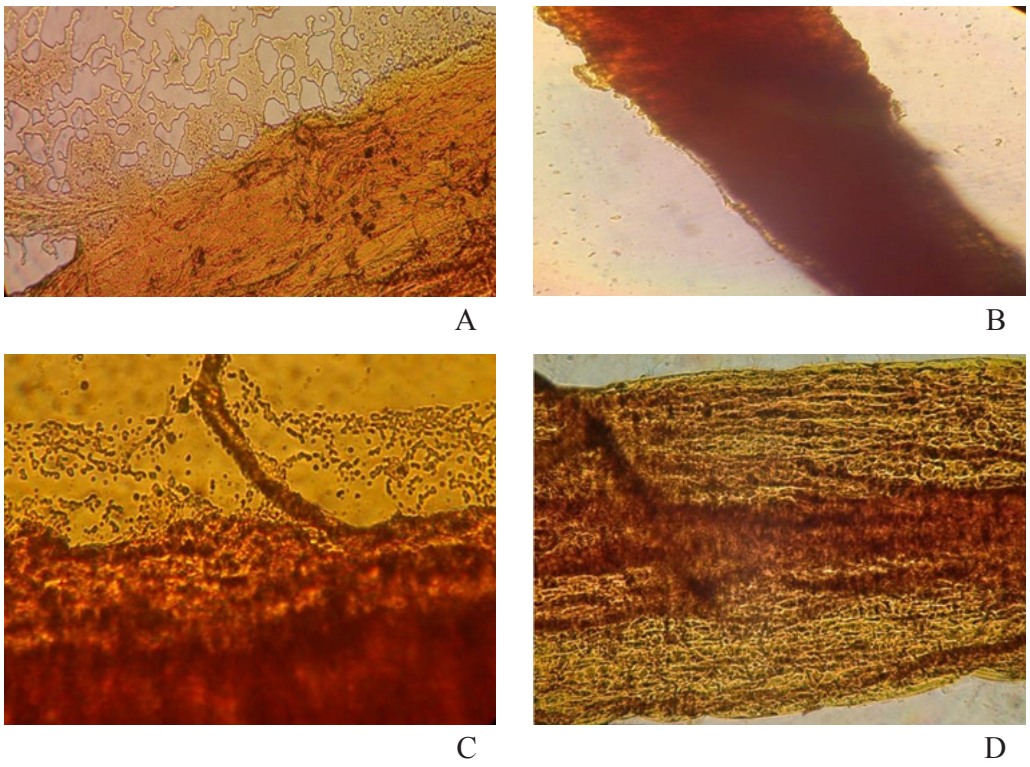

Fig. 1. *L. plantarum* ONU 355 biofilm formation on the surfaces of *Lepidium sativum* roots after inactivation of different groups of adhesins: A – control; B – inactivation of surface polysaccharide; C – inactivation of teichoic acids; D – inactivation of S-layer

Elimination of teichoic acids (Figure 1) resulted in breaches of the *L. plantarum* ONU 355 biofilm. Incubation of the bacterial cells with 30% trichloroacetic acid had no effects on adhesion and microcolony formation, but stopped biofilm maturation. In this case the biofilm had the form of single microcolonies unconnected with each other.

Inactivation of S-layer proteins of *L. plantarum* ONU 355 with 5 M LiCl resulted in total loss of the adhesion ability of bacterial cells on *L. sativum* roots (Figure 1).

Inactivation of S-layer proteins was the crucial – no biofilms were formed. S-layer is a complex of outer proteins in a crystalline form [Mobili et al. 2010]. This structure is located on the outer cell surface and plays several important roles including adhesion determination [Mobili et al. 2010].

Conclusion

Our data suggest that in the system *"L. plantarum* ONU 355 – *Lepidium sativum"* the S-layer plays a key role like an adhesin that causes the primary interaction of the *L. plantarum* cells with the root surface whereas polysaccharides from the cell outer surface are involved in microcolonies formation, and teichoic acids promote biofilm maturation. To fully understand the mechanisms of *L. plantarum* attachment and biofilm formation on plant surfaces further investigations are needed.

References

1. Kos B., Suskovic J., Vukovic S., Simpraga M., Frece J., Matosic S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92 // J. Appl. Microbiol. – 2003. – V. 94. – P. 981–987.
2. Limanska N., Merlich A., Galkin M., Vasylieva N., Choiset Y., Ivanytsia T., Zlatohurska M., Ivanytsia V., Chobert J.-M., Haertlé T. Biofilm formation and genetic diversity of *Lactobacillus plantarum* strains originated from France and Ukraine // Journal of Microbiology, Biotechnology and Food Sciences. – 2019. – 8(6). – P. 1326 – 1331.
3. Limanska N. V., Galkin M. B., Ivanytsia V. O. Effect of *Lactobacillus plantarum* on survival of crown gall agent and tumour formation // Mikrobiologichnyi Zhurnal. – 2019. – 81(1). – P. 22-33.
4. Lorca G., Torino M.I., de Valdez G.F., Ljungh A. Lactobacilli express cell surface proteins which mediate binding of immobilized collagen and fibronectin // FEMS Microbiology Letters. – 2002. – V. 206. – P. 31–37.
5. Mobili P., Gerbino E., Tymczyszyn E.E., Gómez-Zavaglia A. S-layers in lactobacilli: structural characteristics and putative role in surface and probiotic properties of whole bacteria. // Curr. Res. Tech. Ed. Top. in Appl. Microdiol. and Biotech. (Ed) A. Mendez-Vilas. – 2010. – P. 1224–1234.
6. Sengupta R., Altermann E., Anderson R., McNabb W., Moughan P., Roy N. The role of cell surface architecture of lactobacilli in host-microbe interactions in the gastrointestinal tract // Mediators of Inflammation. – 2103. – Article ID 237921. http://dx.doi.org/10.1155/2013/237921
7. Velez M., Keersmaecker S., Vanderleyden J. Adherence factors of *Lactobacillus* in the human gastrointestinal tract // FEMS Microbiology Letters. – 2007. – https://doi.org/10.1111/j.1574-6968.2007.00908.x
8. Zárate G., Morata De Ambrosini V., Chaia A.P., González S. Some factors affecting the adherence of probiotic *Propionibacterium acidipropionici* CRL 1198 to intestinal epithelial cells. // Can. J. Microbiol. – 2002. – V. 48. – P. 449–457.

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CLONAL MICROPROPAGATION OF *PAULOWNIA TOMENTOSA IN VITRO*

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.

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 [4.0 g/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*

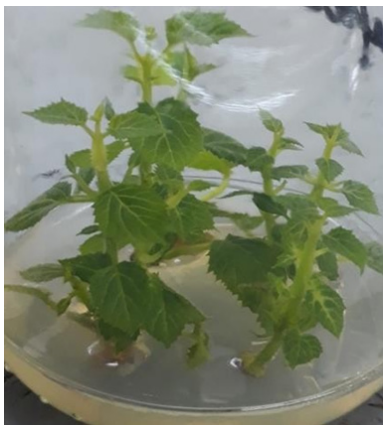

Fig. 2. Rooting and reproduction of microclones on nutrient media

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 (Table 1).

Total vitality on MS(solid) is 40%, total vitality on MS(semi-liquid) is 60%.

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

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 of material from the plants obtained by in vitro gave better results.

References

1. Bhojwani Sant Saran, DantuPrem Kumar. Plant Tissue Culture: An Introductory Text.– 2013. – 318 p.
2. Zelenanska N.N. The technology propagation grape with using culture in vitro. / N.N. Zelenanska, DgaburiaL.V. ,Teslyuk N.I. //. VitiCulture. – 2009.– numb 3 – p. 50–53
3. del Carmen S. J. Histology of the regeneration of *Paulownia tomentosa* (Paulowniaceae) by organogenesis / San José, Mª del Carmen, Cernadas, Mª José, Corredoira, Elena // Revista de BiologÃ•a Tropical.– 2014.– Vol. 62.– pp. 809–818

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DEVELOPMENT OF *LACTOBACILLUS* STRAINS COMPOSITION FOR ENHANCING OF ANTAGONISTIC ACTIVITY AGAINST *SALMONELLA ENTERICA*

Currently, the use of antibiotics for the treatment of most diseases leads to the emergence of the so-called "multiple resistance" in pathogens, which significantly reduces the effectiveness of treatment.

Therefore, controlling infections through a nonantibiotic approach is urgently needed. The potential use of *Lactobacillus* to control typhoid fever represents a promising approach, as it may exert protective actions through various mechanisms.

Lactobacilli have a long history of safe use, especially in the dairy industry [3]. Different *Lactobacillus* strains can function as microbial barriers against gastrointestinal pathogens through competitive exclusion of pathogen binding, modulation of the host's immune system, and production of inhibitory compounds, such as organic acid (e.g., lactic acid and acetic acid), oxygen catabolites (e.g., hydrogen peroxide), proteinaceous compounds (e.g., bacteriocins) and etc [4; 5; 7; 10]. Herewith, the combination of different strains among themselves in different proportions allows to achieve the best result.

The research aim was to investigate change of the antagonistic activity of lactobacilli strains when they are co-cultivated in different combinations.

Materials and Methods

In our study, we used strains of *Lactobaciilus* which were isolated from sea sponges. Determination of antagonistic properties was carried out *in vitro* by a hole-diffuse method [6; 7; 8] in relation to the *Salmonella enterica* NCTC 6017.

At the first stage of optimization, was checked the change of the antagonistic activity provided they were introduced in different combinations, but in equal volume (Table 1).

Table 1

The combination of the investigated strains of lactobacilli

№ experience	Option (combination that was used)	Strains that used
1	**2**	**3**
1	[1+2+3+4]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8+ *Lactobacillus vaccinostercus* ONU2
2	[1+2+3]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8
3	[1+2]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU19.2b

Table continued

1	2	3
4	[1+3]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus parabuchneri* ONU8
5	[1+4]	*Lactobacillus bifermentans* ONU55.1a + *Lactobacillus vaccinostercus* ONU2
6	[4+2]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b
7	[4+3]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU8
8	[4+2+3]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8
9	[4+2+1]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus bifermentans* ONU55.1a
10	[4+3+1]	*Lactobacillus vaccinostercus* ONU2 + *Lactobacillus parabuchneri* ONU8 + *Lactobacillus bifermentans* ONU55.1a
11	[2+3]	*Lactobacillus parabuchneri* ONU19.2b + *Lactobacillus parabuchneri* ONU8

At the second stage, were used the optimization matrices based on the analysis of variance adapted for Greek-Latin squares (Table 2).

Table 2

Matrix for a three-factor experiment for three levels according to the principle of Greek-Latin squares

A	B		
	b1	b2	b3
a1	c1	c2	c3
a2	c2	c3	c1
a3	c3	c1	c2

Calculations to identify the most effective combination, according to the results of an experiment based on analysis of variance, allow us to estimate the influence of existing factors and separate their effects from variability, which is provided by a random [1; 2].

Results

According to the results of an antagonistic study of the investigated strains in previous experiments, we selected four strains that were different in the investigated index relative to the indicator strain *Salmonella enterica* NCTC 6017:

Lactobacillus vaccinostercus ONU2 (low antagonistic activity);

Lactobacillus bifermentans ONU55.1a (high antagonistic activity);

Lactobacillus parabuchneri ONU8 (average antagonistic activity);
Lactobacillus parabuchneri ONU19.2b (average antagonistic activity).

In order to check the change in the level of antagonistic activity of the investigated strains of lactobacilli when co-cultivated, we used the scheme given in Table 1. In this case, the number of nocturnal culture per strain was the same (25.0 μl).

The obtained results showed that there is a change in the antagonistic activity index compared to control in the joint cultivation of strains in relation to *Salmonella enterica*.

The maximum response, with a control over 23.0%, was recorded at pairwise joint cultivation of the strong and second medium strains (variant [1 + 3]), strong and weak strains (option [1 + 4]), weak and first medium strains (option [4 + 4]) and both middle strains (variant [2 + 3]) (Fig. 1).

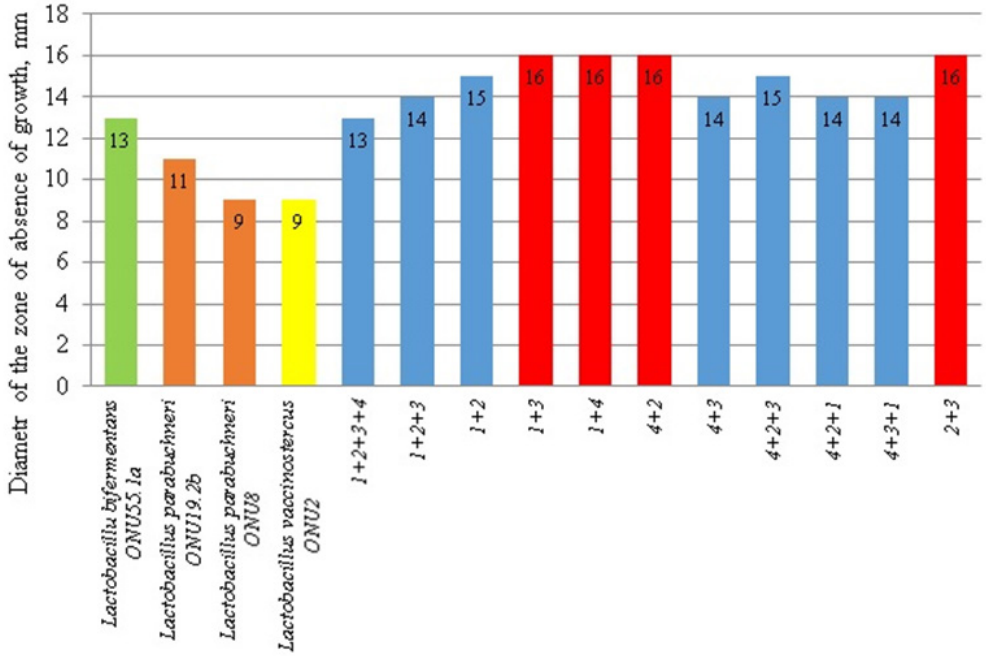

Fig. 1. Antagonistic activity of the investigated strains of lactobacilli in relation to *Salmonella enterica* NCTC 6017 in the joint cultivation of strains

That is, according to the results of the study, it was shown that co-cultivation of lactobacilli strains may increase their antagonistic activity, but for the indicator strain it is necessary to select their combination of strains.

Therefore, in the next series of experiments, we used a mathematical optimization method based on a one-factor dispersion analysis of the Greco-Latin squares matrix adapted to determine the optimal combination of lactobacilli strains, which would increase their aggregate antagonistic activity.

As we can see from the optimization results presented in Figure 2, the maximum effect on the increase of the antagonistic activity of the lactobacillus complex in relation to *Salmonella enterica* NCTC 6017 showed an over-control of over 50.0% in (Fig. 2).

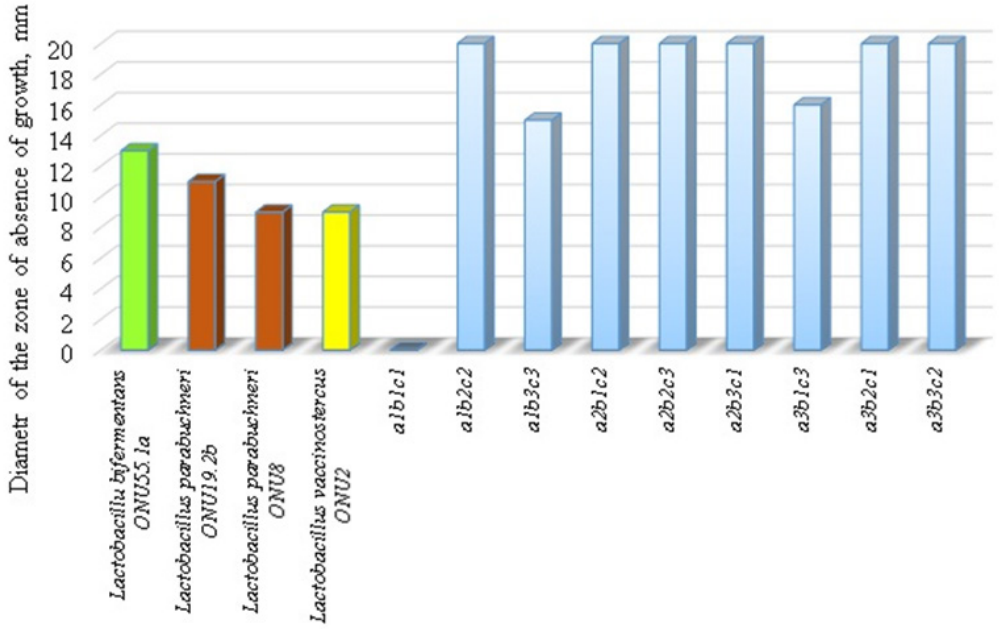

Fig. 2. Antagonistic activity of combinations of investigated strains of lactobacilli in relation to *Salmonella enterica* NCTC 6017 using an optimization matrix

The combination of the strains *Lactobacillus bifermentans* ONU55.1a (25.0 µl), *Lactobacillus parabuchneri* ONU19.2b (25.0 µl) and *Lactobacillus parabuchneri* ONU8 (50.0 µl) in the final volume appeared to be the most effective against the indicator strain *Salmonella enterica* NCTC 6017. 5.0 ml of MRS medium.

That is, losing all the results we can say that the use of compositions lactobacilli significantly increases the level of antagonistic activity against opportunistic bacteria, and mathematical planning helps to find the optimal combination.

References

1. Ахназарова С. Л. Методы оптимизации в химической технологии: учебное пособие для хим.-технол. спец. вузов./ С. Л. Ахназарова, В. В. Кафаров. – 2-е изд. – М.: Высш. шк., 1985. – 327 с.
2. Батрак А.П. Планирование и организация эксперимента: Учебное пособие к изучению теоретического курса для студентов направления / А. П. Батрак – Красноярск: ИПЦ СФУ, 2010. – 60 с.
3. Borriello S. P. Safety of probiotics that contain lactobacilli or bifidobacteria. / S. P. Borriello, W. P. Hammes, W. Holzapfel // *Clinical Infectious Diseases.* – 2003. – Vol. 36, No 6. – P. 775–780.
4. Gänzle M. G. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage / M. G. Gänzle // Curr. Opin. Food Sci. – 2015. – V. 2. – P. 106–117.
5. Magnusson J. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. / J. Magnusson, K. Ström, S. Roos, J. Sjögren, J. Schnürer // *FEMS Microbiology Letters.*–2003. –P. 219, 129–135.

6. Presti I. Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their in vitro effect. / I. Presti, G. D'Orazio, M. Labra // Appl Microbiol Biotechnol –2015.–P. 99, 26.
7. Servin A. L. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. / A. L. Servin // *FEMS Microbiology Reviews*. –2004.–P. 28, 405–440.
8. Schillinger U. (1989) Antimicrobial activity of Lactobacillus sake isolated from meat. / U. Schillinger, F. Lucke // J Appl Environ Microbiol. –1989. –P. 55.
9. Sharma C. Antibacterial effects of *Lactobacillus* isolates of curd and human milk origin against food-borne and human pathogens. / C.Sharma, B. P. Singh, N. Thakur // Biotech. –2017–P. 7, 31.
10. Valerio F. Production of phenyllactic acid by lactic acid bacteria: an approach to the selection of strains contributing to food quality and preservation. / F. Valerio, P. Lavermicocca, M. Pascale, A. Visconti. // *FEMS Microbiology Letters*. –2004– P.233, 289–295.

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SEARCH FOR NEW BIOTECHNOLOGICALLY VALUABLE LACTOBACILLI STRAINS

Abstract

Aim. *The aim of this work was to study the antibacterial potential and salt tolerance of lactobacilli strains isolated from different sources.* ***Methods.*** *The antagonistic activity of lactobacilli was determined by the diffusion method in the agar. The stability to NaCl was carried out by determining the optical density of the microorganisms suspension in the MRS liquid medium with an appropriate concentration of sodium chloride.* ***Results.*** *Lactobacilli strains were observed the highest degree of antagonistic activity to E. coli and P. aeruginosa test cultures. C. albicans was the weakest sensitive culture. The most active were strains isolated from self-fermenting eggplants (Odesa region). The vast majority of lactobacilli was tolerant at 2.5-5.0 % NaCl. The increasing NaCl concentration to 7.5 % has resulted in a decrease of the viable cells number and its growth intensity. The primary source of strain isolation does not affect the final resistance to NaCl and antagonistic activity. The resistance to NaCl is not related to the antagonistic activity of most lactobacilli strains.* ***Conclusion.*** *Increased content of sodium chloride in nutrient medium don't inhibit the growth of the investigated lactobacilli strains and, moreover, don't affect on their antagonistic ability, which is very important and essential for the creation and production of probiotic products with functional purpose.*

Key words: lactobacilli, probiotic properties, antagonistic activity, salt tolerance, clustering.

Lactic acid bacteria belonging to the genus *Lactobacillus* today are one of the most important and perspective biotechnology objects. These microorganisms attract the close attention for a long time, and their careful study are due, first of all, to the variety of positive effects provided on the human body and animals [1, 8, 12]. The results of numerous experimental and clinical studies indicate a pronounced prophylactic and therapeutic efficacy of probiotic and functional nutrition products based on industrial lactobacilli strains [6, 9, 11].

Nevertheless, the search for new strains of bacteria of the genus *Lactobacillus* to create modern probiotic and products of functional nutrition remains actual. The use of lactobacilli new strains in biotechnology for the production of probiotics becomes possible only after a detailed study of their biological properties, in particular antagonistic activity [3, 4]. Moreover, a great attention is paid to a number of technological properties, particularly of salt tolerance [12].

The purpose of this work was to study the antibacterial potential and salt tolerance of lactobacilli strains isolated from different sources.

Material and methods

34 lactobacilli strains isolated from self-fermenting vegetables, raw meat material and children feces, and 6 strains from the culture collection of microorganisms of the Department of Microbiology, Virology and Biotechnology Odesa I. I. Mechnikov National University (*Lactobacillu buchneri* ATCC 4005, *L. acidophilus* ATCC 32200, *L. plantarum* VTCC 0921, *L. plantarum* UCM B 11/16 та *L. plantarum* UCM B 2209) were used in experiments.

The antagonistic activity of lactobacilli was determined by the diffusion method in the agar [2]. Eukaryotic and prokaryotic microorganisms were used as the test cultures: *Candida albicans* UCM У 2501^{T}, *Esherchia coli* UCM B 906, *Bacillus subtilis* ONU-24, *Pseudomonas aeruginosa* ONU-211, *Staphylococcus aureus* ONU-223. The evaluation of the results was carried out by measuring the diameter of the lack of growth zone, oriented towards the zone of complete suppression of visible growth.

The degree of antagonistic activity was determined by the following criteria: the diameter of the zone of growth absence 1–15 mm means low antagonistic activity; 16–25 mm – medium antagonistic activity; 25 mm and more – high antagonistic activity.

The stability of lactobacilli to NaCl (at concentrations of 2.5 %, 5.0 %, and 7.5 %) was carried out by determining the optical density of the suspension of microorganisms in a MRS liquid medium with an appropriate concentration of sodium chloride. The measurements were carried out by photometric method at λ- 600 nm using a spectrophotometer (model SmartSpecM Plus Spectrophotometer, series 273 BR 05027).

The study was conducted in triplicates. Statistical analysis of the results were performed using *Excel* and *STATISTICA* 8.

Values are reported as the mean ± standard error of the mean (SEM). The Students' t-criterion was used during the comparative analysis of the research results. The p-value < 0.05 was considered statistically significant [5].

Results and discussion

One of the main criteria for the selection of bacterial strains - candidates for the probiotics – is their antagonistic activity to opportunistic and pathogenic microorganisms [7]. The obtained results indicated that the lactobacilli strains inhibited the growth and reproduction of all indicator microorganisms.

However, the degree of antagonistic activity was different and depended predominantly on a test strain and an indicator strain, but not on a source of lactobacilli isolation. *S. aureus* and *C. albicans* were the most resistant to all studied lactobacilli.

The results of determining the degree of lactobacilli antagonistic activity are shown in Table 1.

The most sensitive test cultures were *E. coli* and *P. aeruginosa.* The percentage of antagonistic activity to *E. coli* was 46.2 %, *P. aeruginosa* – 43.7 %.

B. subtilis and *S. aureus* were less susceptible test cultures. The percentage of lactobacilli antagonistic activity to these microoorganisms was only 10.3 % and 7.7 %, respectively. However, the percentage of the medium degree of antagonistic activity was 38.4 % and 33.3 %.

Table 1

Lactobacilli antagonistic activity to test cultures

Test cultures	Degree of antagonistic activity							
	Low		Medium		High		Absence of antagonism	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
E. coli	0	–	9	23.1	18	46.2	12	31.0
B. subtilis	2	5.1	15	38.4	4	10.3	18	46.0
S. aureus	3	7.7	13	33.3	3	7.7	20	51.0
P. aeruginosa	5	12.8	6	15.2	17	43.7	11	28.0
C. albicans	0	–	1	2.6	0	–	38	97.0

Lactobacilli isolated from self-fermenting eggplants (Odesa region) accounted for the highest percentage (56.0 %) among all examined strains with high degree of antagonistic activity to at least one of the test cultures (Fig. 1).

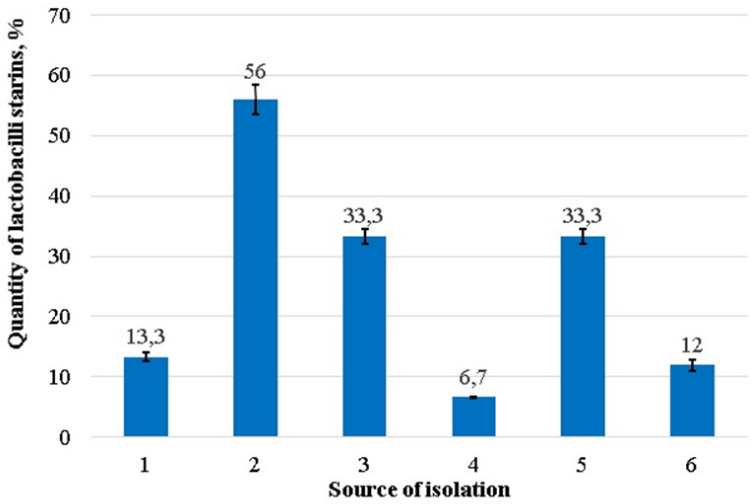

Fig. 1. Lactobacilli strains with high antagonistic activity, isolated from different sources. 1 – children's feces, 2 – self-fermenting eggplants (Odesa region), 3 – raw meat materials (Odesa region), 4 - self-fermenting vegetables (Vietnam), 5 – self- fermenting cucumbers (Sweden), 6 – typical collection strains

Note: high antagonistic activity of lactobacilli strains was expressed in relation to at least one of the test cultures

The lactobacilli strains from raw meat materials (Odesa region) and self-fermenting cucumbers (Sweden) exhibited an equally high degree of antagonistic activity (33.3 %). The percentage of strains from children feces (Odesa region) and typical collection lactobacilli was 13.3 % and 12.0 %, respectively. The smallest percentage (6.7 %) of strains with a high antagonistic activity was selected among lactobacilli isolated from self-fermenting vegetables (Vietnam).

Lactobacilli strains were observed the highest degree of antagonistic activity to *E. coli* and *P. aeruginosa* test cultures.

B. albicans was the weakest sensitive culture. The most active were strains isolated from self-fermenting eggplants (Odesa region).

Probiotic bacteria must adupt well in the gastrointestinal tract. Therefore, the lactobacilli strains ability to survive at different values of NaCl was investigated *in vitro*. The obtained results have confirmed the assumption that lactic acid bacteria undergo stress in an aggressive environment which affects on their viability and survival. Testing for a selective feature – resistance to increased NaCl concentration – is due to the fact that sodium chloride is necessary for the formation of hydrochloric acid, which is an integral part of gastric juice. The vast majority of lactic acid bacteria was tolerant at 2.5–5.0 % NaCl. The increasing NaCl concentration to 7.5 % has resulted in a decrease of the viable cells number and its growth intensity. The validity of the difference between the averaged indices of resistance of the investigated strains to different concentrations of NaCl was performed using a non-parametric analogue of the Student Criterion – a Wilcoxon rank test for two independent samples. It has been shown that the calculated criteria (Table 2) with high probability confirm the difference of the growth rates of strains at concentrations of NaCl 5.0 % and 7.5% compared with control and between each other.

Table 2

Comparison of averaged stability indexes of investigated strains to different NaCl concentrations according to Wilcoxon criteria

Indexes	Control	2.5 % NaCl	5.0 % NaCl	7.5 % NaCl
Control	W = 84.5, p-value = 1	W = 81, p-value = 0.8776	W = 165.5, p-value = 3.623e-05	W = 169, p-value = 1.628e-05
2.5 % NaCl	W = 81, p-value = 0.8776	W = 84.5, p-value = 1	W = 165.5, p-value = 3.634e-05	W = 169, p-value = 1.622e-05
5.0 % NaCl	W = 165.5, p-value = 3.623e-05	W = 165.5, p-value = 3.634e-05	W = 84.5, p-value = 1	W = 163.5, p-value = 5.599e-05
7.5 % NaCl	W = 169, p-value = 1.628e-05	W = 169, p-value = 1.622e-05	W = 163.5, p-value = 5.599e-05	W = 84.5, p-value = 1

The average growth rate of strains in the presence of 2.5 % NaCl also significantly differed from that for experiments with 5.0 % and 7.5 % NaCl. However, for a concentration of NaCl of 2.5 %, the calculated Wilcoxon criterion indicated for the need to adopt a null hypothesis, that is, the absence of a difference between growth indices in control and experiment.

Graphical representation of the averaged indicators of stability of the investigated strains to different concentrations of NaCl has shown at Figure 2.

Just as in the determination of antagonistic activity, it was noted that the primary source of strain isolation does not affect the final resistance to NaCl. For example, among the most resistant strains are present as isolated from self-fermenting vegetables (*Lactobacillus sp.* B4, *Lactobacillus sp.* B1), meat products (*Lactobacillus sp.* M6) and children's feces (*Lactobacillus sp.* 175).

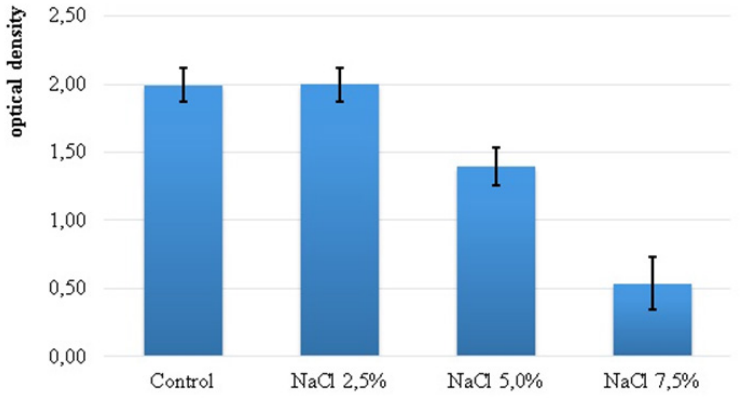

Fig. 2. The averaged indices of investigated lactobacilli resistance to various concentrations of NaCl

To confirm, the clasting with a binary dendrogram construction ("dendextend" package) was performed [10]. This way of visualizing the similarities and differences is to draw two dendrograms like root trees and reflect connecting lines between vertices that correspond to each other in two trees.

The resistance to NaCl is not related to the antagonistic activity of most lactobacilli strains. Only for some strains was placement in parallel (on topology of trees) clusters (*Lactobacillus sp.* M6, *Lactobacillus sp.* M2, *Lactobacillus sp.* M3 and *Lactobacillus sp.* B6, *Lactobacillus sp.* B3) (Fig.3).

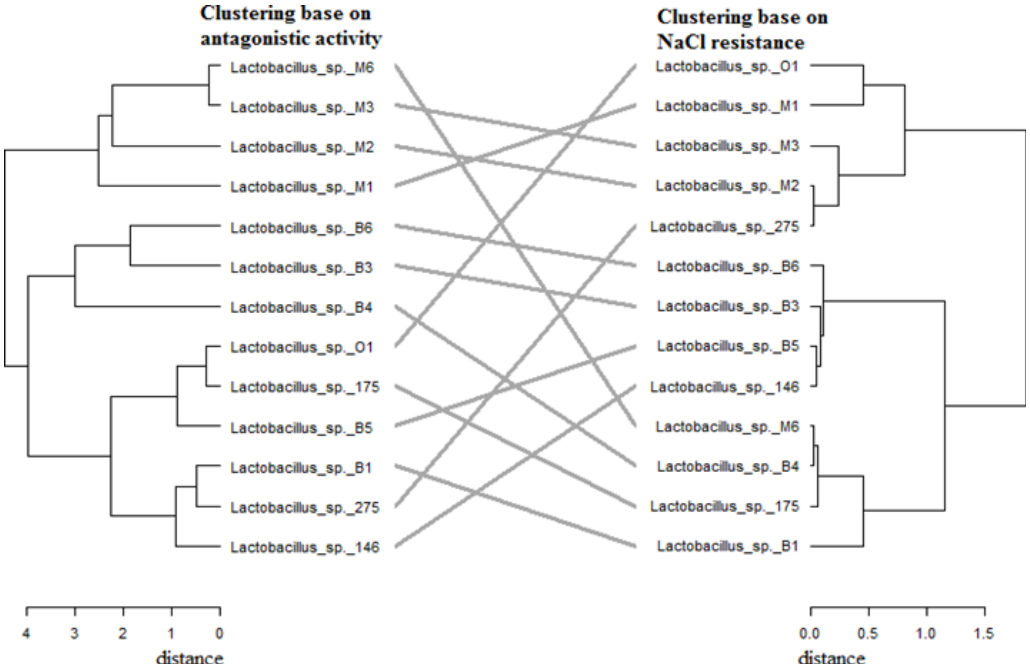

Fig. 3. A binary dendrogram illustrating the trees topology, constructed on the basis of the results of clusterization of the parameters of antagonistic activity and NaCl tolerance (distance matrix – "canberra", mode of aggregation – "complete")

The performed experimental studies and mathematical calculations show that increased content of sodium chloride in nutrient medium don't inhibit the growth of the investigated lactobacilli strains and, moreover, don't affect on their antagonistic ability, which is very important and essential in the creation and production of probiotic products with functional purpose.

References

1. Kitaevskaya S. V. Modern trends in the selection and identification of probiotic strains of lactic acid bacteria // Bulletin of Kazan Technological University. – 2012. – V.15, № 17. – P. 184 – 188 (in Russian).
2. Netrusov A. I., Egorova M. A., Zakharchuk L. M. Workshop on Microbiology: a training manual for students of higher institutions. – Moscow: Academy, 2005. – P. 467 – 478 (in Russian).
3. Ramonova E. V. Isolation and identification of local strains of lactic acid microorganisms and their use as probiotics: Author. diss. cand. biol. sciences. - Vladikavkaz, 2011. – 19 p. (in Russian).
4. Rzhevskaya V. S., Oturina I. P., Teplitskaya L. M. Studying the biological properties of lactic acid bacteria strains // Scientific notes of the V. I. Vernadsky Tauride National University. – 2014. – V. 27, № 1. – P. 145–160 (in Russian).
5. Sidelev S. I. Mathematical methods in biology and ecology: an introduction to elementary biometry: A manual. – Yaroslavl: YarSU, 2012. – 140 p. (in Russian).
6. Solov'eva I. V., Tochilina A. G., Novikova N. A., Belova I. V., et al. Studying the biological properties of new strains of the genus *Lactobacillus* // Bulletin of the N. I. Lobachevsky Nizhny Novgorod University. – 2010. – №. 2 (2). – P. 462 – 468 (in Russian).
7. Tochilina A. G., Belova I. V., Solovyova I. V., Novikova N. A., Ivanova T. P., et al.. Study of the biological properties of strains of the genus *Lactobacillus* // Modern problems of science and education. – 2015. – № 5. (in Russian).
8. Fedorova O. V., Yunusova Z. S., Shurbina M. Yu., Valeeva R. T. Probiotic preparations: characteristics, criteria, requirements for them // Bulletin of the Technological university. – 2016. –Vol. 19, № 7. – P. 142 –145 (in Russian).
9. Conway P. L. Prophylactic treatment of piglets with *Lactobacillus* strains of porcine origin // In: Probiotics: prospects of the use in opportunistic infections. Old Herborn University Seminar Monograpg. Inst. Microbiol. Biochem. Herborn Dill, Germany, 1995 – P. 89–100.
10. Galili T. Dendextend: an R package for visualizing, adjusting and comparing trees of hierarchical clustering// Bioinformatics. – 2015. –Vol. 31(22). – P. 3718–3720.
11. Giraffa G., Chanishvili N., Widyastuti Y. Importance of lactobacilli in food and feed biotechnology // Res. Microbiol. – 2010. – V.161, N 6. – P.480 – 487.
12. Saarela M., Morgensen G., Fondén R., Mättö J., Mat-tila-Sandholm T. Probiotic bacteria: safety, functional and technological properties // Journal of Biotechnology. – 2000. –Vol. 84. – P. 197–215.

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

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.

Key words: 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.

References

1. Girin V. M., Dzyublyk I. V., Rotavirus gastroenteritis // Ukr. med. magazine – 1998. – № 2. – pp. 146–150.
2. Distribution of rotavirus in water objects in the Ukraininan environment / I. V. Zyublyk, O. V. Obertinskaya, I. G. Kostenko et al. // Infectious diseases. – 2008. – No. 4. – pp. 38 – 43.
3. Bulavka L. V., Bondarenko V. I., Zadorozhna V. I. The role of environmental facilities in the spread of rotavirus infection // Environment and Health. – 2002. – No. 2. – pp. 35 – 38.
4. Kovalchuk L. Y., Mokienko A. V. Estimation of the hygienic quality of viral contamination of water objects of the Ukrainian Pridunav // Current problems of transport medicine. – 2014. – No. 4, t. 2. – pp. 41–48.
5. The problem of viral contamination of water bodies in the Republic of Belarus / T. V. Amvrosyeva, Z. Bogush, O. N. Kazinets, N. V. Poklonskaya, P. I. Grinkevich, A. N. Khilo // Water: hygiene and ecology. – 2013. – №1 (1). –pp. 35– 44.
6. Stepanova O. A. Ecology of allochthonous viruses of the Black Sea. – Sevastopol: Express print, 2004. – 307 p.
7. Water use of the Odesa region: to the analysis of the risks of contamination by viruses of water bodies and drinking water / A. V. Mokienko, N. F. Petrenko, A. A.Polishchuk, L. I. Filling, L. S. , A. V. Skopenko, N. P.Isakova // Actual problems of transport medicine. – 2009. – № 1 (15). – pp. 136 – 145.
8. Virus contamination of water bodies with pathogens of socially significant high-grade infections / T. V. Amvrosyeva, Z. F. Bogush, O. N. Kazinets, N. V. Poklonskaya, K. L. Dedyulya // Health and ambient environment. – 2014. – № 24, t. 1. – p. 3–7.
9. Kramarev S. O., Zakordonets L. V. Rotavirus infection in children, epidemiology and prophylaxis // Medical practice.– 2007.– № 1, t.55.–pp.21 – 23.
10. Guidelines. Epidemiology and prophylaxis of rotavirus infection / I. V. Zyublyk, V. I. Zadorozhna, V. V. Gavura and others. – K., 2003. – 22 p.

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THE EFFECT OF CULTURE MEDIA COMPONENTS ON THE CHANGE OF THE LEVEL OF ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA STRAINS

It is known that lactic acid bacteria have a pronounced antagonistic activity relative to pathogenic and opportunistic microorganisms, as well as to other species and strains of lactobacilli. The antagonism of lactic acid bacteria in relation to microorganisms is due to the formation of lactic acid and the production of other antimicrobial and antibiotic substances: lysozyme [2; 3], hydrogen peroxide [2; 4], bacteriocin [5; 6] or fatty acids [1].

The research aim was to study the effect of culture media components of various quantitative and qualitative composition on the change in the level of antagonistic activity of lactic acid bacteria.

Materials and methods

In our study, we used *Lactobacillus* strains which were isolated from sea sponges. Determination of antagonistic properties was carried out *in vitro* by a hole-diffuse method [7–9] in relation to the *Bacillus subtilis* ATCC6633. The study of the influence of culture media components on the antagonistic activity index was carried out using statistical methods (correlation analysis and the principal component method). The work was performed in the program R 3.4.0 using the following libraries: library(ggfortify), library(factoextra), library(ggbiplot), library(ggcorrplot), library(RColorBrewer), library(corrplot).

Results

As we see from the data presented in the figure 1, the *Lactobacillus vaccinostercus* ONU2 strain had a weak antagonistic effect on the growth of the indicator test-strain *Bacillus subtilis* ATCC 6633. The maximum growth inhibition zone of this strain was recorded during its preliminary cultivation on the Brigs medium (Fig. 1). This strain showed a lower effect in terms of antagonistic activity when using MRS and Lactic agar2 media (fig. 1).

The stronger strain in terms of its antagonistic activity *Lactobacillus bifermentans* ONU 55 inhibited the growth of the indicator test-strain *Bacillus subtilis* ATCC 6633 when using all types of media. The maximum effect was recorded under the condition of preliminary cultivation of this strain on Lactic agar2 and Lactic agar4 media (fig. 1).

Analyzing the effect of culture media components on the change in the level of antagonistic activity of the studied strains, we used several statistical methods. First of all, the influence of the quantitative and qualitative composition of the media on the antagonistic activity index was estimated using the method of correlation analysis using the Piersson correlation coefficient *(r)*.

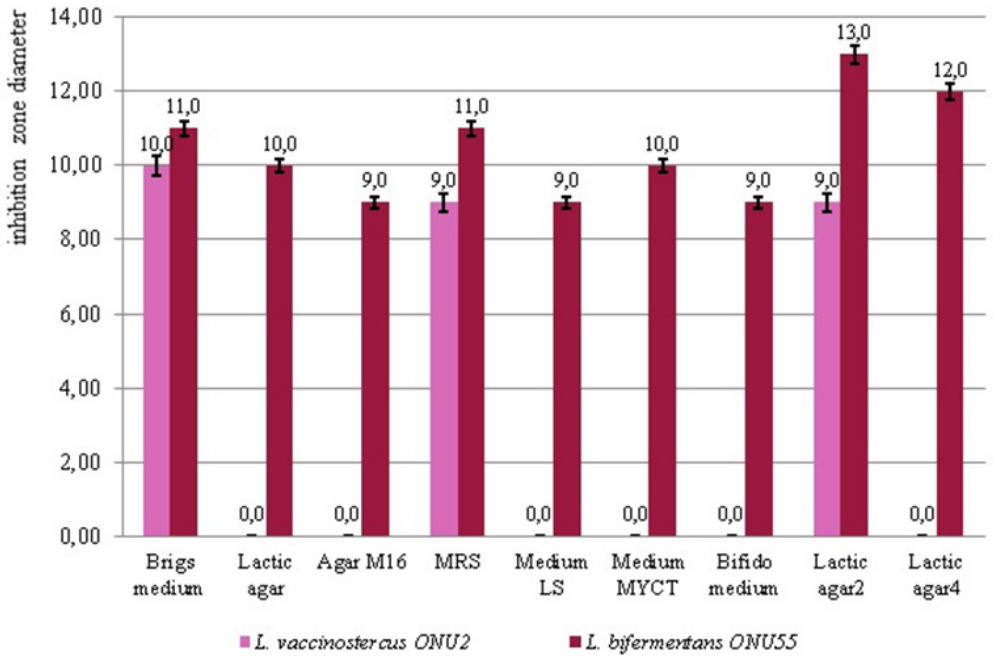

Fig. 1. Changes in the level of antagonistic activity of lactic acid bacteria strains using different culture media

The results of statistical processing at a confidence level of p=0.05 are presented as graphic material in the figure 2. As can be seen from the data presented, the level of antagonistic activity of *L. vaccinostercus ONU2* was maximally affected by the presence of peptone (r = 0.77), glucose (r = 0.75), and sodium acetate (r = 0.68) in the medium. Lower linear positive correlation values were obtained when calculating the effect on the antagonistic activity index of *L. vaccinostercus* ONU2 strain and the presence of ammonium citrate (r = 0.68) and two-displaced potassium phosphate (r = 0.59) in the medium. Insignificant positive correlation was found between the level of antagonistic activity of *L. vaccinostercus* ONU2 strain and the presence of ethanol (r = 0.47), tomato juice (r = 0.40) and yeast extract (r = 0.37) in the medium (fig. 2).

The level of antagonistic activity of *L. bifermentans* ONU55 strain was influenced by several other culture media components (fig. 3). Thus, the maximum linear Pearson correlation was calculated between the level of investigated trait and the presence of sodium acetate (r = 0.79) and ethanol (r = 0.67) in the medium.

Ammonium citrate, dibasic potassium phosphate, yeast extract and maltose had an average effect on the change in the level of antagonistic activity of *L. bifermentans* ONU55 strain, which was expressed in terms of a linear correlation varying from 0.47 to 0.31 (fig. 3).

The use of the principal component method (PCA) confirms that the change in the level of antagonistic activity of strains that were initially different according to this trait is affected by different components of culture media (fig. 4–5).

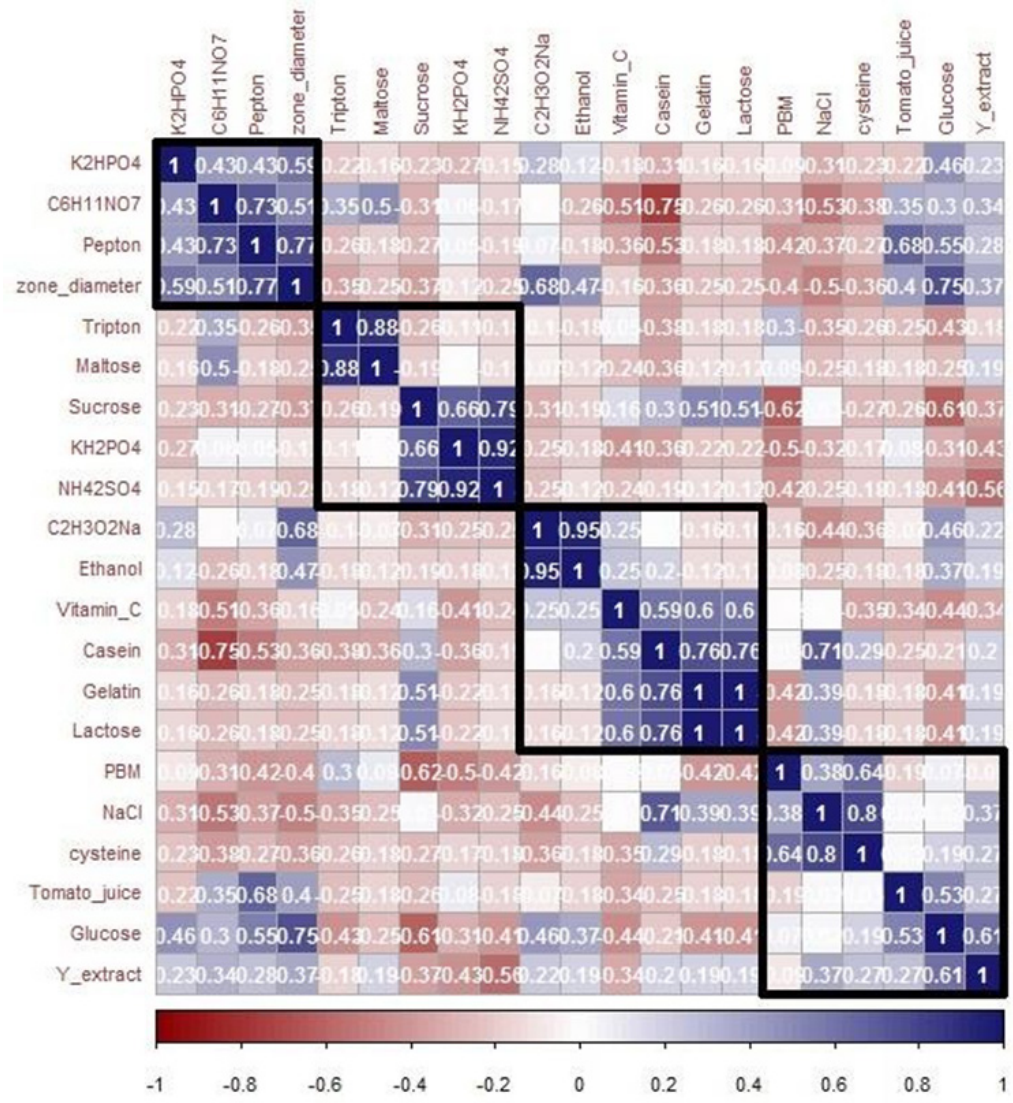

Fig. 2. Pearson correlation coefficients *(r)* between the antagonistic activity index (zone_diameter) of *L. vaccinostercus* ONU2 strain and culture media components

Thus, it was shown that the change in the level of antagonistic activity of lactic acid bacteria strains depends largely on the culture media components. At the same time, the set of significant components varies, depending on the initial level of strain activity. For weaker strains in terms of antagonistic activity (*L. vaccinostercus* ONU2), more significant are those components of culture media, which contribute to an increase in the number of strains. For initially more active strains (*L. bifermentans* ONU55), priority is given to components that not only stimulate growth, but also contribute to low pH levels.

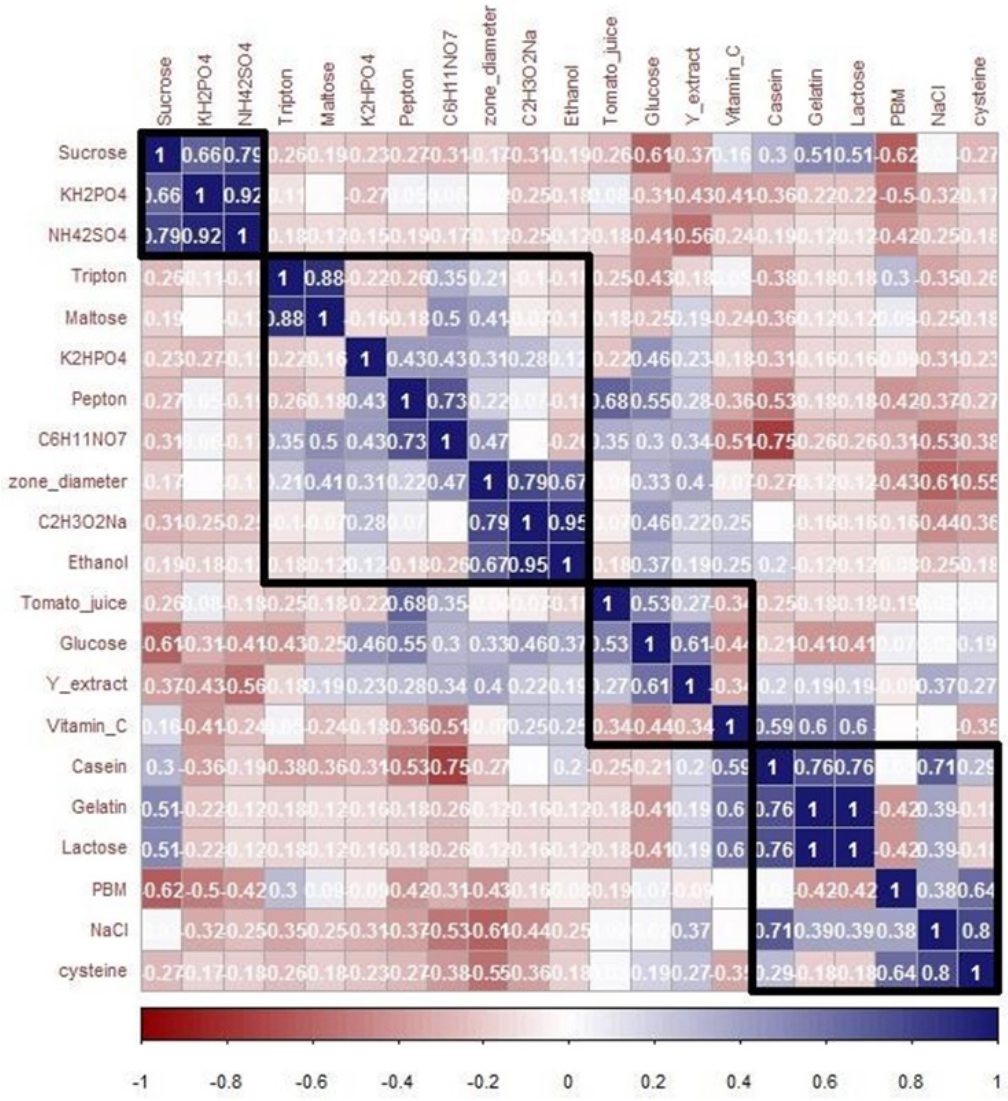

Fig. 3. Pearson correlation coefficients *(r)* between the antagonistic activity index (zone_diameter) of *L. bifermentans* ONU55 strain and culture media components

References

1. Алиев Э. А. Выращивание овощей в гидропонных теплицах – Киев: Урожай. – 1985. – 160 с.
2. Ленцнер А. А., Ленцнер Х. П., Микельсаар М. Э. Лактофлора и колонизационная резистентность // Антибиотики и медицинская биотехнология. – 1987. – Т. 32, № 3. – С. 173–179.
3. Максимов В. И., Миловзорова Т. А., Молодова Г. А. О специфичности микробных лизоцимов // Успехи биологии и химии. – 1988. – Т. 29. – С. 218–230.

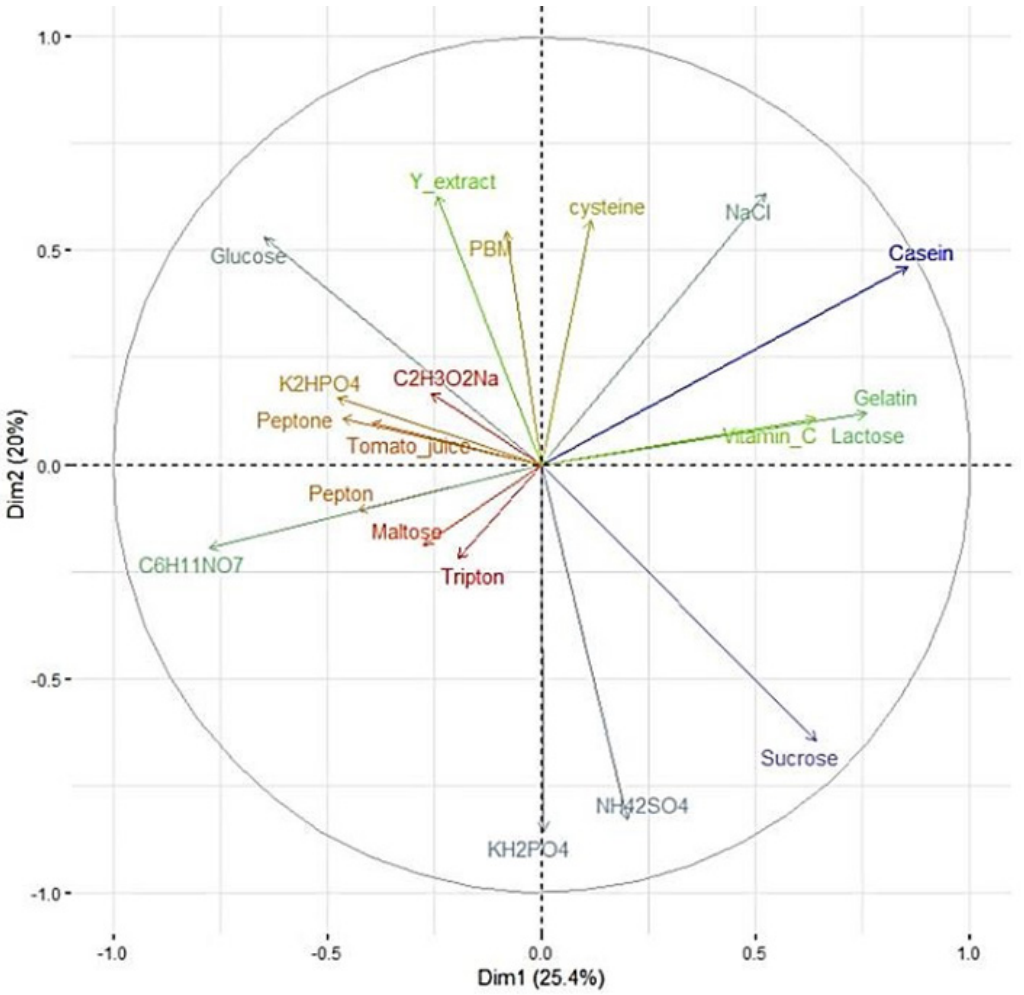

Fig. 4. PCA analysis of the influence of culture media components on the antagonistic activity index of *L. vaccinostercus* ONU2 strain (dispersion distribution characterizes the main components of PCA1 and PCA2)

4. Сорокулова И. Б.,Резник С. Р., Качан А. Ф. Дополнительный подход к дифференциации споровых бактерий *B. subtilis* и *B. cereus* // Микробиол. журн. − 1978. − № 4. − С. 448–453.
5. Смирнов В. В., Резник В. Р., СорокуловаИ. Б. Методические рекомендации по выделению и идентификации бактерий рода *Bacillus* из организма человека и животных / – Киев: Наукова думка. − 1983. − 51с.
6. Andersson R. E., Daeschel M. A., Hassan H. M. Antibacterial activity of plantaricin SIK-83, a bacteriocin produced by *Lactobacillus plantarum* // Biochimie. – 1988. – Vol. 70, № 3. – P. 381 – 390.

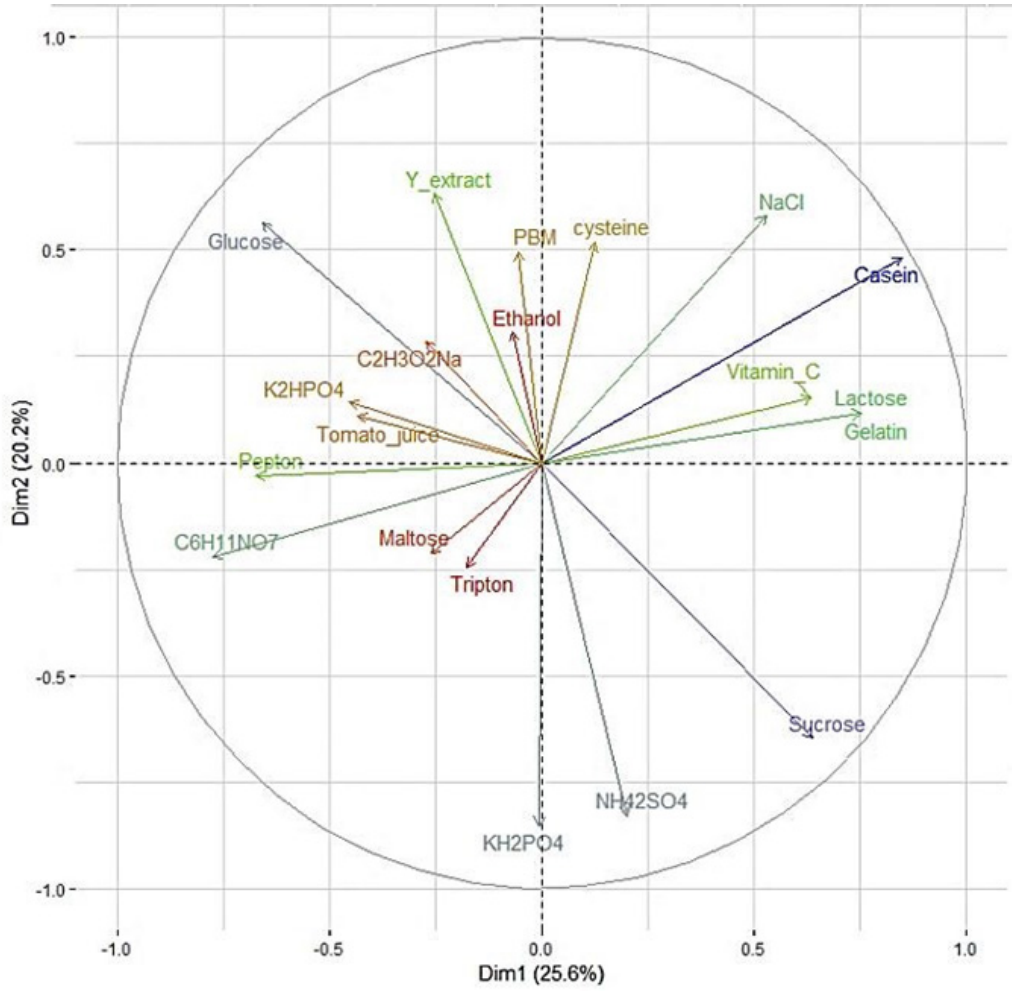

Fig. 5. PCA analysis of the influence of culture media components on the antagonistic activity index of *L. bifermentans* ONU55 strain (dispersion distribution characterizes the main components of PCA1 and PCA2)

7. Presti I., D'Orazio G., Labra M. Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their in vitro effect //Appl Microbiol Biotechnol. – 2015. – Vol. 99, No 13. –P. 5613-5626.
8. Servin A.L. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens // *FEMS Microbiology Reviews*.– 2004. – Vol. 28, No 4. – P. 405–440.
9. Schillinger U., Lucke F. Antimicrobial activity of Lactobacillus sake isolated from meat //J Appl Environ Microbiol. – 1989. – Vol. 55. – P. 1901–1906.

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

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.

Key words: 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 5^{th} 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.

References

1. Beifuss U. Methanophenzine and other natural biologically active phenazines / U. Beifuss, M. Tietze // Top Curr. Chem. – 2015. – Vol. 44. – P. 77– 133.
2. Bloemberg G. Phenazines and their role in biocontrol by Pseudomonas bacteria / G. Bloemberg, B. Lugtenberg // New Phytologist. – 2010. – Vol. 157. – P. 503–523.
3. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats / M. Mazzola, R. Cook, L. Thomashow, D. Weller, L. Pierson // Applied and Environmental Microbiology. – 2015. – Vol.58. – P. 2616–2624.
4. Davies J. *Pseudomonas aeruginosa* in cystic fibrosis: pathogenesis and persistence / J. Davies // Paediatr. Respir. Rev. – 2012. – Vol. 3. – P. 128–134.
5. *In vitro* inhibition of lymphocyte proliferation by Pseudomonas aeruginosa phenazine pigments / R. Sorensen, J. Klinger, H. Cash, P. Chase, D. Dearborn // Infection and Immunity. – 2008. – Vol. 41. – P. 321–330.
6. Kobayashi H. Pathogenesis and clinical manifestations of chronic colonization by *Pseudomonas aeruginosa* and its biofilms in the airway tract / H. Kobayashi ,

O. Kobayashi, S. Kawai // J. Infect. Chemother. – 2009. – Vol. 15. – P. 125–142.
7. Laursen J. Phenazine natural products: Biosynthesis, synthetic analogues, and biological activity / J. Laursen, J. Nielsen // Chemical Reviews. – 2014. – Vol. 104. – P. 1663–1685.
8. Levitch M. Study of the biostnthesis of phenozine-1-carboxylic acid / M. Levitch, E. Stadtman // Arch. Biochem. Biophys. – 1964. – Vol. 106. – P.194–198.
9. Maddula V. Altering the ratio of phenazines in *Pseudomonas chlororaphis (aureofaciens)* strain 30-84: effects on biofilm formation and pathogen inhibition / V. Maddula, E. Pierson, L. Pierson // J. Bacteriol. – 2012. – Vol. 19. – P. 2759–2766.
10. Pearson A. Expression cloning of dSR-CI, a class C macrophage- specific scavenger receptor from *Drosophila melanogaster* / A. Pearson, A. Lux, M. Krieger // Proc. Nat. Acad. Sci. – 2013. – Vol. 92. – P. 4056–4060.
11. Schoonbeek H. Fungal ABC transporters and microbial interactions in natural environments / H. Schoonbeek, J. Raaijmakers, M. De Waard // Mol. Plant Microbe Interact. – 2012. – Vol. 15. – P. 1165–1172.
12. Structural and functional analysis of the pyocyanin biosynthetic protein PhzM from *Pseudomonas aeruginosa* / J. Parsons, B. Greenhagen, K. Shi, K. Calabrese, H. Robinson, J. Ladner // Biochemistry. – 2016. – Vol. 46. – P. 1821– 1828.
13. Turner J. Occurrence, biochemistry and physiology of phenazine pigment production / J. Turner, A. Messenger // Advances in Microbial Physiology. – 2014. – Vol. 27. – P. 211–275.

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LACTIC ACID BACTERIA ON PLANT SURFACES

Abstract
Literature data about lactic acid bacteria as representatives of plant microbiota, ways of their dissemination and colonization of the plants, diversity of species and influence on food industry and agriculture are discussed.

Key words: lactic acid bacteria, plants, sources of colonization, lactobacilli.

How do lactobacilli penetrate plants? How do they become the members of plant microbiome? Davies G.H.G. in 1955 suggested that primarily lactobacilli inhabit intestinal tract and after getting into environment they survive under harsh conditions. Indeed, lactobacilli are common dwellers of intestinum of vertebrate and invertebrate animals. They could be found in marine fish (L. plantarum) [Alonso, 2018], in insect guts [Groenewald, 2006], guts of birds [Videnska, 2013], animals [Endo et al., 2010] and human [Attri, 2018].

The same hypothesis suggested Stirling A. C. and Whittenbury R. in 1963. They studied the sources of lactic acid bacteria inhabiting silage. Few lactobacilli as well as other lactic acid bacteria could be found on undamaged tissues of growing plant, but while plants were harvested for ensiling, amount of LAB on damaged and crashed plants significantly increased. Lactobacilli are known to be fastidious microorganisms with high demands in nutrients and their survival on plant surfaces depends on availability of carbon-containing nutrients [Lindow, 2003]. Plant sap delivering through the damaged sites becomes their natural enrichment medium. Indeed, in studies of Stirling A.C. and Whittenbury R. (1963) LAB were found on leaves damaged by insects, on sheaths at the base of grasses and on withered blades of grass, but not on the intact inflorescences or seeds. Random occurence of lactobacilli allowed authors to suggest that plant surfaces are not the primary source of LAB.

Nilsson G. and Nilsson P.E. (1956) have found that the microbiota on the surface of some fodder plants was different depending on a stage of maturity which could be explained by the difference in temperatures of the seasons. Thus, lactobacilli were isolated from cereals, clovers and grasses after the beginning of July. But not only the summer temperature could play such a role in increasing LAB population. It could be suggested that contamination of plant surfaces by representatives of insect microbiota occured just in the beginning of summer, and after the microbe population on flowers and leaves increases till the detectable level.

Mundt J. O. and Hammer J. L. (1968) described lactobacilli from the surfaces of plants growing in moist subtropical area. In 54% of the samples lactobacilli were found in a quantity less than 10 cells per one gram of plant material. The most frequently isolated were *L. fermentum, L. plantarum, L. brevis.* Other speceis such as *L. casei, L. viridescens, L. cellobiosus, L. salivarius* and *L. buchneri* were isolated from less amount of samples. Lactobacilli were not prevalent in LAB population

of plant tissues – the quantity of streptococci and *Leuconostoc mesenteroides* was much higher. Only in samples of corn the amount of lactobacilli exceeded 4.6 x 10^2 per gram.

L. brevis was isolated from such plants as *Pteridium aquilinum, Linum usitatissium, Ficus benjamina, Aloe barbadensis,* and *L. delbrueckii* – from *Linum usitatissimum* [Anacorso et al., 2015].

In silage *Lactobacillus paraplantarum* (4.5%) and *Lactobacillus plantarum* (27.3%) were found [Pang, 2011].

On corn and alfalfa lactic acid bacteria presented less than 5% of the total microbial population [Lin, 1992]. Nevertheless, chopping plants with the resulting sap bleeding leads to the increased proliferation of LAB [Chunjian, 1992]. The total quantity of epiphytic LAB in ensiled grass reached 105 CFU/g [Sharp, 1992]. *L. plantarum* becomes the predominant species among lactobacilli [Chunjian, 1992], and the total amount of lactobacilli among other LAB constitutes approximately 10^3 cells per gram [Cai, 1998].

When genetically modified L. plantarum in an inoculum 10^6 CFU/g was applied for silage treatment, these bacteria survived in silage at least for 30 days [Sharp, 1992].

From plant surfaces LAB get into the food products where these microorganisms become responsible for plant fermentation and subsequent preservation [Daeschel, 1987].

Lactobacilli are the most widely used probiotic bacteria in production of food of vegetable origin [Martins, 2013].

L. plantarum together with *L. pentosus* are the main species found in fermented table olive [Hurtado, 2012; Ercolini, 2006; Con, 2009].

Korean rice wine contained *L. plantarum* in 27% of samples [Jin, 2008]. LAB population in sauerkraut during fermentation and storage reached 10^3–10^7 CFU/ml. *L. brevis, L. plantarum, L. fermentum* were found [Ram Kumar, 2010].

Conclusion

Presence of lactic acid bacteria on plants leads to the possibility of using them in functional nutrition based on fermented plant material.

References

1. Alonso S. et al. 2018. Isolation and partial characterization of lactic acid bacteria from the gut microbiota of marine fishes for potential application as probiotics in aquaculture. Probiotics and Antimicrobial Proteins.doi:10.1007/s12602-018-9439- 2
2. Anacorso I. et al. 2015. Isolation and identification of lactic acid bacteria from plants and other vegetable matrices and microbial recombination with *Enterococcus* spp. American Research Thoughts. 1:7 http://dx.doi.org/10.6084/m9.figshare.1425170
3. Attri S. et al. 2018. Development and diversity of lactic acid producing bacteria and bifidobacteria in healthy full term Indian infants from Himachal Pradesh. Intest Res. 16: 529 – 536.
4. Cai et al. 1998. Influence of Lactobacillus spp. from an inoculants and of

Weissella and Leuconostoc spp. from forage crops on silage fermentation. Appl Env Microbiol. 64: 2982 – 2987.
5. Con A.H. et al. 2009. Determination of antagonistic starter cultures for pickles and olive fermentation processes. Czech. J. Food Sci. 27: 185 – 193.
6. Daeschel M.A. et al. 1987. Microbial ecology of fermenting plant materials. FEMS Microbiol. Reviews. 46: 357 – 367.
7. Davis G.H.G. 1955. The classification of lactobacilli from the human mouth. J Gen. Microbiol. 13: 481 – 493.
8. Endo A. et al. 2010. Diversity of *Lactobacillus* and *Bifidobacterium* in feces of herbivores, omnivores and carnivores. Anaerobe. 16:590-6.
9. Ercolini D. et al. 2006. Fluorescence in situ hybridization detection of *Lactobacillus plantarum* group on olives to be used in natural fermentation. Int J Food Microbiol. 2: 291 – 296.
10. Groenewald W.H. et al. 2006. Strains of *Lactobacillus plantarum* in grape must are also present in the intestinal tract of vinegar flies. S. Afr. J. Enol. Vitic. 27: 46 – 50.
11. Hurtado A. et al. 2012. Lactic acid bacteria from fermented table olives. Food Microbiol. 31:1. doi: 10.1016/j.fm.2012.01.006. Epub 2012 Feb 2.
12. Jianbo J. et al. 2008. Diversity analysis of lactic acid bacteria in Takju, Korean rice wine. J. Microbiol. Bitechnol. 18: 1678 – 1682.
13. Lin C. et al. 1992. Epiphytic lactic acid bacteria succession during the pre-ensiling and ensiling periods of alfalfa and maize. J. Appl bacteriology. 73: 375 – 387.
14. Lin C. et al. 1992. Epiphytic microflora on alfalfa and whole plant corn. J. Dairy Sci. 75: 2484 – 2493.
15. Lindow S. et al. 2003. Microbiology of the phyllosphere. Appl Environm Microbiol. 69: 1875 – 1883.
16. Martins E.M.F. et al. 2013. Products of vegetable origin: a new alternative for the consumption of probiotic bacteria. Food Research International. 51: 764 – 770.
17. Mundt J. et al. 1968. Lactobacilli on plants. Appl Microbiol. 16: 1326 – 1330.
18. Nilsson G. et al. 1956. The microflora on the surface of some fodder plants at different stages of maturity. Arch Mikrobiol. 24: 412 – 422.
19. Pang H. et al. 2011. Natural populations of lactic acid bacteria associated with silage fermentation as detrmined by phenotype, 16S ribosomal RNA and recA gene analysis. Syst Appl Microbiol 34(3) 235-41. doi: 10.1016/j.syapm.2010.10.003. Epub 2011 Feb 1.
20. Ram Kumar Pundir et al. 2010. Change in microflora of sauerkraut during fermentation and storage. World J. Dairy Food Science. 5: 221 – 225.
21. Sharp R. et al. 1992. Growth and survival of genetically manipulated Lactobacillus plantarum in silage. Appl Environm Microbiol. 58: 2517 – 2522
22. Stirling A.C. et al. 1963. Sources of the lactic acid bacteria occurring in silage. J Appl Bact. 1963: 86 – 90.
23. Videnska P. et al. 2013. Chicken faecal microbiota and disturbances induced by single or repeated therapy with tetracycline and streptomycin. BMC Veterinary Research 39: 30 https://doi.org/10.1186/1746-6148-9-30

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

Abstract

The biological properties of phenol destruction bacteria Bacillus subtilis ONU551 and Aeromonas ichthiosmia ONU552 are studied. The strain of Bacillus subtilis ONU551 is presented by gram-positive rods that form subterminal spores. A strain of Aeromonas ichthiomia ONU552 is gram-negative direct rod. The features of fat-acid composition of strain of Aeromonas ichthiomia ONU552 ONU552, B. subtilis ONU551 of destructions – to the phenol, that distinguish them from other bacteria – in cellular lipids each of strain presence of fat acids : 16: 1 w7c alcohol, 17: 0 iso, 17: 0 anteiso.

Key words: 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). 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

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.

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

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 growat 20–30 °C, pH 7.0 a simple nutrient media – MAA.

The spectrum of fatty acids of strain *Aeromonas ichthiomia* ONU552 is presented in Table 2.

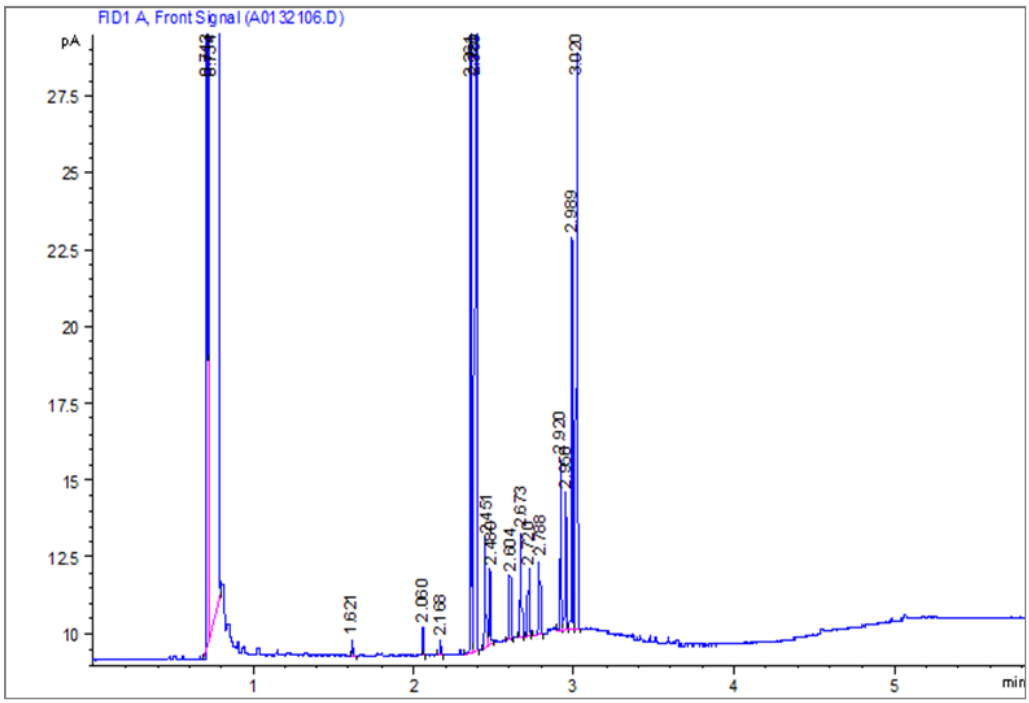

Fig. 1. Fatty acid spectrum of *Bacillus subtilis* ONU551 strain

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		

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

Conclusions

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

References

1. Севастьянов О. В. Исследование удаления анилина и фенольных соединений путем соокисления с помощью тирозиназы Agaricus bisporus // Вісн. Одес. нац. ун. Сер.: Хімія. – 2012. – №17(4). – С. 55–60.
2. Шестеренко Ю.А. и др. Удаление фенолов из водных растворов с использованием иммобилизованной на полимерных носителях тирозиназы и неорганических коагулянтов // Химия и технология воды. – 2012. – № 34(2). – С. 179–186
3. Филипчук В. Л. Очистка фенолових стічних вод виробництва консервної жерсті // Экотехнологии и ресурсосбережение. – 2003. – № 2. – С. 47–52.

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

Abstract
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 *B. 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).

References

1. Bibel D. Elie Metchnikoff's bacillus of long life / D. Bibel // ASM News. – 2008. – Vol. 54. –P. 660 – 680.
2. Chervinets Yu. V., Bondarenko V. M., Shabanova N. A., Samoukina A. M., Chervinets V. M. Bacteriocinogenic high-antagonistic strains of lactobacilli // Microanalysis biology. – 2006. – № 7. – P. 114.
3. Fijan S. Influence of the growth of *Pseudomonas aeruginosa* in milk fermented by multispecies probiotics and Kefir microbiota / S. Fijan // J. Probiotics Health. – 2016. – Vol. 3. – P. 136 – 140.
4. Glushanova N. A., Blinov A. I., Bakhaev V. V. About antagonism of probiotic lactobacilli // Epidemiology and infectious diseases. – 2004. – № 6. – P. 114 – 117.
5. Coman M. *In vitro* evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501, *Lactobacillus paracasei I*MC 502 and SYNBIO against pathogens / M. Coman, M. Verdenelli, C. Cecchini // Journal of Applied Microbiology. – 2004. – V. 117, № 5. – P. 518 – 527
6. *Lactobacilli* as Probiotics and their Isolation from Different Sources / F. A. Arshad, R. Mehmood, S. Khan, A. Rubina, S. Hussain, A. Khan, O. Farooq // British J. of Research. – 2018. – Vol. 5. – P. 1 – 35.
7. Lin T.-H. Characterization of an antimicrobial substance produced by *Lactobacillus plantarum* NTU 102 / T.-H. Lin, T.-M. Pan // Journal of

Microbiology, Immunology and Infection. – 2019. – Vol. 52, № 3. – P. 409 – 417.
8. Millette M. *In vitro* growth control of selected pathogens by *Lactobacillus acidophilus* and *Lactobacillus casei* fermented milk / M. Millette, F. M. Luquet, M. Lacroix // Lett. Appl. Microbiol. – 2007. – Vol. 44. – P. 314 – 319.
9. Nowroozi J. Study of *Lactobacillus* as Probiotic Bacteria /J. Nowroozi, M. Mirzaii, M. Norouzi // Iranian J. Publ. Health. – 2004. – Vol. 33, № 2. – P. 1 – 7.
10. Prabhurajeshwar C. Probiotic potential of *Lactobacilli* with antagonistic activity against pathogenic strains: An in vitro validation for the production of inhibitory substances / C. Prabhurajeshwar, R. Kelmani // Biomedical journal. – 2017. – Vol. 40, № 5. – P. 270 – 283.
11. Ratsep M. Effect of *Lactobacillus plantarum* strains on clinical isolates of *Clostridium difficile in vitro* / M. Ratsep, P. Naaber, S. Koljalg, I. Smidt, E. Shkut, E. Sepp // J. Probiotics Health. – 2014. – Vol. 2. – P. 119 – 129.
12. The effect of a multispecies probiotic on the composition of the faecal microbiota and bowel habits in chronic obstructive pulmonary disease patients treated with antibiotics / C. J. Koning, D. Jonkers, H. Smidt, F. Rombouts, H. J. Pennings, E. Wouters, E. Stobberingh // Br. J. Nutr. – 2010. – Vol. 103. – P. 1452 – 1460.

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NEW BIOLOGICALLY ACTIVE COMPOUND OF *BACILLUS* GENUS

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.

Key words: 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.aminosugarsneotregalosodiamine 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 endosporeformingfacu ltativeanaerobic 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

The results of analysis of the second metabolites of bacilli are listed in Tables 1–3.

In order to simplify the understanding, we will later name the compoun ds found in bacilli, but previously not known for this group, as "new compounds".

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%), *Bacillus megaterium*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, there vealed compounds are very diverse. There are present antimicrobial, antifungal compounds, cytostatic sandone 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	270,20613	269,198854	3-Aminodihydro-2(3H)-furanone; (S)- form, N-Undecanoyl	269,199094	-0,89	Pseudomonas aeruginosa	Bacteria	
4,42	326,21825	325,210974	Sperabillin C	325,21139	-1,28	Prod. by Pseudomonas fluorescens	Bacteria	Active against Gram- positive and-negative bacteria
5,34	1035,54086	1034,533584	Micropeptin 88Y	1034,532452	1,09	cyanobacteria Microcystisaeru ginosa	CyanB	Chymotrypsi n inhibitor Depsipeptideantibiotic
1,99	328,12267	327,115394	KimbeamideA	327,115668	-0,84	Isol. from a consortium of marine cyanobacteria,	CyanB	Moderate sodium channelblocker
5,08	1021,52469	1020,517414	Insulapeptolide E	1020,516802	0,60	Nostocinsulare	CyanB	Potent inhibitor of human leukocyte elastase
6,03	1063,56976	1062,562484	Micropeptin 88E	1062,563752	-1,19	Prod. by Microcystisaeru ginosa NIES-88	CyanB	Chymotrypsi n inhibitor
6,33	1491,83724	1490,829964	MIXED PEACK	1490,831944	-1,33	From Tolypothrixbys soide	CyanB	Antifungal agent
7,02	398,227800	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	357,22889	356,221614	Octalactin A.	356,21989	4,84	by a marine Streptomyces sp.	Strpmc	Cytotoxic
7,63	640,39952	639,392244	Antibiotic M 2847	639,398249	-9,39	Streptomyces platensis	Strpmc	Active against Gram-positiveorganisms
4,77	380,16556	379,158284	Kasugamycin	379,159082	-2,10	Prod. by Streptomyces kasugaensis, also from Streptomycesrutgersensis, Streptomycesalbulus and Nocardiasp	Strpmc	Inhibitor of the fungus responsible for rice blast
4,61	312,21728	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 actinomycete Amycol atopsis sp.	Actmct	Herbicide
3,27	370,234590	369,227314	Pyrrolysine Derivative : Nα-tert- Butyloxyc arbonyl, Me este	369,226372	2,55	methanogenic bacteria Methanosarcinabarke ri	Bacteria	
8,51	1068,719380	1067,712104	Azalomyc in 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.

References

1. Bycroft B. W. (ed.) Dictionary of Antibiotics & Related Substances CRC Press: London New York, 1988. 962 p.
2. Cochrane S. A., Vederas J. C. Lipopeptides from *Bacillus* and *Paenibacillus* spp.: A Gold Mine of Antibiotic Candidates // Med Res Rev. – 2016. –36, N 1 – P. 4–31.
3. Fajardo-Cavazos P., Maughan H., Nicholson W. L. Evolution in th *eBacillaceae* // Microbiol. Spectrum.– 2014. – 2, N 5. – Режим доступу: https://www.ncbi.nlm.nih.gov/pubmed/26104365
4. Hamdache A., Lamarti A., Aleu J., Collado I. G. Non-peptide metabolites from the genus *Bacillus* // J Nat Prod. –2011. –74, N 4. –893–899.
5. Harunari E., Komaki Y., Igarashi Y. Biosynthetic origin of butyrolactol A, anantifungal polyketide produced by a marine-derived *Streptomyces* //Beilstein Journal of Organic Chemistry. – 2017. –13,N 1.– P. 441–450.
6. Llewellyn N. M., Li Y., Spencer J. B. Biosynthesis of Butirosin: Transfer and Deprotection of the Unique Amino Acid Side Chain // Cell Chemical Biology.– 2007. –14, N 4. –379–386.
7. Mondol M.A.M., Kim J. H., Lee M., Tareq F. S., Lee H.-S., Lee Y.-J., Shin H. J. Ieodomycins A–D, Antimicrobial Fatty Acids from a Marine *Bacillus* sp.// J. Nat. Prod. – 2011. –74, N 7. –P. 1606–1612.
8. Stein T. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions // Molecular Microbiology. – 2005. – 56, N 4. – P. 845–857.

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THE INFLUENCE OF N-BENZIMIDAZOLE SULFONAMIDE DERIVATIVES ON C*ANDIDA ALBICANS* BIOFILMS

Aim: *to study the effects of N-benzimidazole sulfonamide derivatives on biofilm formationby C. albicans and on mature biofilm, compare them with the activity of N-benzimidazole, well-known sulfonamide drug streptocide and antifungal agent fluconazole.* ***Materials and methods.*** *Antifungal activity of six N- benzimidazole sulfonamide derivatives: N-(1H-benzoylamidazol-2-yl)-4-chloro- benzenesulfonamide, N-(1H-benzoyl-amidazol-2-yl)-4-nitro-benzene-sulfon- amide, N-(1H-benzoylamidazol-2-yl)–benzene-sulfonamide, N-(1H-benzoyl- amidazol-2-yl)-4-methyl-benzenesulfonamide,N-(1H-benzoylamidazol-2-yl)-4- bromo-benzenesulfonamide, benzoylamidazole-sulfo-phenyl-acetamide, N- benzimidazole, fluconazole and streptocide towards opportunistic yeasts C. albicans was evaluated using microtiter plate essay at concentration range 0,4; 4; 40; 80 μM/ml.* ***Results.*** *N-(1H-benzoyl-amidazol-2-yl)-4-nitro-benzenesulfonamide and benzoylamidazole-sulfo-phenyl-acetamide at all concentrations showed the highest activity towards planktonic culture and forming biofilm. Against the formed biofilm, in general, the smallest number of compounds acted: benzimidazole and compound VI, with benzimidosol better activity. Fluconazole showed the best effect on the formed biofilm (up to 25%).* ***Conclusion.*** *Sulfonamides can inhibit the growth of planktonic cells and biofilm formation by C. albicans. Biofilm formation is not always accompanied by switching to hyphal forms.*

Key words: C. albicans, mature biofilm, hyphal forms, antifungal activity.

Nowadays many countries face the problemof increasing tendency to antifungal drug resistance in fungi of the genus *Candida.* It creates serious difficulties in the treatment of candidiasis and requires constant correction of treatment regimens. In this regard, studing of new compounds with antifungal activity is an urgent task [Karhanin N.P, 2008].

The process of antimicrobial drugs interaction with microorganisms that are part of biofilms has not been sufficiently studied yet. Benzimidazoles and imidazoles are already known aseffective fungicides against *C. albicans* and *Aspergillus niger* [Elnima et al., 1981]. They inhibit fungal cytochrome P450 3A- dependent C14-α-demethylase, which is responsible for the conversion of lanosterol to ergosterol, thus decrease ergosterol in the membrane of fungal cells. The interaction of benzimidazoles with cytochrome P450 can cause disturbance of many fungal enzymes [Khabnadideh et al., 2012]. It is known that inbiofilms of *C.albicans* and some other fungi, the mechanism of sulfonamide drugs action, such as sulfamethoxazole, is based on the prevention of tetrahydrofolatesynthesis by inhibiting dihydropteroatinase [Clayton et al., 2017], which affects biosynthesis of purines and thymidines. However, the influence of sulfonamides on biofilm formation by yeast-like fungi has not been studied yet.

The objective of this work was to study the effects of N-benzimidazole sulfonamide derivatives on biofilm formationby *C. albicans* and on mature biofilmand compare them with the activity of N-benzimidazole,well-known sulfonamide drugstreptocide and antifungal agent fluconazole.

Materials and methods

Antifungal properties of the following compounds were evaluated:N-(1H-benzoylamidazol-2-yl)-4-chlorobenzenesulfonamide (I), N-(1H-benzoylamidazol-2-yl)-4-nitrobenzenesulfonamide (II), N-(1H-benzoyl-amidazole)-2-yl)-benzene-sulfon-amide (III), N-(1H-benzoylamidazol-2-yl)-4-methylbenzenesulfon-amide (IV),N-(1H-benzoylamidazol-2-yl)-4-bromobenzenesulfonamide (V), benzoyl-amidazole sulfophenyl acetamide (VI), N-benzimidazole, sulfonamide and fluconazole.

As a test object, *Candida albicans* ATCC 18804 strain was used. Antifungal properties of compounds were evaluated by microtiter plate assay method in *Sabouraud broth* in 96-well plates. Studied compounds were evaluated in concentration range 0.4; 4; 40; 80 μM. Each concentration was used in 3 replicates. In the control wells, the medium without compounds was added. The plates were incubated at 37 °C 24 h. Afterwards, the optic density of the culture was measured in a plate reader (Quant BioTek, USA) at 600 nm.

Results and discussion

Compounds II and IV in all concentrations were characterized by the greatest activity regarding the planktonic culture that grew during the formation of the biofilm (data is not presented). Benzimidazole showed lower activity than its derivatives, but also exhibited an effective inhibitory effect. Fluconazole, on the contrary, in most cases, stimulated culture growth. However, at highest concentration of the drug (80 μM) inhibitory effect about 30.2% was observed. Streptocide also showed insignificant activity.

Planktonic culture above mature biofilm was less susceptible to studied compounds than that one growing over forming biofilm. Fluconazole, as in the previous case, was inactive, moreover, it stimulated biomass accumulation. Benzimidazole and its derivatives III, IV, V, and VI were able to inhibition the growth of planktonic culture by 80% (data not shown).

Fluconazole and streptocide were also ineffective towardsgrowing biofilm. Compounds III and IV were the most active. They caused the biofilm growth decrease from 33% to 55.4%. Thus, compound IV, was able to suppress effectively both the growth of the planktonic culture and biofilm formationby 51.0–52.8% (fig. 1).

N-benzimidazole sulfonamide derivatives were in most cases ineffective towards mature biofilm. Only compound II at concentration 4μM reduced the growth of biomass by 7.5% (fig. 2).

Towards mature biofilm, only N-benzimidazole and compound VI were activeat all concentrations. Fluconazole showed the best effect on the formed biofilm (up to 25% of inhibition).

Observing the microscopic picture allowed visual assess of the activity of studied compounds.

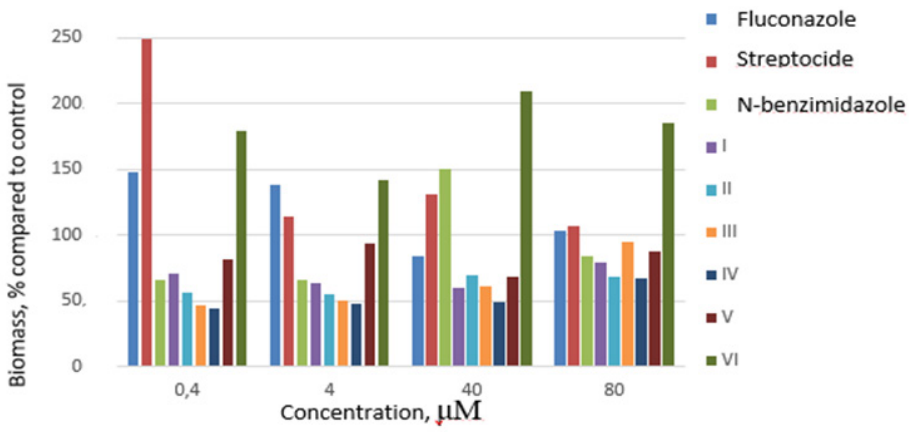

Fig. 1. The influence ofstudied compounds on *C. albicans* biofilm formation

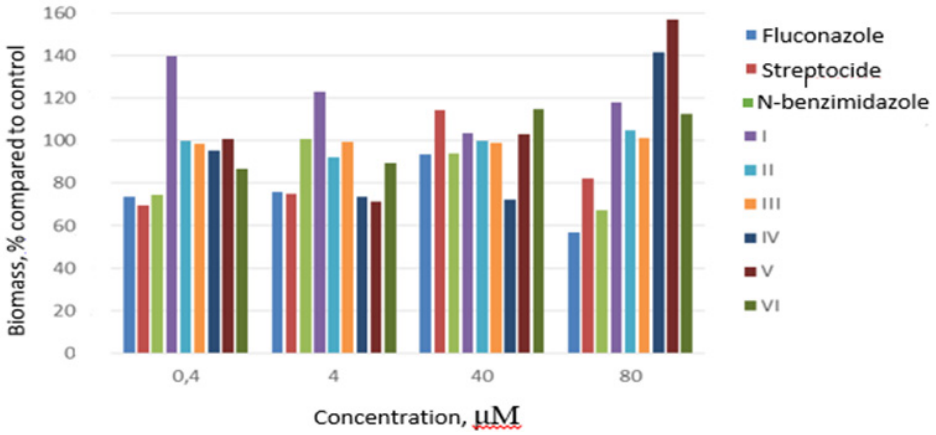

Fig. 2. The influence of studied compounds on mature C. albicans biofilm

Biofilm in control wells contained both yeast-like and hyphal forms.

In the presence of compound V, N-benzimidazole and fluconazole, hyphal forms predominate, but in the presence of compounds I, II, III and IV mixed forms were detected (fig. 3).

Assessing the influence of N-benzimidazole sulfonamide derivatives on the mature *C. albicans* biofilm the existence of hyphal formswas observed.

Only in the presence of compound III at the concentration of 0.4 μM and in the presence of streptocide in a similar concentration, yeast-like cells were observed (Fig. 4).

It should be noted that during the formation of dense biofilm the transition from yeast form to the hyphal is not always observed.

Compound III at a concentration of 0.4 μM did not affect the biofilm formation, but microscopy revealed a dense yeast-like cells layer.

Thus, in spite of the widespread opinion that *Candida* biofilm formation is always accompanied by hyphal forms, we found out the opposite phenomenon [Pavlova et al., 2017]. It is believed that chemical agents that can block the switching

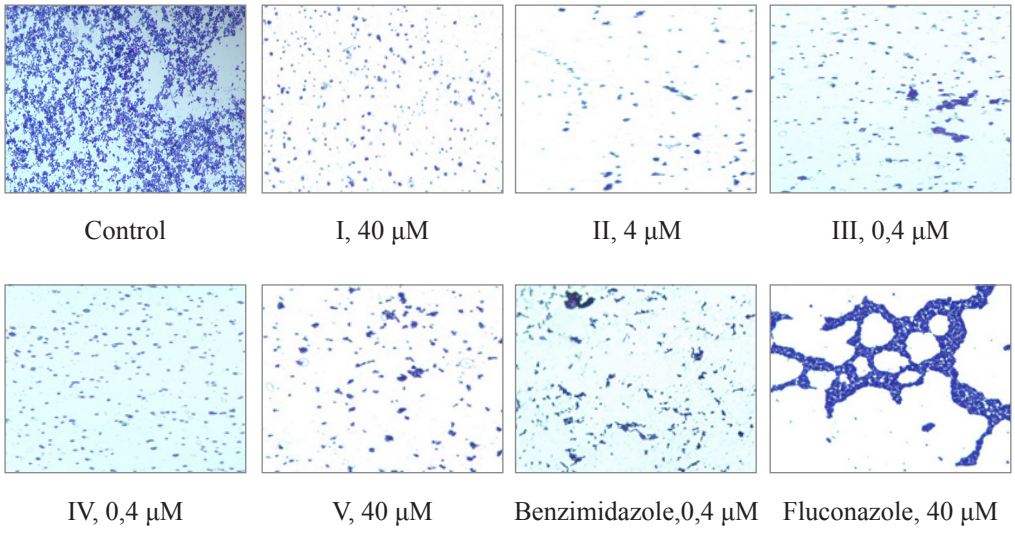

Fig. 3. Biofilms formation by *C. albicans* in the presence of studied compounds

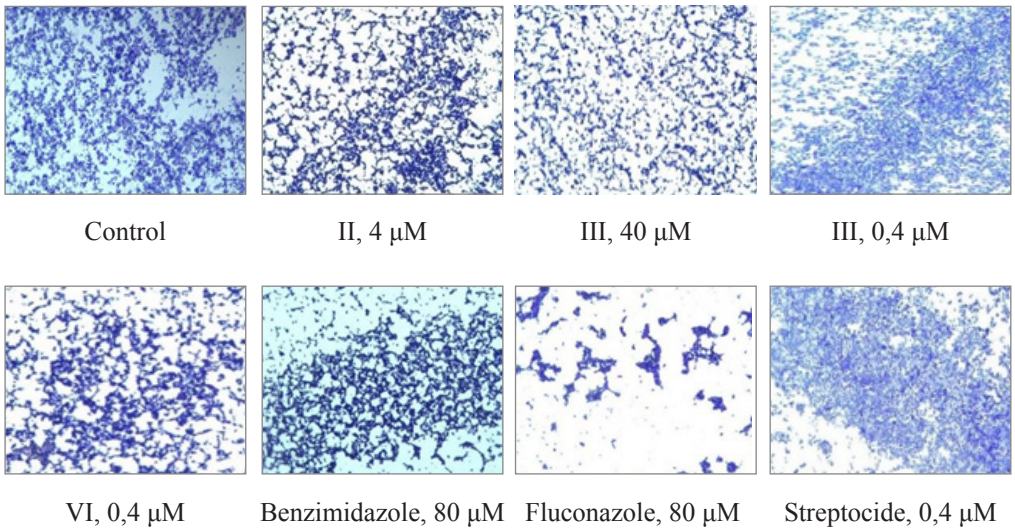

Fig. 4. Influence of studied compounds on mature *C. albicans* biofilm

between yeast-like and hyphal forms suppress the biofilm formation. However, the data we received does not confirm it.

Conclusion

To sum up, it was found out that investigated compounds have an inhibitory effect on *C. albicans*. N-benzimidazole sulfonamide derivatives affect biofilm formation and planktonic culture growth above it.

The values of plankton inhibition range from 60.9% to 80.3%, biofilm inhibition –12.8%–55.4%. Mature biofilm is less susceptible to studied agents. The values of biofilm inhibition ranged between 4.7% and 28.7%.

Unlike fluconazole and streptocide, the derivatives of N-benzimidazole sulfonamide showedhigheractivity towards *C. albicans* cells and biofilms.

References

1. Clayton W. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria / W.Clayton, H.Thien-Fah Mah// *FEMS Microbiology Reviews.* – 2017. – V. 41. – №3. – P. 276–301.
2. Elnima E. I. Antibacterial and antifungal activities of benzimidazole and benzoxazole derivatives/ E. I. Elnima, M. U. Zubair, A. A. Al-Badr // Antimicrob Agents Chemother. – 1981. – V. 19. –№1. – P. 29–32.
3. Karhanin N. P. Medicinal resistance of fungy genus Candida, associated with violations of endoecology of the vagina and intestines / N. P. Karkhanin, M. E. Abdalkin, A. E. Bilov, A. V. Gestkov, O. S. Sergeev, E. N. Abdalkina // Modern science-intensive technologies. – 2008. – №3. – P. 86–86.
4. Khabnadideh S. Synthesis and antifungal activity of benzimidazole, benzotriazole and aminothiazole derivatives/ S. Khabnadideh, Z. Rezaei, K. Pakshir// Res Pharm Sci. – 2012. – V. 7. – № 2. – P. 65–72.
5. Pavlova E. V. Modern methods of sanitary-microbiological control of raw materials and products of animal origin / I. B. Pavlova, A. N. Antonova, E. M. Lenchenko // Development of the Food and Processing Industry of Russia: Personnel and Science. " – M.: MGUPP. – 2017. – P. 146–148.

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ANTAGONISTIC EFFECT OF SAPROPHYTIC *BACILLUS MEGATERIUM* ON PHYTOPATHENIC *AGROBACTERIUM SPP.*

Abstract
*The **aim** of investigation was to find out how B.megaterium interacts with phytopathogenic Agrobacterium spp. **Materials and Methods.** Six strains of Agrobacterium sp. isolated from vineyards of Odesa region, two museum strains Agrobacterium tumefaciens and two strains Bacillus megaterium. To determine the antimicrobial activity of bacilli in relation to agrobacteria, method of diffusion into agar was used. For verification of the antagonistic interaction test plant species Kalanchoe daigremontiana Mill was used. **Results.** It has been established that bacilli inhibited the activity of agrobacteria, caused the delay of growth of their colonies. The study of interaction of bacteria by inoculation with mixtures of test plants was carried out, which showed positive results in preventing the development of tumors and reducing their total amount in 62–80%. The mentioned results allowed to conclude that further studies of the antagonistic properties of bacilli are perspective for protection of plants against crown gall disease.*

Key words: antagonism, crown gall disease, Agrobacterium, Bacillus, Kalanchoe.

Introduction

Crown gall is a dangerous disease of plants with systemic character. The causative agent of a disease is a pathogenic *Agrobacterium spp.,* which, when entering the plant, transfers a segment of its genome using the Ti-plasmid. The symptoms of this disease are galls that are formed on the plant organs. Tumors affect the growth of plants and reduce resistance to the environment and also can lead to the death of plants due to poor supply of water and nutrients [5].

The main methods of biological control of crown gall disease are the restriction of the spread of agrobacteria, the diagnosis of plant material for the presence of phytopathogens using PCR, thermal therapy, biological and chemical methods. Perspective is the use biological agents based on bacterial antagonists to Agrobacterium spp. for plant protection [3].

Among the microbial interactions one of the most important is antagonistic relation in which one of the microorganisms suppresses the other.

Bacteria of the genus *Bacillus* are often used to suppress phytopathogens because some species of them are safe for humans [1]. Bacilli are able to synthesize antimicrobial substances against pathogens, and these substances are successfully used in agriculture. These include cyclic lipopeptides, surfactin, iturin, macrolactin, subtilin [2; 4].

Materials and methods

The study were conducted using 6 strains of Agrobacterium spp. (A4, A17, A18, A19, A20, A21), *Agrobacterium tumefaciens* (C58, pJZ) and 2 strains of Bacillus megaterium (ONU 500, ONU 484). Nutrient medium potato agar 1% was used. Sowed on nutrient medium culture of agrobacteria in two repeats and made holes in agar. The cultures and supernatants of *B. megaterium* were dropped into the holes.

Bacillus megaterium supernatants were obtained using a syringe filter Millipore (0.2 mcm). Agars with cultures of *Bacillus megaterim* ONU 500 and ONU 484 (500 µl) put in the holes were incubated at 28 ° C.

To study the effect of Bacillus antagonists on the manifestation of agrobacterial infection, equal amounts (50 µl each) of cultures of agrobacteria and bacilli were mixed and this mixture was inoculated into the leaves and stems of Kalanchoe.

Results and discussion

Among the agrobacteria, strains *A. tumefaciens* pJZ (Fig. 1, A) and *A. tumefaciens* C58 strains were the most susceptible to B. megaterium and also the average sensivity was detected in strains of *Agrobacterium sp.* A4 and *Agrobacterium sp.* A19.

All other strains showed moderate sensivity to the action of secondary metabolites of bacilli.

A B

Fig. 1. Zones of growth retardation of *Agrobacterium* cultures under the influence of *B. megaterium:* A – pJZ (influence of bacilli culture); B – pJZ (influence of bacilli supernatant)

The most resistant and sensitive strains of agrobacteria depending on the strain of bacilli are demonstrated in table 1.

As it was studied by the method of diffusion into agar, among bacilli, *B. megaterium* strain ONU500 showed the greatest antagonistic activity against agrobacteria both in culture and in supernatant.

B. megaterium strain ONU 484 has not shown an antagonistic effect on agrobacteria, except the strains *Agrobacterium sp.* A19 and *A. tumefaciens* C58, where low antagonistic activity was observed.

Table 1

Antagonistic effect of *B. megaterium* on *Agrobacterium spp.*

Bacillus strain	Variant	Diameter of zone of growth inhibition of the Agrobacterium spp., mm							
		A4	A17	A18	A19	A20	A21	C58	pJZ
ONU 484	culture	–	–	–	1–2	–	–	–	–
	super-natants	–	–	–	–	–	–	1	–
ONU 500	culture	12–15	1–2	2–3	6–7	3–4	1–2	9–10	17–20
	super-natants	5–6	1–2	–	–	–	–	2–3	8–10

To prove the antagonistic action of bacilli against *Agrobacterium in vivo* inoculation of test plant Kalanchoe was carried out (Fig. 2).

After observing the plants for two months, tumors formed on the stems of the test plants were evaluated.

On a variant of *Agrobacterium sp.* pJZ + *B. megaterium* ONU 484 the number of tumors was less than in the control variant, but more than in the variant *A. tumegaciens* pJZ + *B. megaterium* ONU 500.

The ratio of tumors to bacterial mixtures is shown in table 2.

Fig. 2. Stems of test plants *Kalanchoe daigremontiana* Mill. inoculation with bacteria: A – *A. tumefaciens* pJZ + LB; B – *A. tumefaciens* pJZ + *B. megaterium* ONU 484; C – *A. tumefaciens* pJZ + *B.megaterium* ONU 500

Table 2

Manifestation of crown gall on Kalanchoe

Variant	Number of tumors on the stem and leaves of the plant	
	Absolute	%
A. tumefaciens pJZ + *B. megaterium* ONU 484	13	37.1
A. tumefaciens pJZ + *B. megaterium* ONU 500	7	20
A. tumefaciens pJZ + LB	35	100

When inoculation with a mixture of *A. tumefaciens* pJZ strain with *B. megaterium* ONU 500 strain was carried out, a decrease in the number of tumors on the test plant in 80% was noted. Thus, the strain of *B. megaterium* ONU 500 was the most effective.

Conclusions

Saprophytic *B. megaterium* ONU 500 and *B. megaterium* ONU 484 showed antagonistic activity against phytopathogenic *Agrobacterium spp.,* inhibiting their growth on the nutrient medium.

Inoculation of *B. megaterium* ONU 484 reduced the number of tumors on test plants infected with A. tumefaciens pJZ in 62.9%, and inoculation with *B. megaterium* ONU – in 50 - 80%.

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References

1. Beric T., Kojic M., Stankovic S., Topisirovi L., Degrassi G., Myers M., Venturi V., Fira D. Antimicrobial activity of *Bacillus sp.* natural isolates and their potential use in the biocontrol of phytopathogenic bacteria // Food Technology and Biotechnology. – 2012. – V.50, № 1. – P. 25 – 31.
2. Chen Y., Yan F., Chai Y., Liu H., Kolter R., Losick R. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation // Environ. Microbiol. – 2013. – Vol. 15, № 3. – P. 848–864.
3. Ferrigo D., Causin R., Raiola A. Effect of potential biocontrol agents selected among grapevine endophytes and commercial products on crown gall disease // BioControl. –2017. –V. 62, №6, p. 821– 833.
4. Huang J., Wei Z., Tan S., Mei X., Shen Q., Xu Y. Suppression of bacterial wilt of tomato by bioorganic fertilizer made from the antibacterial compound producing strain *Bacillus amyloliquefaciens* HR62// J. Agric. Food Chem. – 2014. – Vol. 62.– P. 10708 – 10716.
5. Matthysse A. G. The genus *Agrobacterium* // The Procariotes: Proteobacteria: alpha and beta subclasses. – 2006. – V. 5. – P. 91 – 114.

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L'INFLUENCE DE *LACTOBACILLUS PLANTARUM* A L'AGENT CAUSATIF DE FUSARIOSE DU PIN *(PINUS SYLVESTRIS L.)*

Les bacteries du genre Lactobacillus se caractérisent par une fotre activité antagoniste, y copris contre les agents pathogénes de fusariose des plantes. les bactéries de genre L. plantarum peut etre considéré comme micro-organisme prometteur pour l'améliorer la similitude de graines du pin et protection contre les agents pathogènes Fusarium.

Mots clés: L. plantarum, le mycélium, la macroconidies et microconidies.

Introduction

Plus de 33.0–40.0% des jeunes plantes de pépinières de cultures forestières, cultivées en Ukraine et dans d'autres pays du monde chaque année, sont traitées avec des fongicides, pourtant les maladies continuent à se développer affectant la qualité du pin et générant des pertes de rendement. Cette maladie dangereuse est provoqué par les micromycètes des genre *Alternaria, Botrytis, Pythium, Rhizoctonia, Phytopthora,* mais le plus souvent – le représentants du genre *Fusarium* [Ahangar, 2011].

La protection biologique des plantes par des souches de micro-organismes – est une alternative applications à l'utilisation d'agents chimiques à action antofongiques. Les produits biologiques ne violent pas l'équilibre dans la biocénose, qui devient un avantage sur les dommages environnementaux causés par des substances nocives. Les lactobacilles appartiennent au micro-organismes de GRAS ("Generally Recognized as Safe"), ils sont absolument sans darger pour la santé humaine et animale, ce qui constitue un avantage supplémentaire de l'utilisation de tels micro-organismes.

L'objectif de ce travail consiste à isoler de l'agent pathogène du pin et etudier de l'effet de lactobacillus sur la fusariose, identifier la souche de lactobacillus, le plus active contre le *Fusarium spp.*17 lors d'expériens sur le milieu de culture et l'assout de la plante *Pinus sylvestris.*

Matériel et méthodes

Les semis de pins ont été obtenus, par voie en semencement dans un sol tourboux commercial non stérile. La germination a été realisé dans une serre (avec une température de croissance optimale de 18–22°, l'éclairage 12 heures).

La culture de micromycètes nous isolons par une méthode à partir affectée des semis lunaires du *Pinus sylvestris.* Les semis touchées nous plaçons sur une boite de Petri, avec la milieu artificiel Saburo et laissons à 25° pendant 5 jours, jusqu'à l'apparition de mycélium du champignon autour des plantules.

Pour la détection et l'indentification la culture nous étudions les propriétes

culturelles est morphologiques du mycromycète (la couluer, la consistance, les pigments dans la milieu de sélection).

A B C

Img. 1. Les propriétes culturelles est morphologiques des micromycetes *Fusarium spp.* 17: A – la culture du champignon sur le milieu nutritif Saburo, la colonie, B – micélium de pathogen; C – macroconidies et microconidies du champignon

Les caractéristiques morphologiques du mycélium sont: blanc-rose, bien développé, sur certains sites – immergé dans la gélose nutritive; le substrat sont coloré en vin-rouge couleur *[Nelson, 1983]*.

La culture de micromycètes nous cultivons sur la gélose nutritive Saburo, pendant 2 semaines et sélectionné un fragment de milieu avec le mycélium, qui nous plaçons dans un flacon avec de l'eau stérile distillée et secouons pour obtenu la suspension, dans laquelle plus tard nous calculons les conidies avec le microscope optique.

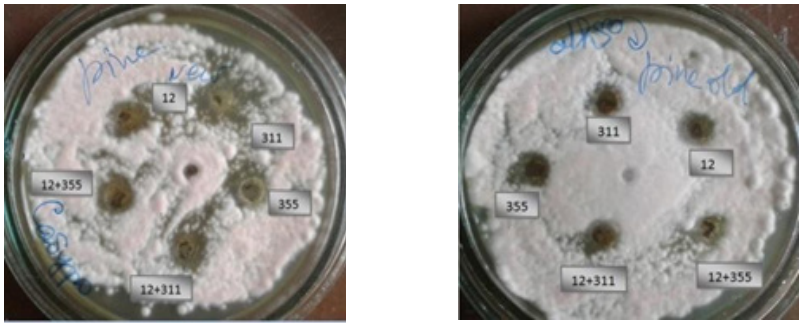
L'influence de la culture et accellulaires à chaine ramifiée, qui ont reçu pendant la centrifugation à 10000 tours/min pendant 20 minutes. Nous avons semé par application la methode "le pelouse" avec le milieu Saburo. Après dans la gélose semé nous faisons les trous avec le pointe stérile. Dans les trous ont été introduite la culture ou la culture accellulaire de lactobacilles. La boite Petri avec la récoltes sont laissé pendant 7–10 jours avant l'apparition du mycélium *[Ahangar, 2011]*.

Résultat et discussion

Les souches de lactobacilles *L. plantarum* ONU 12, ONU 311, ONU 355 de la collection la chaire microbiologie, virusologie et biotechnologie à l'universite Mechnikov et leurs consortiums *L. plantarum* ONU 12 et *L. plantarum* ONU 311 cultivaient dans le milieu MRS avec la t° 37 °C pendant la journée et trois jours.

Notre étude de l'influence des cultures de lactobacilles sur la croissance de. Fusarium spp.17 à montré, la cultures quotidienne *L. plantarum* ONU 12, ONU 311, ONU 355 et leurs consortiums *L. plantarum* ONU 12 et *L. plantarum* ONU 311 provoquent les zones de retard de croissance le mycélium.

A l'étape suivante nous étudions l'influence la souche *L. plantarum* ONU 311, parce que les bactéries de cette souche très déprimé à l'agent causal de fusariose (la zone de suppresion de croissance 5 – 6 mm).



A B

Img. 2. Les zones de retard de croissance *Fusarium spp.* 17 sur le milieu Saburo à l'influence des cultures de *L. plantarum* (le trou central – le controle négatif, sans introduit de culture)

Conclusion

L'introduction dans le sol le culture de chene *L. plantarum* ONU 311, augmenter la germination des graines du pin infecte *Fusarium spp.* 17 avec 17.3%, et la survie des plantules – 7%. L'introduction dans le sol le culture quotidienne *L. plantarum* ONU 311 aussi positivement influencé sur la croissance des plantules de pin, augmenter la hauteur avec 8%.

Donc les bactéries de genre *L. plantarum* peut etre considéré comme micro-organisme prometteur pour l'améliorer la similitude de graines du pin et protection contre les agents pathogènes *Fusarium.* Avec ça, doit etre respecté certaines conditions de traitement avec des bactéries: l'introduction du fluide de culture dans le sol avec des cellules, qui ont grandi pour un jour.

Liste bibliograhique

1. Ahangar M. A., Dar G. H., Bhat Z. A., Sofi N. R. Fungi associated with root rot of *Pinus wallichiana* seedlings in Kashmir // Plant Pathology Journal. – 2011. – V. 10 (1). – P. 42 – 45.
2. Gordon T. R., Swett C. L., Wingfield M. J. Management of *Fusarium* diseases affecting conifers // Crop protection . – 2015. – Vol. 73. – P. 28 – 39.
3. Nelson P. E., Toussoun T. A., Marasas W. F. O. *Fusarium* species: an illustrated manuel for identification, Pennsylvania, 1983, 193 p.

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Міжнародна конференція молодих вчених

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