MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
ODESA NATIONAL I. I. MECNIKOV UNIVERSITY

BIOLOGY, BIOTECHNOLOGY, BIOMEDICINE

XVI International SummerSchool Conference

Proceedings

22th June – 5th July 2021, Odesa

ODESA
ONU
2021

UDC [579+60] (063)
M 78

M 78 **Biology, Biotechnology, Biomedicine:** materials of young scientists international conference 3-5 July 2021 / Ed. board : M. B. Galkin, N. V. Limanska, V. O. Ivanytsia – Odesa : «Odesa I. I. Mechnikov National University», 2021. – 68 p.
ISBN 978-617-689-376-9

This collection contains the materials of the International Summer School-Conference « Biology, Biotechnology, Biomedicine».

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

UDC [579+60] (063)

ISBN 978-617-689-376-9

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UDC 575.87:577

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CULTURAL PROPERTIES OF ACTINOBACTERIA ISOLATED FROM BLACK SEA SHELL ROCKS

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Abstract. *The ability of strains of actinomycetes isolated from sea shell rocksto grow on ISP series media, to use different carbon sources and tolerance to different salt concentrations (NaCl) was studied. It was shown that the studied strains had the ability to grow on all media of the ISP series, and some are able to form melanoid pigments. For most strains, the optimal salt concentration was a concentration of up to 7.0%. The ability of actinomycete strains to ferment different carbon sources was shown. Lactose, glucose and galactose were the most favorable.*

Keywords. *Actinomycetes, BlackSea, morphologicalcharacteristics*

Introduction

Among gram-positive bacteria, actinomycetes show the richest morphological differentiation, which is one of the main stages of their polyphasic classification [Xiu, 2016; Barka et al., 2016]. This variability allows you to quickly adapt them to changing environmental conditions. Beginning studies of morphological variability, we first study morphological features that investigate the type of mycelium, its color, the presence of pigment, the formation of chains of spores and sporangia, the structure of hyphae and ultrastructure of spores or sporangia under different growth conditions on different media [Qinyuan, 2016]. Hereditary variability of actinomycetes obeys the law of homologous series [Ludwig et al., 2012]. In populations of any species, along with the basic form, there are options: oligosporous, asporogenic, dwarf, cardioid, colored. In general, a unique combination of morphological features of eukaryotic fungi with molecular, chemical and physiological features of prokaryotes are characteristic of actinomycetes [Zhi et al., 2009]. In addition, the interest in morphological variability is due to the fact that it is most often associated with the ability to antibiotics. Also, the degree of manifestation of the process of variability depends on the genetic nature of the organism and environmental conditions Actinobacteria [Zhi et al., 2009; Nouioui et al., 2018]. The aim of the study was to investigate the change in morphological characteristics of actinomycete strains isolated from the fouling zone of shell rock immersed in the Black Sea.

Materialsandmethods

Morphological characteristics of the strains were studied with using ISP media according to the International Streptomyces Project (ISP). Observations of growth characteristics were evaluated after 3, 7, 14 and 27 days of cultivation at

30 °. ISP-6 and ISP-7 media were used to detect the production of melanoid pigments [Basavaraj et al., 2010; Kuznetsova et al., 1990; Гаузе и др., 1983].

Tolerance of the strain to sodium chloride (NaCl) was studied using MPA medium to which were added various concentrations of NaCl (1.0, 2.0%, 5.0%, 7.0%, 9%, 12.0%). Incubation was performed in a thermostat at a temperature of 28 - 30 ° C for 14 days. The presence of growth was recorded visually [Kumar et al., 2014; Benhadj et al., 2019].

Utilization of carbon sources by strains was performed using the minimum environment ISP 9 [Kumar et al., 2014; Benhadj et al., 2019]. Carbon sources such as glucose, fructose, galactose, glycerol, lactose and xylose were added separately at a rate of 1% to the volume of the medium. The presence of growth was recorded visually.

Results

When cultured on media of different composition, we observed a slight change in pigmentation and type of air mycelium. In general, most strains had a dark substrate mycelium color with light aerial mycelium. Isolated strains were characterized by various morphological properties, including morphological. The morphology of colonies of actinomycetes isolated from shell rock using ISP media revealed changes in the pigmentation of the substrate mycelium, the formation and pigmentation of air mycelium, and the production of melanoid pigments (ISP-6 medium), which reflects the nutrition preference of selected microorganisms(table 1, Fig. 1-2).

Strains Lim 3.1 and Lim 3.4 and Lim 12.2, had the ability to repay assessment (Table 1).

For most strains, the composition of the growth medications but strains Lim 7.1 and Lim 7.2 showed more active growth on media with mineral composition.

According to the results of the study of the tolerance of actinomycete strains to sodium chloride (NaCl), it was shown that almost all our strains were able to grow at high concentrations of NaCl (Fig. 3). According to growth indicators, we divided the strains into two groups: the first included strains that showed growth from the first days , and the second included strains that showed good growth after 14 days. The only strain that did not grow on NaCl-containing medium was Lim 9.2. The Lim 9.1 strain stopped growing in the presence of 5.0% NaCl in the medium. In general, according to the results of the study of tolerance of our strains to NaCl, it was shown that 73.3% of all strains were resistant to the maximum concentration (9.0 and 12.0%), but they grew with growth retardation, to a concentration of 7.0% were resistant 80.0% of all strains (26.7% grows after 7 days, and 53.3% grows after 14 days) and 93.3% were resistant to concentrations below 7.0% NaCl.

From table 2 and figure 4 we see that the studied strains of actinomycetes show a fairly high variability on the basis of the ability to ferment carbohydrates.

Table 1

Characteristics of actinomycetes isolated strains growth from shell rock on ISP series media

	ISP-1	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9
Lim 2.1	+	+	+	+	+	-	+	+*
Lim 2.2	+	+	±	+	+	-	+	+
Lim 3.1	+*	+*	+*	+*	+	+**	+	+
Lim 3.2	+*	+*	+*	+*	+	-	+	+
Lim 3.3	+*	+*	+*	+*	+	+	+	+
Lim 3.4	+*	+*	+*	+*	+	+**	+	+
Lim 5.1	+	+	+	+	+	+	+	+
Lim 6.1	+	+	+	+	+	±	+	+
Lim 6.2	+	+	+	+	+	+	+	+
Lim 7.1	±	±	±	+	+	-	+	-
Lim 7.2	+	±	±	+	+	-	+	+
Lim 9.1	+	+	+	+	+	-	+	+
Lim 9.2	+*	+	+	+	+	+	+	+
Lim 12.2	+*	+*	+	+	+	+**	+	+
Lim 12.3	+	+*	+*	+	+	-	+	+

Note: "+" -presence of dense growth; "+" - weak growth; "-" - lack of growth; * - the ability to form a soluble pigment; ** - ability to form melanoid pigment

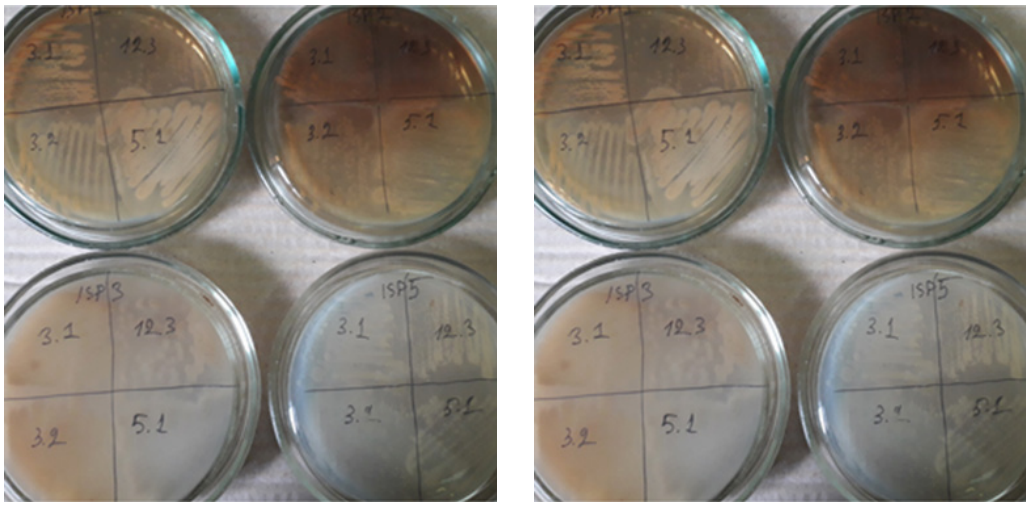

Fig. 1. Photographs of the substrate mycelium of strains of actinobacteria isolated from shell rock after cultivation on media of the ISP series (27 days of observation)

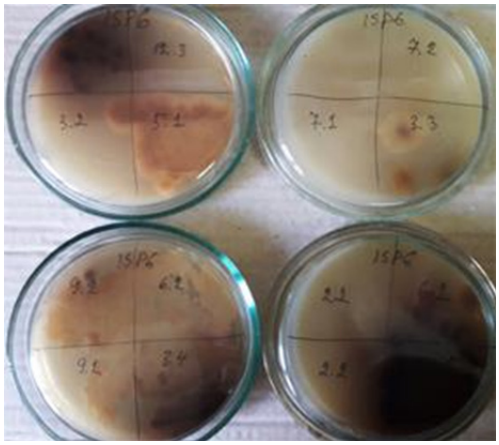

Fig. 2. Photographs of the substrate mycelium of strains of actinobacteria isolated from shell rock after cultivation on ISP-6 medium

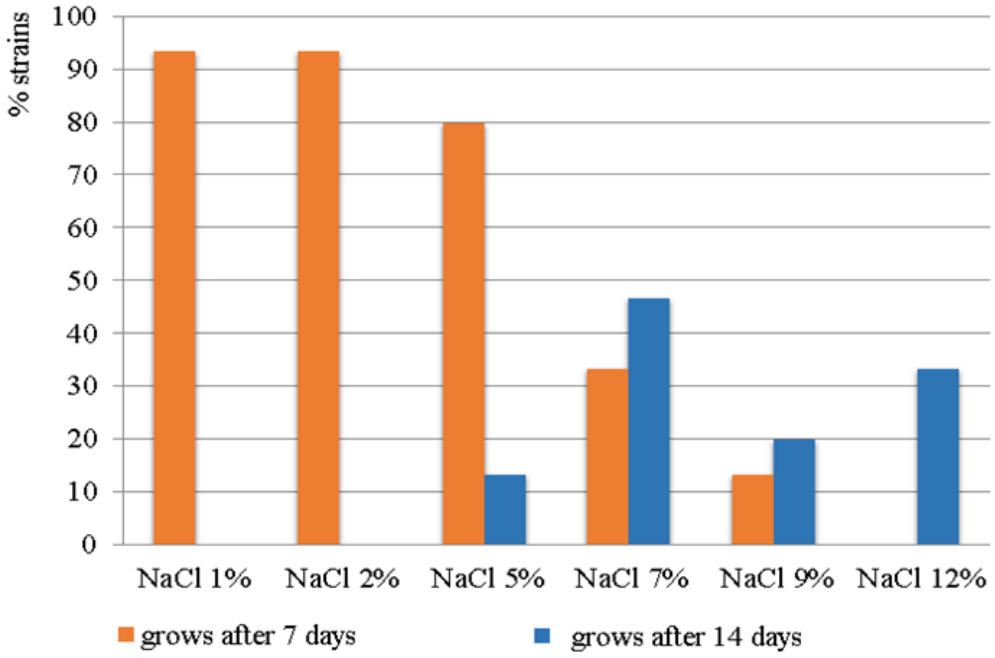

Fig. 3 Indicators of tolerance to NaCl of the studied strains of actinomycetes

Table 2

The ability of actinomycete strains to ferment different carbon sources

Strain	Carbonsubstrate					
	Glucose	Fructose	Xylose	Lactose	Glycerol	Galactose
Lim 2.1	-	-	-	+	+	-
Lim 2.2	+	-	-	+	+	+
Lim 3.1	+	-	-	+	+	+
Lim 3.2	+	+	-	+	+	+
Lim 3.3	+	-	-	+	-	+
Lim 3.4	+	+	+	+	+	+
Lim 5.1	+	+	+	+	+	+
Lim 6.1	+	-	-	+	+	+
Lim 6.2	+	+	-	+	+	+
Lim 7.1	+	-	-	+	-	+
Lim 7.2	+	+	+	+	+	+
Lim 9.1	+	+	-	+	+	+
Lim 9.2	+	+	-	+	+	+
Lim 12.2	+	+	+	+	+	+
Lim 12.3	+	+	+	+	+	+

Note: "+" the presence of growth; "-" - lack of growth.

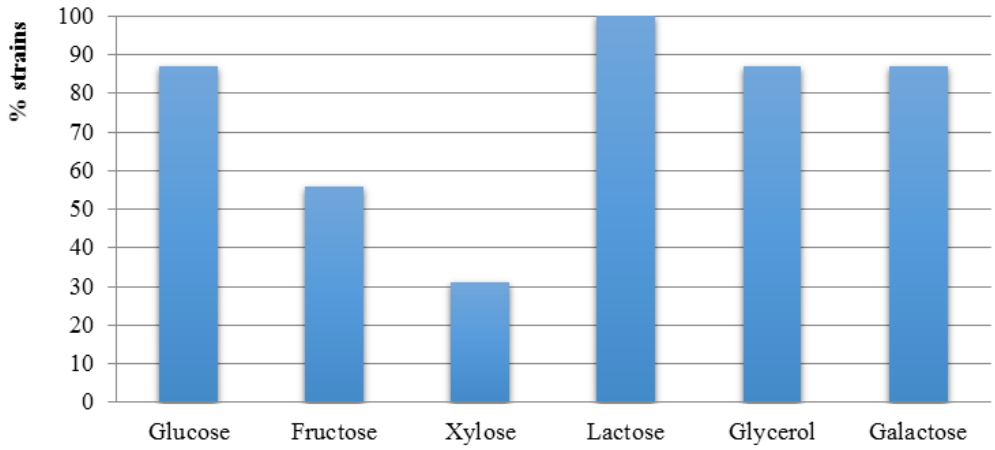

Fig. 5. Indicators of fermentation of various carbon substrates by strains of actinomycetes isolated from shell rocks

Based on the obtained data, according to the degree of utilization by actinobacteria, the studied carbon substrates can be arranged in the following order: lactose > glucose / glycerol > galactose > fructose > xylose.

Conclusion

Studies have shown that actinomycetes isolated from sea shell rocks have variable morphotypes that depend on the type of medium used. The study of colony morphologies of actinomycetes isolated from shell rocks using ISP series media revealed changes in the pigmentation of the substrate mycelium (shades of brown and brown), formation and pigmentation of air mycelium, and production of melanoid pigments (black on ISP-6 medium).

According to our data on the ability of actinobacteria to ferment carbohydrates, it was shown that all studied strains had the ability to ferment lactose. 93.3% fermented glucose and galactose. according to the minimum number of strains was capable of fermentation of xylose (31.1% of strains).

In the study of tolerance to NaCl, it was shown that most strains showed good growth at a concentration of not more than 7.0%. With increasing concentration, growth retardation (up to 14 days) was recorded. A small proportion of actinomycetes (6.6% of all strains) grew well at a concentration of 9.0% NaCl.

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UDC 604.6:577.13

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EFFECT OF *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION ON THE ANTIOXIDANT STATUS OF TILESIUS' WORMWOOD "HAIRY" ROOTS

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The aim *of the study was to investigate the effect of Agrobacterium rhizogenes-mediated transformation on the antioxidant status of Tilesius' wormwood "hairy" roots.* **Materials and methods.** *Lines of "hairy" roots of Artemisia tilesiiobtained after agrobacterial transformation with A. rhizogenes A4 wild strain and with A. rhizogenes carrying the human interferon-α2b gene were used for the study. Total flavonoid content and antioxidant activity were determined.* **Results.** *Thetotal flavonoid content of "hairy" root lines varied from2,57±0,28 mg RE/g FW to 9,47±1,97 mg RE/g FW comparing to 1,90±0,34 mg RE/g FW of the control. The antioxidant activity expressed in* EC_{50} *was 3,21…9,96 mg FW comparing to 8,57 mg FW of the control.* **Conclusions.** *Agrobacterium rhizogenes-mediated transformation affected the antioxidant status of the "hairy" root lines of Artemisia tilesii taken for the study, resulting in the increase of total flavonoid content and antioxidant activity.*

Key words: *"hairy" roots, flavonoids, antioxidant activity, Artemisia tilesii.*

Introduction

Natural antioxidants, especially phenols, flavonoids, carotenoids and vitamins (vitamins E and C), are common in medicinal plants and have a wide range of effects, including anti-inflammatory, rejuvenating, antiatherosclerotic and antitumor ones [1].Moreover, it is recognized that the addition of exogenous antioxidants or strengthening endogenous antioxidant defenses of the body is a promising method of counteracting the undesirable effects of oxidative stress [2].

The *Artemisia* L. genus is one of the largest genera in the *Asteraceae* family, which includes more than 500 species [3].They are widespread mainly in the northern hemisphere – a temperate zone of Europe, Asia, North America and North Africa. Plants of this genus contain ascorbic acid, inulin, sugars, fatty oils, carotene, tannins, resins, essential oils, flavonoids, coumarins, sesquiterpenes, tannins, glycosides, cineole, thujone, fenhon, *p*-cimen, camphene, borneol, pinene, amyrin, nerol, murolol, scopoletin [4, 5]. Due to such an abundance of chemicals that are biologically active, a lot of species from *Artemisia* genus are considered as medicinal plants.

A. tilesii (Tilesius' wormwood) is poorly-studied, as it is only found throughout the northern parts of Alaska, Japan, Siberia and Canada.This species has long

been used by Native Americans for wound healing and treatment of various illnesses of the cardiovascular system, as well as for prevention of infectious diseases [6]. The plant is a perennial herb, unpretentious to environmental conditions and able to grow on different types of soil. In the wild, the shoot of the plant reaches 80 cm, and the leaves – from 2 to 6 cm in length, toothed and divided;leaf color is green with a whitish tinge [7, 8].

Taking into account the abovementioned, it would be very useful to boost the production of secondary metabolites in Tilesius' wormwood for the investigation of its antioxidant potential. To do so, "hairy" roots formation by *Agrobacterium rhizogenes*-mediated transformation can be used. As, according to the literature, cultures of "hairy" roots can accumulate biologically active substances in much larger quantities than the original untransformed plants, which is due to the transfer of foreign genes into the genome of the plant by agrobacterial transformation [9].

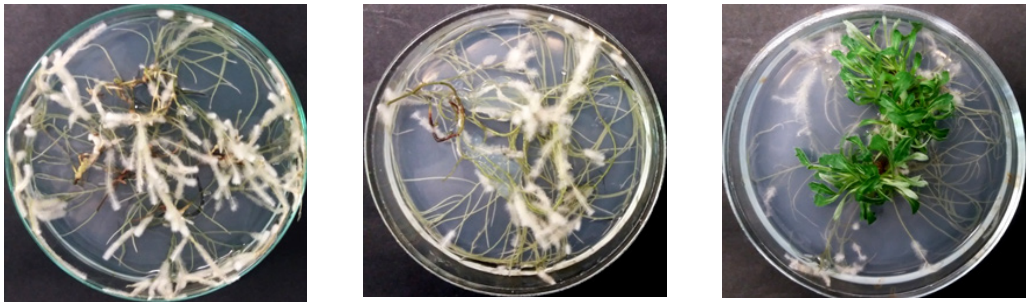
Materials and methods

For the conduction of experiments such lines of *A. tilesii* were used: 4 lines obtained by agrobacterial transformation with *A. rhizogenes* A4 wild agropine strain (fig.1, *a*), 4 lines obtained by agrobacterial transformation with strain of *A. rhizogenes* carrying the target gene of human interferon-α2b (fig.1, *b*) under the control of 35S promoter of cauliflower mosaic virus (pCB124) and 1 control non-transformed plant for the reference (fig.1, *c*). All lines of "hairy" roots were produced in the previous studies as a result of agrobacterial transformation by a modified method [10] in the Institute of Cell Biology and Genetic Engineering of NASU.

As it can be seen in figure 1, "hairy" root lines of the same species differ significantly from each other and from the control plant: changes are not only in the structure of roots and their branching, but also in their color (in some lines the appearance of green color was observed, i.e. the ability for photosynthesis);as well, "hairy" root lines showed increased rate of growth and biomass accumulation over time.

To determine the effect of genetic transformation on the antioxidant status of plants, total flavonoid content and antioxidant activity were studied. Total flavonoid content was calculated by the method of Pekal and Pyrzynska [11] spectrophotometrically on a spectrofluorimeter Fluorate-02-Panorama. To prepare the extracts, the roots were separated from the agar, washed with distilled water, dried on filter paper, weighed 0.3 g and ground in a porcelain mortar in 3 ml of 70% ethanol. The resulting homogenate was centrifuged in an "Eppendorf Centrifuge 5415 C" at 15.000 g for 10 minutes. The reaction mixture in the cuvette contained 0.25 ml of extract supernatant, 1 ml of deionized water, 0.075 ml of 5% $NaNO_2$ solution. Incubation duration was 5 minutes. Then 0.075 ml of 10% $AlCl_3$ solution was added and kept for another 5 minutes. After that, 0.5 ml of 1M NaOH and 0.6 ml of deionized water were added. Optical density was determined at $\lambda = 510$ nm. The reference solution contained all the reagents abovementioned, but the supernatant was replaced by deionized water. Calibration graph was constructed using rutin in the reaction mixture ($y = 0.8842x$, $R^2 = 0.998$).The flavonoid content was determined in mg/g of fresh root weight in rutin equivalent according to the formula:

$$C_1 = C \cdot V / m, \quad (1)$$



a *b* *c*

Fig.1. *Artemisiatilesii* "hairy" roots *(a and b),* and control plant (c)

where C_1 is the concentration of flavonoids in 1.0 g of fresh weight of plant material, mg/g;C – concentration of flavonoids in ethanol extracts, mg/ml;V –volume of ethanol used to prepare the extract (3 ml);m – weight of plant material used for the experiment (0.3 g).

Antioxidant activity (AOA) of ethanol extracts of "hairy" roots was measured using the DPPH test [12] spectrophotometrically on a spectrofluorimeter Fluorate-02-Panorama. The reaction was performed in cuvettes, successively decreasing the concentration of ethanol extracts, adding DPPH at a concentration of 0.0001 M. Incubation duration was 20 minutes. Control solution was DPPH, reference solution – 96 % ethanol.The percentage of DPPH radical reduction was determined by the formula:

$$AOA = [(OD_1\text{-}OD_2) / OD_1] \cdot 100\%, \quad (2)$$

where OD_1 – optical density of DPPH, units;OD_2 –optical density of the reaction mixture after incubation, units. AOA was expressed in terms of the effective concentration (EC_{50}) in mg of fresh weight of "hairy" roots required to reduce 50% of DPPH in the sample.

Results and discussion

Total flavonoid content in the investigated lines of "hairy" roots of *A. tilesii*varied greatly (fig. 2), but for all the samples this content was higher than in the control line: from 2,57±0,28 mg RE/g FW to 9,47±1,97 mg RE/g FW comparing to 1,90±0,34 mg RE/g FW of the control.

However, the variation among the two groups of "hairy" roots (one group was obtained from agrobacterial transformation with *A. rhizogenes* A4 wild strain and another – with *A. rhizogenes* carrying the target gene of human interferon-α2b pCB124) showed that the presence of the target gene of human interferon-α2b does not affect the increase or decrease in secondary metabolites production of Tilesius' wormwood "hairy" roots.

Such a result may be due to the fact that the production of flavonoids by "hairy" roots is affected by the presence of *A. rhizogenesrol* genes in the plant genome after the transformation, and not by the presence of the target genes that this bacterium can carry in the plasmid.

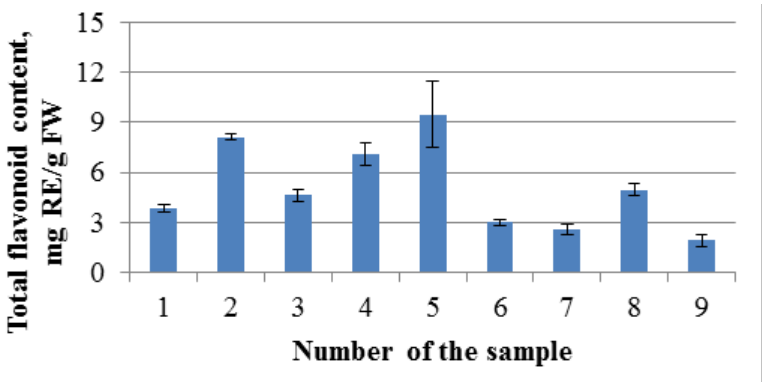


Fig.2. Total flavonoid content in ethanol extracts of *A. tilesii,* 1-4 – "hairy" roots transformed by A4 wild strain, 5-8 – "hairy" roots transformed by *A. rhizogenes carrying ifn-α2b* gene, 9 – roots of the control plants

Antioxidant activity of ethanol extracts of *A. tilesii* expressed in EC_{50} (fig. 3) varied from 3,21 mg FW to 9,96 mg FW comparing to 8,57 mg FW of the control. I.e., AOA of 7 out of 8 "hairy" root lines was better than in the control plant, as the lower value of EC_{50} corresponds to the higher antioxidant activity. The EC_{50} parameter mostly correlated to the total flavonoid content: higher content of flavonoids corresponded to the lower EC_{50} values for most of the lines. In the same way as for the flavonoids content, there was no significant difference between the two groups of the "hairy" roots.

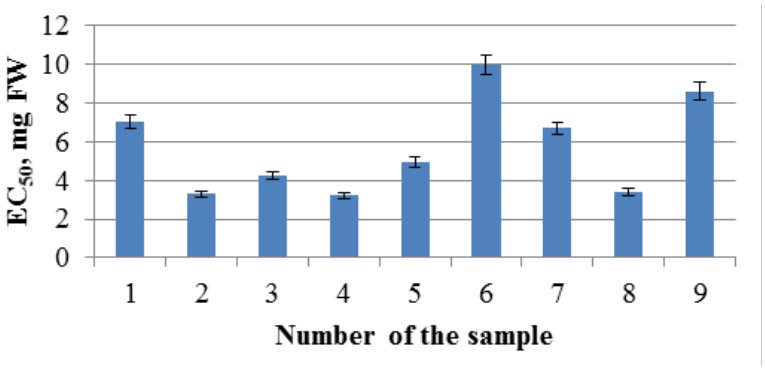


Fig. 3. Antioxidant activity of ethanol extracts of *A. tilesii,* 1-4 – "hairy" roots transformed by A4 wild strain, 5-8 – "hairy" roots transformed by *A. rhizogenes carrying ifn-α2b* gene, 9 – roots of the control plants

Conclusions

Agrobacterium rhizogenes-mediated transformation affected the antioxidant status of the "hairy" root lines of *Artemisia tilesii*. The difference in total flavonoid content between the "hairy" root lines and control line for some samples was even 4- and 5-fold, that is a great increase. The same effect of transformation was on the antioxidant activity of the studied ethanol extracts. Moreover, the values correlated for most of the samples. However, there was no significant difference in activity

between the lines obtained by agrobacterial transformation with *A. rhizogenes* A4 wild agropine strain and with *A. rhizogenes* carrying the target gene of human interferon-α2b.

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UDC 615.849.19:616.36

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FREE RADICAL PROCESSES IN THE MITOCHONDRIAL FRACTIONS OF RATS' LIVER UNDER THE CONDITIONS OF BISPHENOL A ADMINISTRATION AND LOW-LEVEL LASER IRRADIATION

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Abstract. The aim of this study is to investigate the effect of low-level laser irradiation on oxidative stress parameters in hepatic mitochondrial fractions of rats under the conditions of BPA administration. Short-term BPA-exposure results in the induction of free radical photopolymerization O_2• –and decreased activity of antioxidantenzymes. At the same time, low level laser irradiation reduces the prooxidant antioxidant enhancemen of the antioxidative which is primarily associateded a short-termincreaseinthetemperatureoflight-absorbingbiomolecules. This effects observations notes LLLI after BPA exposure.

Keywords: *bisphenol A, free radical processes, low lever laser irradiation, mitochondrial fraction, antioxidant system*

Introduction

Oxidative stressis characterized by the imbalance between anincreased generation of free radicalsand a reduced antioxidant capacity**.**The mitochondria are much more susceptible to oxidative stress as the site of the electron transport chain for adenosine triphosphate (ATP) production and the main source of ROS[1]. Many xenobiotics may induce the damage of mitochondria due to the enhanced generation of reactive oxygen species. These compounds include bisphenol A (BPA), the xenoestrogen and monomer in polycarbonate synthesis (plastic containers, water bottle, protective and corrective eyewear), plasticizer in the production of epoxy resins, a cold developer in a thermal paper, that can induce mitochondrial dysfunction via the shift in the balance between proxidants and antioxidants[2, 3].

The application of laser irradiation to correct pathological lesions of various etiologies has become extremely relevant. Among many possiblemechanisms potentially responsible for the above,are increases in energy metabolism and ATP synthesis,stimulation of defences against oxidative stress[4]**.** These effects of low level laser irradiation (LLLI) are the result of primary reactions involving the absorption of light of a certain wavelength by specific chromophores in the cell, in particular components of the mitochondrial respiratory chain, such as cytochromes, cytochrome oxidase and flavine dehydrogenase, which leads to a change in the redox state of the cytoplasm. The conversion of laser energy into useful energy for cells, due to photochemical and photophysical reactions, can stimulate mitochondrial production of adenosine triphosphate, cell proliferation and protein synthesis [5].

In the current study, we decided to evaluate the effect of low level laser irradiation on BPA-induced free radical processes in the mitochondrial fractions of rats' liver.

Materials and Methods

Male Wistar rats (10-12 weeks of age and with body weight 120±10 g) were maintained at 22°C with a 12-h light/dark cycle and had free access to normal combination water. Animal hand llangadwaladr llangedwyn llangernyw 26 of the Law of Ukraine No 3447-IV 21.02.2006 "On the protection functionality "The European Convention for the Protection of Vertebrate Animalsusedfor Experimental and characterization (Strasbourg, 1986),"General Ethical Considerations for Animal Experimentation"establishedby First Ukrainian Congresson Bioethics, and withregardto NIH Guideforthe Careand Use of Laboratory Animals[6].

The rats were divided into five groups, each consisting of eight-nine animals. The first group consisted of intact rats (control group, C), the animals of the second group received BPA bygavage for 3 days once a day (BPA group). The third and fourth groups were formed by animals which received BPA and irradiated with laser after each (BPA+LLLI group) or last (BPA+$LLLI_a$group) administration of xenobiotic.

BPA was dissolved in corn oil and administered at 50 mg/kgbodywt which corresponded to the lowest observable adverse effect level (LOAEL) dosage [7]. LLLI was performed once a dayfor3 days after each or last bisphenol A exposure. Alaserdiode (50mW) with 650 nmcontinuouswavelengthwasappliedtotheskinsurfaceattheanatomicalsiteoftheliver. Theexperimentalgroupswereirradiatedwith a dosage1,5 J/cm^2 and power density 12,5 mW/cm^2, andthedurationofirradiationwasselected 120 s.Thedistancebetweenthelasersourceandthe skin surfacewas 10 cm.

Theratswereanesthetizedunderlightetheranesthesiaafter a 24-h fast. Theliverwasexcised, rinsedwithphysiologicalsaline, weighed, flash-frozeninliquidnitrogen, andstoredat −80°C until.

The procedure yields mitochondria and is performed according to the methods described by the *Hamilton et al.* and *Frezza et al.*[8, 9]. The catalytic activity of catalase (CAT) was assessed by the spectrophotometric method according to *Goth*[10], which measures the stable complex formation of hydrogen peroxide with ammonium molybdate. Gluthatione peroxidase activity (GPx) was measured based on thenonenzymaticoxidationofreducedglutathione (GSH)[11].Superoxide dismutase (SOD) activity was measured by the method of *Misra* and*Fridovich*[12],based on the ability of SOD to inhibit the autoxidation of adrenaline to adrenochrome in a alkaline medium.

Thedetectionofthe intensityofsuperoxideanionradicalgenerationinmitochondrialfractionsbasedonthereductionofnitrobluetetrazoliumbysuperoxideanionradical. Thetechniquehasbeendescribedpreviouslyindetail[13]. The intensity of hydroxyl radical was determined by the method of *Halliwell et al.*[14]. The assay is based on the quantification of the deoxyribose degradation product, which forms a pink chromogen upon heating with TBA. Determination of hydrogen peroxide concentration was determined by the method of *Gupta*[15]. The basic principle of this method is oxidation of ferrous ions by the sample oxidizing agents to ferric ions, which bind with xylenol orange to give a colored complex.

Theproteinconcentrationinallsampleswasdeterminedbythemethodof*Lowry et al*.usingbovineserumalbumin (BSA) asstandard[16].

Eachresultisexpressedasmean±SDfromthreeexperiments (n=3). Differencesamonggroupswerethenevaluatedbyone-wayanalysisofvariance (ANOVA) followedbyTukey's HSDposthocanalysisformultiplecomparisons.Theresultswereconsideredstatisticallysignificantat$P<0,05$.

Resultsand Discussion

Results of current study were shown that the administration of 50 mg/kg body wt of BPA was accompanied by the decrease in SOD activity in rats' hepatic mitochondria (Fig. 1A). This fact is emphasis to inhibition effect of BPA and enhanced of free radical processes by used xenobiotic. Irradiation animals for correction of prooxidant effect of BPA did not have the positive results in case different irradiation modes. The enzymes activity in both irradiated rats' groups did not statistically significant differ from corresponding values in BPA-exposed group. The obtained results were unexpected, as our previous results [17] were shown the increase in total SOD activity in liver under the simultaneously LLLI and exposure of BPA. In addition, it was evidenced [18] that the main mechanism of correction effect of LLLI is the activation of enzymes containing metal ions (metalloenzymes). The obtained results possibly associated with different effects of red light on the activity of different isoforms of this enzyme, primary due to the metal in active center of enzymes. This suppose is partly confirmed by the fact that the SOD activity in the animal of LLLI-group was at the level of control group (Fig. 1A).

The short-term administration of high dose of BPA also accompanied with the decrease of hydrogen peroxide degradation enzymes CAT and GPx activity, which values were 1.7 and 1.4 times lower than the corresponding indicators of the control group.The obtained results were explained bythefree radical damage of mitochondria under the BPA-exposure. In addition, as known from sources of literature [19], BPA may reduce the expression of genes encoding major antioxidant enzymes.

The irradiation of animals with diode laser after each administration of BPA did not effect on the antioxidant enzymes' activities, these indicators statistically significant differ from corresponding values in BPA-exposed group and were lower than in control group (Fig. 1). At the same time, the increase of CAT and GPx activities in hepatic mitochondria were identified after the change of irradiation mode (the irradiation of animals with diode laser after last administration of BPA). This LLLI effect is primarily due to conformation changes, which are induced by a short-term increase in the temperature of biomolecules, which absorb the light. This, in turn, can induce/initiate activation or inhibition of enzymes [20].

The low SOD activity in hepatic mitochondrial fraction of BPA-exposure rats explains the establishedenhanced generation of superoxide radical in this fraction (Fig. 2A), that was accompanied by the dysfunction of respiratory chain as an effect of xenobiotic-induced lipid peroxidation processes. The irradiation of the animals after each administration of contaminant had little effect on the intensity of generation of this ROS, probably due to the synergistic effect of the action of BPA and LLLI. It is known that the absorption of red-spectrum photons can speed up the

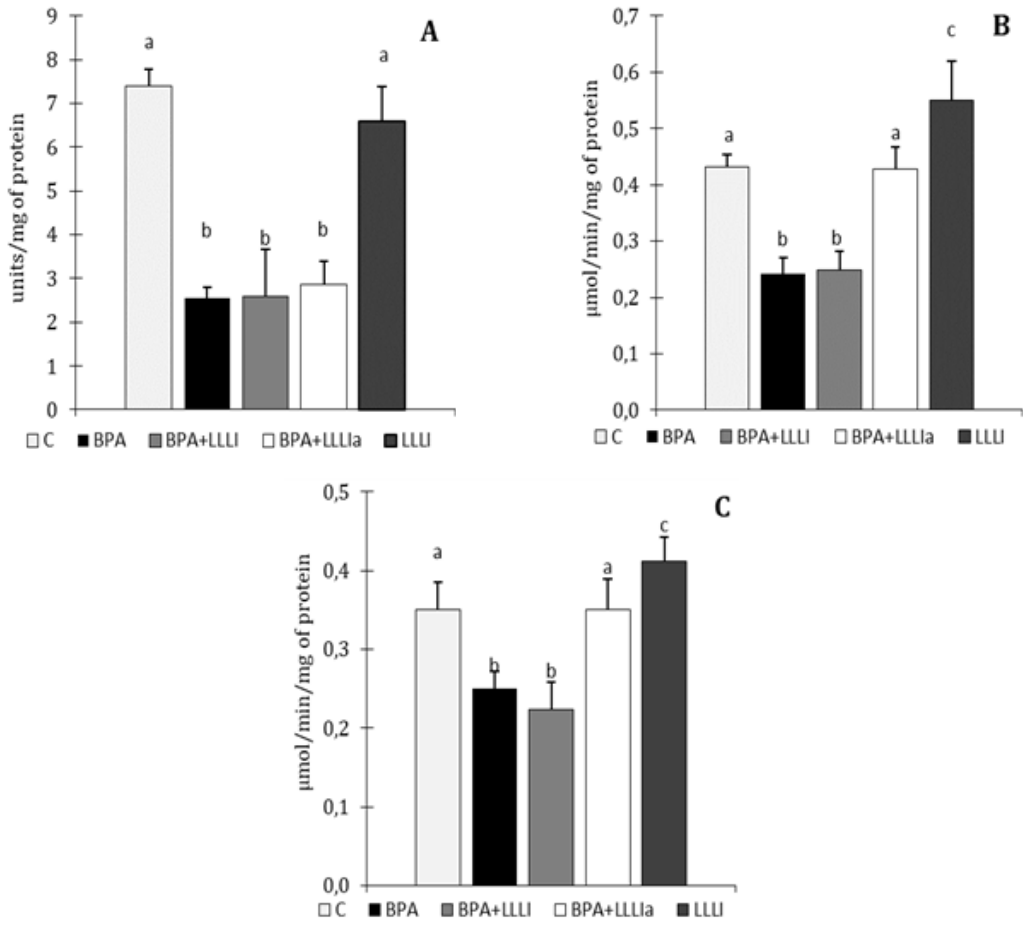

Fig. 1. The SOD (A), CAT (B) and GPx (C) activities in mitochondrial fractions of rats' liver under the BPA-exposure and LLLI. Values marked with different letters (a, b, c) are statistically different, P<0.05. All values are given as the mean ± SD

transfer of electrons across the respiratory chain by changing the oxidative properties of its components and increase the concentration of superoxide radical due to the activation of respiratory chain. This explanation is confirmed by the results obtained in the group of animals that were irradiated with diode laser alone. At the same time, the change in the mode irradiation has contributed to a greater reduction in the intensity of the generation of superoxide radical in the mitochondrial fraction.

ThelowSODactivity in mitochondrial fraction will predetermine the decrease content of hydrogen peroxide, as the product of reaction, in this hepatic subcellular fraction of BPA-exposure rats (Fig. 2B.). On the other hand, these results were explained by the conversation H_2O_2 into hydroxyl radical in Haber–Weiss reaction. The irradiation of the animals after each administration of contaminant did not had effect on the content of hydrogen peroxide in hepatic mitochondria, probably due to the decreased activity of SOD in this animals' group. At the same time, the change in the mode irradiation has contributed to a greater reduction in the content of the hydrogen peroxide in the mitochondrial fraction.

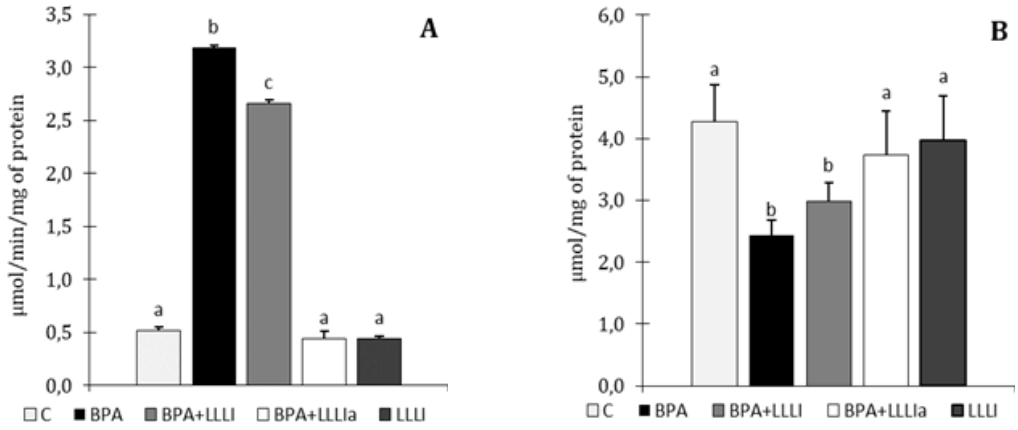

Fig. 2. The intensity of superoxide (A) radical generation and content of hydrogen peroxide (B) in mitochondrial fractions of rats' liver under the BPA-exposure and LLLI. Values marked with different letters (a, b, c) are statistically different, P<0.05. All values are given as the mean ± SD

Thus, short-term BPA exposure by high dosage accompanied by the induction of free radical processes in hepatic mitochondria and the LLLI effect of on these processes is mode irradiation dependent.

Conclusions

1. The administration of bisphenol A was accompanied by the induction of free radical processes in the mitochondrial fraction of the liver due to increased generation of free radicals and a decrease in the main enzymatic activities of the antioxidant system.

2. Irradiationofanimalsaftereachadministrationofxenobioticsdidnotsignificantlyaffecttheactivityofantioxidantenzymesandtheintensityoffreeradicalgeneration, whichisprobablyduetothesynergisticeffectof BPA and LLLI.

3. The effect of low-level laser irradiation of the red spectrum on free radical processes in the mitochondrial fraction of the liver is mode dependent.

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UDC 575.87:577

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ANTAGONISTIC ACTIVITY OF LACTIC ACID STRAINS AND COCCI CONSORTIA

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Abstract. *The composition of the consortium of lactic acid bacteria and cocci was optimized in order to increase their antagonistic activity against gram-positive opportunistic indicators strains. It was shown that the best results were obtained with the combined use of lactic acid bacteria and cocci. Exceeding the benchmarks reached 70.0%.*

Keywords: *lactic acid bacteria, lactic acid cocci, antagonistic activity*

Introduction

Lacticacidbacteriaandcoccihavelongbeenofinteresttobiotechnologistsduet otheirabilitytohaveanantagonisticeffectonpathogenicmicroorganisms, evenifthe concentrationoflacticacidbacteriaintheenvironmentislow[Bernardeauetal., 2006; Bernardeauetal., 2008]. Thedevelopmentofnewcombinationsorconsortiaoflacti cacidbacteriaandcocciisbasedonpureculturesofmicroorganismsthathavespecifie dproperties, withimprovedorganoleptic, technologicalcharacteristics, andhave a positiveimpactonhuman, animal, orplantprotection. [Klaenhammer et al., 2012; Barrettet al., 2014]. Bacteriawithhighgrowthrateandacid-formingactivity, producingantimicrobial, aromaticcompounds, polysaccharides, vitamins, en zymesandotherbiologicallyactivecompoundsareusedforthispurpose. [Емцев, 2012]. Inordertoimprovetheresultsinbiotechnologyusingmethodsofmathematical processoptimization, amongwhichoneofthemostpopularistheanalysisofvariance, adaptedfortheplanontheGreco-Latinsquares [Батрак, 2010].

Theaimofthisstudywastoinvestigatethechangeofantagonisticactivityofdiff erentconsortiumsofstrainsoflacticacidbacteriaandcocciintheirjointcultivationag ainstgram-positiveopportunisticstrainsofindicators.Materialsandmethods.

Materials and methods

Inourstudy, weusedstrain *L. bifermentans* ONU55.1a, *L. parabuchneri* ONU19.2b, *L. parabuchneri* ONU8, *L. vaccinostercus* ONU2, *Lactococcus sp* ONU7, *Lactococcus sp* ONU6.

Determination of antagonistic properties was carried out in vitro by a hole-diffuse method [Presti et al., 2015; Servin, 2004; Schillinger, 1989] in relation to opportunistic microorganisms. The following indicator strains were used in the work *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633. To carry out the mathematical optimization of the experiment, we used a standard matrix for Greco-Latin squares of dimension 3 x 3 [Батрак, 2010].

Strains of lactic acid bacteria and cocci grown in MRS medium were used as a factor. Concentrations 0.0 µl, 250.0 µl and 500.0 µl were selected as factor levels for the optimization matrix.

Optimization matrix compositions for each experiment №1:
Factor A – *L. bifermentans* ONU55.1a;
Factor B – *L. parabuchneri* ONU19.2b;
Factor C – *L. parabuchneri* ONU8.
Optimization matrix compositions for each experiment № 2:
Factor A – *L. vaccinostercus* ONU2;
Factor B – *L. parabuchneri* ONU19.2b;
Factor C – *L. parabuchneri*ONU8.
Optimization matrix compositions for each experiment № 3:
Factor A – *L.bifermentans* ONU55.1a;
Factor B – *L. parabuchneri* ONU19.2b;
Factor C – *L. vaccinostercus* ONU2.
Optimization matrix compositions for each experiment № 4:
Factor A – *L. bifermentans* ONU55.1a;
Factor B – *L. parabuchneri* ONU8;
Factor C – *L. vaccinostercus* ONU2.
Optimization matrix compositions for each experiment № 5:
Factor A – *Lactococcus sp* ONU7;
Factor B – *L. parabuchneri* ONU8;
Factor C – *L. parabuchneri* ONU19.2b.
Optimization matrix compositions for each experiment № 6:
Factor A –*Lactococcussp*ONU6;
Factor B – *L. parabuchneri* ONU8 ;
Factor C – *L. parabuchneri* ONU19.2b.
Optimization matrix compositions for each experiment № 7:
Factor A –*Lactococcussp*ONU7;
Factor B – *Lactococcus sp* ONU6;
Factor C – *L. parabuchneri*ONU19.2b.

Results

Strainsthathadantimicrobialactivityagainstbothgram-positiveandgram-negati vemicroorganismswereselectedforthestudy. Thediameterofthegrowthinhibitionzon erangedfrom 9.0 to 20.0 mm. Manifestationoftheindicatorstudiedwasvariableandd ependedonthestrain(table 1).

Thus, itwasshownthatthestrain*Lactobacillusparabuchneri* ONU8 show edminimalantagonisticactivityincontrasttothestrain*Lactobacillusbifermentans* ONU55.1a, whichinhibitedthegrowthofmoststrainsofindicators. Otherstudiedstrain soflactobacillishowedaveragelevelandspectrumofantagonisticactivity(table 1).

Optimization of th econsortiumagainst *B. Subtilis* ATCC 6633 usingexperimentaloptimizationmatrices. Theobtainedresultsturnedouttobequiteinterest ing. Aswecansee, weachievethedesiredresultwhenusing a strainwithweakantagoni sticactivityandstrainswithmediumantagonisticactivity. Moreover, theinclusionof a

Table 1

Antagonistic activity of studied strains

Studied strains	*E. coli* ATCC 5922	*P. vulgaris* ATCC 6896	*K. pneumoniae* ATCC 10031	*S. enterica* NCTC 6017	*P. aeruginosa* ATCC 27853	*B. subtilis* ATCC 6633	*M. luteus* ATCC 469	*E. faecalis* ATCC 29212	*S. aureus* ATCC 25923
L. vaccinostercus ONU 2	0	0	0	9	9	0	20	0	0
L. parabuchneri ONU8	12	9	13	9	11	11	20	12	11
L. parabuchneri ONU 19.2b	11	12	11	11	10	12	20	13	13
L. bifermentans ONU 55.1a	16	14	14	13	13	14	20	14	13
Lactococcus sp. sp. ONU6	10	10	11	10	8	6	12	9	10
Lactococcus sp. sp. ONU7	12	10	12	12	10	8	14	12	10

weakstrainis a necessarycondition, whichisconfirmedbyFisher'scalculationcriteriaf ortheoptimizationmatrix № 2 (table 2). Exceedingthecontrolindicators, inthiscase, amountedto 45.0% (Fig. 1).Inthecaseofusingtheoptimizationmatrix № 3, wh ensimultaneouslyusedstrainswithlowantagonisticactivity (*L. vaccinostercus* ONU 2), medium (L. parabuchneri ONU 19.2b) andstrongantagonisticactivity (*L. bifermentans* ONU55.1a) wasshowntoexceedthecontrolvaluesby 27.0% forcombinations a2b2c3 and a3b2c1 (Fig. 1).

Whenusedintheconsortiumoflacticacidbacteriaandcocciagainst*Bacillussubt ilis* ATCC 6633, themaximumeffectwasachievedwhenusingthematrix № 6 (Fig. 2) intheco-cultivationofstrainsof*L. parabuchneri* ONU8*, L. parabuchneri* ONU19.2b and *Lactococcussp.* ONU6. Inpercentageterms, theexcessofcontrolreached 70.0% whenusingcombinationsof a3b1c3 and a3b2c1 inthecorrespondingmatrix (Fig. 2).

CalculationsofFisher'stestconfirmedthattheseconsortiapromoteanincreasei ntheantagonisticactivityoflactobacilliandlacticacidcocciagainst*B. subtilis* ATCC 6633 whenco-cultivated (table 2).

Optimization of the consortiumagainst *S. Aureus* ATCC 25923 usingexperimentaloptimizationmatrices.

Theresultoftestingtheantagonisticpropertiesoftheconsortiumoflactobacilliag ainst S. aureus ATCC 25923 showedanexcessofcontrolvaluesby 63.0% inthecase ofusingtheoptimizationmatrix № 4 (Fig. 3). Inthiscase, themostfavorablewasthec omposition a3b3c2 - simultaneouscultivationofstrainswithstrong, mediumandwe akantagonisticactivity.ThesignificanceoftheobtainedresultsisconfirmedbyFisher'sc alculationcriterion (table 3). Wealsoobtainedanincreaseinantagonisticactivitycom paredtothecontrolusingtheoptimizationmatrix № 1. Inthiscase, thecombinationsof a2b2c3 and a2b3c1 provedtobethemosteffectivewhenthecontrolvalueswereexceed edby 50.0% (Fig. 3).

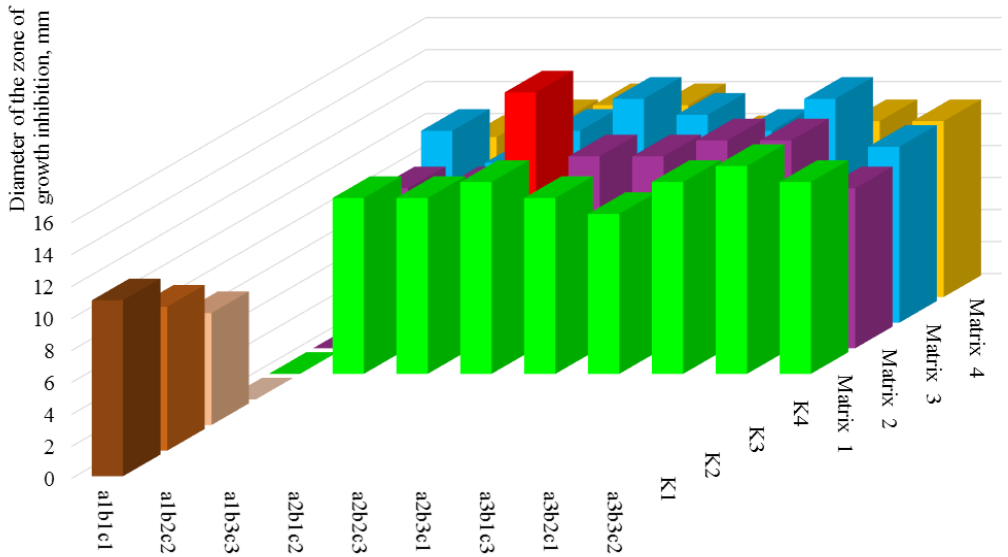

Fig. 1. Antagonistic activity of the studied lactobacillus strains lactobacillus against *Bacillus subtilis* ATCC 6633 usinganoptimizati on matrix

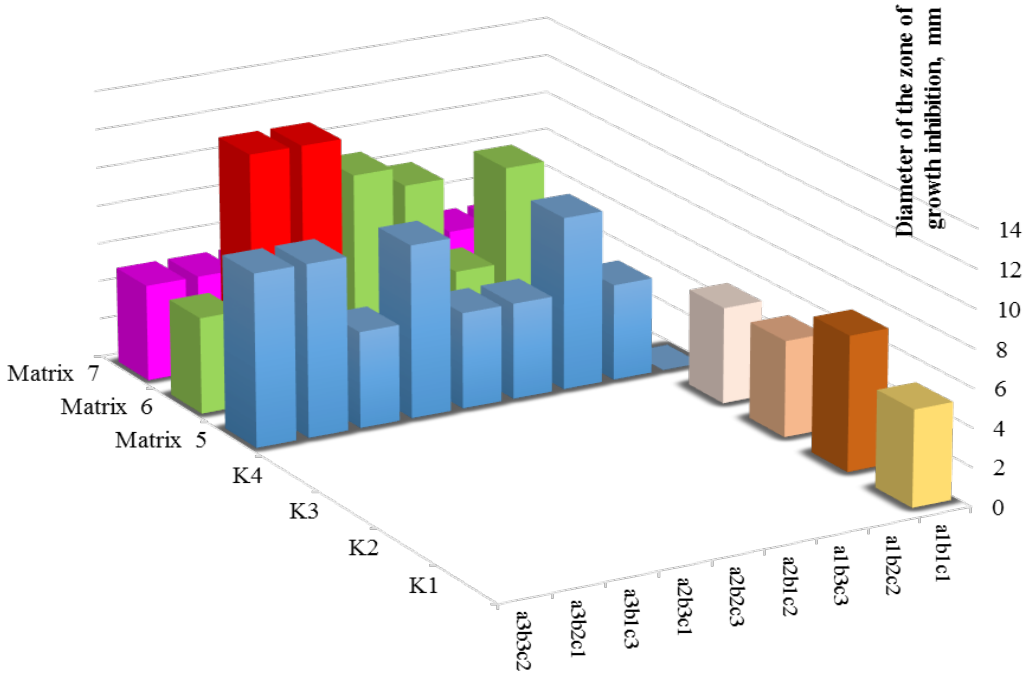

Fig. 2. Antagonisticactivityof a consortiumofinvestigatedstrainsoflacticacidbacilliandc occiagainst *B. subtilis* ATCC 6633 usingoptimizationmatrices (indicatorsofantagonisti cactivityofstrains: К1 – *L. parabuchneri*ONU8,К2 – *L. parabuchneri*ONU19.2b, К3 – *Lactococcussp*. ONU7, К4 – *Lactococcussp*. ONU6)

Table 2

Indicators of Fisher'stestinoptimizingthecombinationoflactobacilliandcocciin theirco-cultivationtoincreaseantagonisticactivityagainst *Bacillussubtilis*ATCC 6633 (F_{st}=3.49, при p=0,05)

Optimizationoption	Fisher's criterion for the factor A	Fisher's criterion for the factor B	Fisher's criterion for the factor C
Matrix 1	**3,91**	2,22	2,87
Matrix 2	**5,69**	**4,53**	1,38
Matrix 3	**4,33**	0,512	1,19
Matrix 4	**4,86**	2,81	**4,86**
Matrix 5	2,21	**6,97**	0,03
Matrix 6	**8,46**	**3,22**	**3,54**
Matrix 7	1,89	0,27	0,79

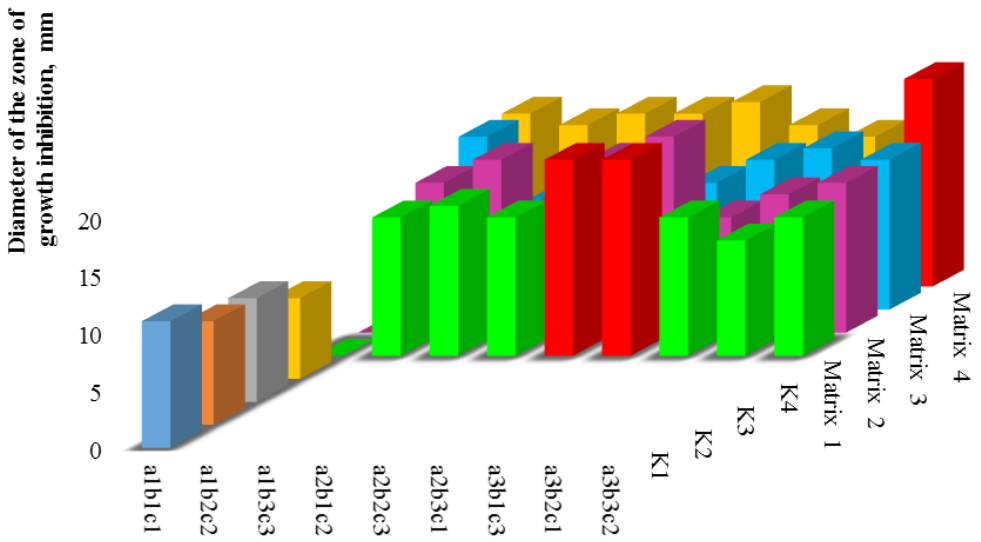

Fig. 3 Antagonistic activityofthestudiedlactobacillistrainscombinationsagainst*S. Aureus* ATCC 25923 usinganoptimizationmatrix

Whenusedco-cultivationin a consortiumofcocciandlacticacidbacteria, th ecombinationturnedouttobethemostfavorablecombinationthatcorrespondstoth eoptimizationmatrix № 6 (Fig. 4) andincludesstrainsof *L. parabuchneri* ONU 8, *L. parabuchneri* ONU 19.2b and*Lactococcussp.* ONU 6. Inpercentageterms, theexcessofcontrolreached 75.0% whenusingcombinationsof a3b1c3 and a3b3c2 (Fig. 4).

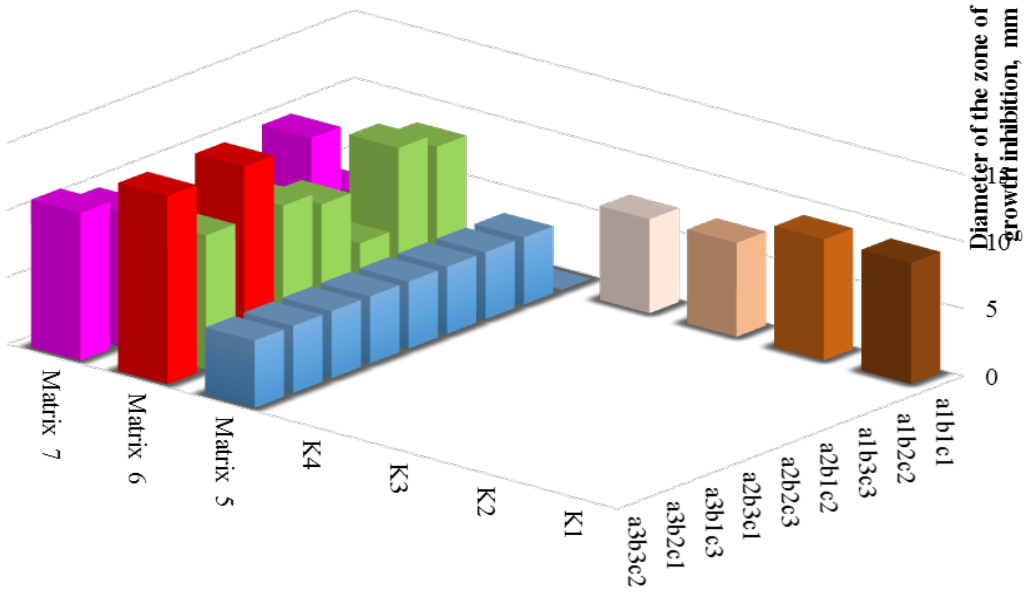

Fig. 4. Antagonisticactivityof a consortiumofinvestigatedstrainsoflacticacidbacilliandcocciagainst*S. aureus*ATCC 25923 usingoptimizationmatrices (indicatorsofantagonisticactivityofstrains: К1 – *L. parabuchneri*ONU8,К2 – *L. parabuchneri*ONU19.2b, К3 – *Lactococcussp*. ONU7, К4 – *Lactococcussp*. ONU6)

Calculations of Fisher'steststhowedthatwhenusingthematrix № 6allfactorsarevalid (table 3). Thatis, forfurtherstudies, wechoosethematrix № 6 tocreate a consortiumoflactobacilliandlacticacidcoccitoincreasetheirantagonisticactivityagainst*S. aureus* ATCC 25923 (table 3).

Table 3

IndicatorsofFisher'stestinoptimizingthecombinationoflactobacilliandcocciintheirco-cultivationtoincreaseantagonisticactivityagainst *S. aureus*ATCC 25923(F_{st}=3.49, прир=0,05)

Optimizationoption	Fisher's criterion for the factor A	Fisher's criterion for the factor B	Fisher's criterion for the factor C
Matrix 1	**9,25**	**7,75**	**4,75**
Matrix 2	**4,09**	**11,64**	2,35
Matrix 3	**9,6**	**5,45**	**3,75**
Matrix 4	**7,00**	**7,46**	**7,46**
Matrix 5	1,34	1,37	2,0
Matrix 6	**3,54**	**4,57**	**3,39**
Matrix 7	0,27	1,91	2,17

Conclusion

During the work, it was shown that the consortia of lactic acid bacteria and cocci have a greater antagonistic activity of individual strains. The best results were obtained when lactic acid bacteria and cocci were used in the consortium at

the same time. In such cases, the excess of the control indicators reached 70.0% in relation to the indicator strain *B. subtilis* ATCC 6633 and 75.0% in relation to indicator strain *S. aureus* ATCC 25923

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UDC 579.22:579.64

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THE USE OF BACTERIA OF THE GENUS *PSEUDOMONAS* FOR WATER PURIFICATION FROM HEAVY METALS AND OIL HYDROCARBONS

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Abstract. *A screening assessment of the oil-oxidizing and matalaccumulating activity of the activity of 3 strains of bacteria of the genus Pseudomonas and their associations was carried out. It has been shown that an association of bacteria consisting of the strains P. cepacia ONU327, P. fluorescens ONU328, and P. maltophilia ONU329 has a high sorption-accumulating ability with respect to heavy metal ions Pb (II), Cd (II), Zn (II) and oxidizing ability to petroleum products. When using bacteria of the genus Pseudomonas for water purification from Pb (II), Cd (II), Zn (II), the residual concentration of heavy metals was in the range of 0.02-0.1 mg / dm^3, which is lower than their maximum permissible concentration for discharge purified water into the sewer. The investigated strains had a high destructive potential in relation to petroleum hydrocarbons. The association of bacteria, consisting of P. cepaciaONU327, P. fluorescens ONU328 and P. maltophilia ONU329 strains, had the highest oil-oxidative activity - which, after 10-30 days, utilized 75.6-85.8% of oil hydrocarbons (the concentration of hydrocarbons in water decreased from 500 mg / l up to 122.0 ± 5.0 - 71.0 ± 9.0 mg / l). The association of bacteria of the genus Pseudomonas can be recommended for use in biotechnology for the purification of multicomponent wastewater from toxic pollutants.*

Key words: *bacteria-destructors, water purification, heavy metal ions, petroleum hydrocarbons.*

Introduction

The ecological situation in Ukraine is characterized by a high anthropogenic load on water resources / 1-3 /. Domestic and industrial wastewater is one of the main sources of environmental pollution byt pollutants of organic and inorganic nature. Wastewater discharge leads to the pollution of surface water bodies with various toxic chemical pollutants (oil products, heavy metal ions, etc.) [1].

Microbiological methods of purification of industrial and domestic wastewater treatment are most environmentally friendly, because they based on natural self-cleaning processes. Heterotrophic microorganisms are able to use pollutants as a food source [2, 3, 4]. A promising direction is the use of universal strains of bacteria for wastewater treatment, which have metal-accumulating and oil-oxidizing activity.

The aim of the work is to evaluate the efficiency of using bacteria of the genus *Pseudomonas* for water purification from heavy metals and oil hydrocarbons.

Materials and methods

Non-pathogenic strains of bacteria of the genus Pseudomonas: *P. cepacia* ONU327 (isolated from soil), *P. fluorescens* ONU328 and *P. maltophilia* ONU329 (isolated from sea water), stored in a collection of microorganisms of the Department of Microbiology, Virology and Biotechnology, Odesa National I.I. Mechnykov University. Identification of bacteria was carried out by fatty acid composition using the method of gas chromatography, the system of identification of microorganisms MIDI Sherlock. Bacterial cultures were grown on a nutrient medium of the composition (g/dm^3): KH_2PO_4 - 1.5; Na2HPO4-3; NaCl-5; NH4Cl-1; peptone - 10; glucose-2; yeast extract - 5 at pH 7.0-7.2 and temperature 28 0C for 48 hours to achieve a crop density of at least 5 g/dm^3 on dry biomass. A prepared bacterial association (bioreagent) composed of strains of P. *cepacia* ONU327, *P. fluorescens* ONU328 and *P. maltophilia*ONU329 (1: 1: 1 by volume) was mixed with contaminated water and solutions of hydrogen peroxide and calcium chloride were introduced. The content of heavy metal ions in aqueous solutions before and after microbiological purification was determined by the atomic absorption method on a flame atomic absorption spectrophotometer "Saturn" in the flame of an air-propane-butane mixture. The effectiveness of the proposed microbiological method is estimated by the degree of extraction from the cations of heavy metals Pb (II), Cd (II), Zn (II) [5].The analysis of the content of oil hydrocarbons in water was carried out infrared spectrometry according to [5].

Results and discussion

The results of water purification from ions of heavy metals Pb (II), Cd (II), Zn (II) using immobilized bacteria of the genus *Pseudomonas* are presented in table 1.

The degree of extraction of Pb (II), Cd (II), Zn (II) from concentrated solutions reached 93.00-99.85% with their residual content in solution (0.03-4.9) mg/dm3. The use of strains of *P. fluorescens* ONU328, *P. maltophilia* ONU329, *P. cepacia* ONU327 in the developed association method provides the greatest efficacy.When processing technogenic solutions with immobilized bacterial cells in the composition of bioflecula, the residual concentration of Pb (II), Cd (II), Zn (II) was within 0,02-0,1 mg/dm3, which is much lower than their maximum allowable concentration for discharge cleaned solutions into the sewage system.

The results of evaluating the oil-oxidizing activity of bacteria of the genus Pseudomonas are presented in table 2.

It was found that the non-pathogenic strains of bacteria of the genus *Pseudomonas* used in the method, along with a high adsorption-storage capacity, also have a high destructive potential in relation to oil hydrocarbons.

The association of bacteria, consisting of *P. cepacia* ONU327, *P. fluorescens* ONU328, and *P. maltophilia* ONU329 strains, had the highest oil-oxidative activity – which, after 10-30 days, utilized 75.6-85.8% of oil hydrocarbons (the concentration of hydrocarbons in water decreased from 500 mg / l up to 122.0 ± 5.0 - 71.0 ± 9.0 mg / l).The association of bacteria of the genus *Pseudomonas* can be recommended for use in biotechnology for the purification of the purification of multicomponent wastewater from toxic pollutants.

Table 1

Efficiency of water purification from heavy metal ions by immobilized bacteria of the genus *Pseudomonas*

Efficiency of water purification from heavy metal ions	Strain			
	P. fluorescens ONU 328	*P.maltophilia* ONU329	*P. cepacia* ONU327	Association *P. cepacia, P.fluorescens, P.maltophilia*
Concentration of Pb (II) in water after purification, mg/dm^3	≤0,05± 0,002	≤0,05± 0,002	≤0,05± 0,003	≤0,03± 0,001
Degree of water purification from Pb (II), %	99,92	99,92	99,92	99,95
Concentration of Cd (II) in water after purification, mg/dm^3	≤0,02± 0,01	≤0,08± 0,05	≤0,04± 0,05	≤0,02± 0,001
Degree of water purification from Cd (II), %	99,96	98,84	99,92	99,96
Concentration of Zn (II) in water after purification, mg/dm^3	≤0,03± 0,004	≤0,1± 0,005	≤0,1± 0,005	≤0,1± 0,002
Degree of water purification from Zn (II), %	99,85	99,50	99,50	99,50

Notes: initialconcentrationsofheavymetalionsinwater: Pb (II) – 60 mg/dm^3; Cd (II) - 50 mg/dm^3; Zn (II) – 20 mg/dm^3; pHofwatertreatment 6,8–7,2.

Table 2

Oil-oxidizing activity of bacteria of the genus *Pseudomonas*

Strain	10 days		20 days		30 days	
	residual oil content					
	mg/l	%*	mg/l	%*	mg/l	%*
P. fluorescens ONU 328	190±1,0	38,0	190±5,0	38,0	103±3,0	20,6
P. maltophilia ONU 329	154±3,0	30,8	140±3,0	28,0	100±5,0	20,0
P. cepacia ONU 327	135±1,0	27,0	134±3,0	26,8	116±2,0	23,2
Association *P. fluorescens ONU328, P. maltophilia ONU329, P. cepacia ONU327*	122,0±5,0	24,4	95,0±6,0	19,0	71,0±9,0	14,2

Note: the initial concentration of oil hydrocarbons is 500 mg/l

Conclusions

The bacterial association, consisting of the strains *P. cepacia* ONU327, *P. fluorescens* ONU328 and *P. maltophilia* ONU329, has a high sorption-accumulating ability with respect to heavy metal ions and an oxidizing ability to petroleum products, which allows them to be widely used in biotechnology for cleaning the environment fromtoxic pollutants.

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UDC 579.222:579.841.1

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THE MOTILITY OF MARINE BACTERIA *PSEUDOMONAS AERUGINOSA* AND *ALCALIGENES FAECALIS*

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Abstract

The aim of the work *is to trace the dependence of the motility of microorganisms isolated from the marine environment.* **Materials and methods.** *The wild-type strain Pseudomonas aeruginosa PA01 from the collection of cultures of the Department of Microbiology, Virology and Biotechnology of ONU named after I.I. Mechnikov and strains of P. aeruginosa M1, M3, M4, and also Alcaligenes faecalis strain, were used in our experiment. Cell motility was determined on Petri dishes using media with different agar content: swimming 0.3%, swarming 0.6% and twitching 1.5%.* **Results.** *It has been shown that bacteria of the genus Pseudomonas isolated from the marine environment were able to move only by swimming, in contrast to the bacterium A. faecalis, which also moved well by twitching.* **Conclusions.** *Therefore, wecanconcludethatthestrainsisolatedfromthe marineenvironmentaremoreabletomoveonliquidmedia.*

Key words: *motility, marine bacteria, swimming, swarming, twitching.*

Introduction

The motility of *P. aeruginosa* cells plays a key role in the colonization and spread of bacteria on various surfaces. It also promotes the formation of biofilms – three-dimensional structured communities of microorganisms associated with the surface [5]. *P. aeruginosa* cells are able to perform three types of movements, which are provided by different structures: swimming and swarming using flagella and twitching, depending on the type 4 pils [9].Thestudyofthemicroorganism *A.faecalis* has beenactivelyconductedoverthepast 30 years, mainlybyspecialistsfromJapanandIndia, wherethebiotechnologyindustryiswelldeveloped, althoughthismicroorganismwasfirstdescribedonlyin 1919 [8]. Move with the helpofflagella, intheamountof 1 to 9, whicharelocatedperitrichially.

Bacteria exhibit various forms of social behavior capable of contact and distance communication. A striking manifestation of the integrity of bacterial consortia is the formation of superorganismic structures that do not belong to any of the cells separately, but important for the life of the colony as a whole [7].

In addition to the theoretical significance, the study of the mechanisms of "communication" through which communicative interactions between different groups of bacteria are carried out is an urgent task for specialists in the field of

microbiology, biotechnology, medicine. For the synthesis of virulence factors, antibiotics, the formation of biofilms, bacteria often use quorum sensing reactions.

The motility of *P. aeruginosa* is closely related to the structure of bacterial cell walls, the ratio of components in their composition and virulence, which is an important process that allows bacteria to colonize different media on both biotic and abiotic surfaces, forming a stable biofilm, and the treatment of *P. aeruginosa* infection remains a serious problem [1].

Motility is important for the development of treatments for infections caused by *P. aeruginosa*. Several global analyzes have shown that a wide range of genes, including regulators, metabolites, chemosensors, secretory and other genes, affect bacterial motility [3].

There are six different phenomena of bacterial colony spread, namely swarming, swimming, sliding, twitching, friction, and ejection. The last two types exist technically because they have not been described before [2].

Materials and methods

The wild-type strain *P. aeruginosa* PA01 from the collection of cultures of the Department of Microbiology, Virology and Biotechnology of Mechnikov ONU and strains of *P. aeruginosa* M1, M3, M4, and also *A. faecalis* strain, were used in our experiment. Cultivation was carried out at 37 °C in liquid medium LB with the following composition (g / l): peptone - 15.0, yeast extract - 10.0, sodium chloride - 5.0.

To assess cell motility, a 2 μl overnight culture was added to the center of a Petri dish with agar medium containing meat-peptone broth - 8.0 g / l, glucose - 50.0 g / l and agar with different concentrations, and incubated for 48 h at 37 °C. For different types of movement, agar was added to the final concentrations of 0.3%, 0.6% and 1.5% in the case of swimming, swarming and twitching, respectively. In the study of swimming and swarming, the cell suspension was applied to the surface of the agar, twitching - by injection into the bottom of a Petri dish under agar. In the case of twitching at the end of incubation, the agar was removed, and the cells were stained with 1% crystalline violet. The results were evaluated by the diameter of the cell distribution zone from the point of inoculation [6].

All experiments were performed in 3 independent experiments with 3-6 replicates in each. Statistical processing of research results was performed using conventional methods of variation analysis. The average values of the indicators ($\bar{X}$) and their standard error ($S\bar{X}$) were calculated. The significance of the differences between the mean values was determined by Student's criterion, estimating the probability of the results obtained at a significance level of at least 95% ($p \leq 0.05$). Mathematical calculations were performed using the computer program Excel [4].

Results and Discussions

Determining the nature of the three types of movement inherent in *Pseudomonas* strains and *A. faecalis*, showed that the studied strains differ in swimming in quantitative terms (Table 1). Thus, the propagation distances of *P. aeruginosa* cells M1, M3, M4 were almost the same and were approximately 1.7 times larger than the control strain. While the cells of the strain *A. faecalis* moved more actively and

the diameter of the corresponding zone was 2.3 times larger. With regard to swarming, it was shown that, unlike the control strain PA01, no strain was able to move this type of motility. Cells of the strain *A. faecalis* moved on a solid surface under a layer of agar by twitching at a distance 2.5 times greater than *P. aeruginosa* PA01. Theotherthreestrainsof*P.aeruginosa* M1, M3 and M4 weretwitchingapproximatelythesame,andtheirindicatorswereabout 1.5-1.8 timeslargerthanthecontrolstrain.

In fig. 1 results are presented as a percentage of *P. aeruginosa* PA01

Table 1

Motility zone diameter (mm) of studied strains bacterialcells

	Swimming	Swarming	Twitching
PA01	38 ±3	43 ±3	6 ±0
M1	65 ±5*	9 ±0*	9 ±0
M3	64 ±5*	10 ±1*	10 ±1
M4	62 ±5*	8 ±0*	11 ±1
A. faecalis	87 ±5*	12 ±1*	15 ±1*

Note: * – significant difference ($p \leq 0.05$) compared with *P. aeruginosa* PA01

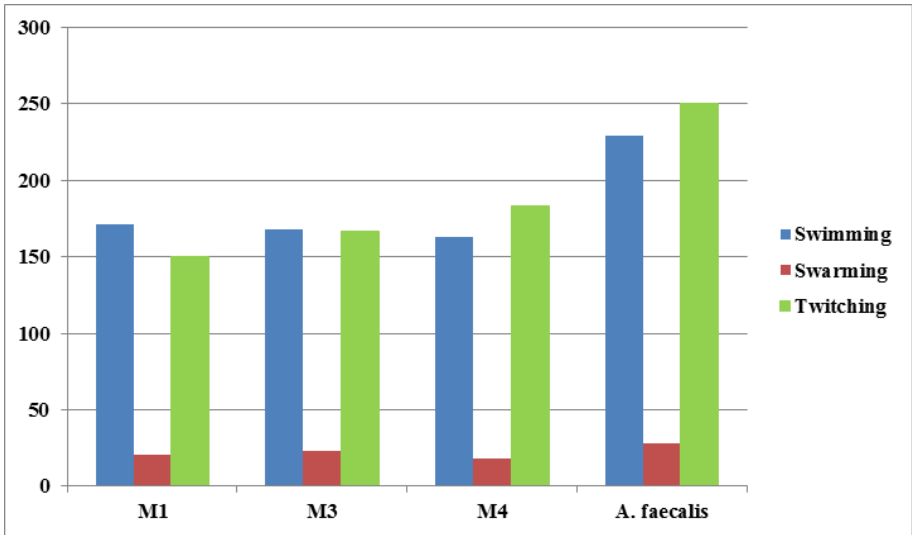

Fig. 1. Marine strains motility versus *P. aeruginosa* PA01 (100%)

Conclusions

Summarizingtheresultsobtained, itcanbenotedthatthestrainof*A. faecalis*showedgreatabilityforeachtypeofmotility. Itshouldalsobenotedthateachmarinestrain, namely 3 strainsof*P. aeruginosa*and*A. faecalis*showedsignificantlygreateractivity whenmovingbyswimming. Therefore, wecanconcludethatthestrainsisolatedfromthemarineenvironmentaremoreabletomoveonliquidmedia.

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SELECTION EFFICIENCY OF DIFFERENT INITIAL EXPLANTS TYPES OF *PHALAENOPSIS* ORCHIDS SPECIES

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Abstract. *The effectiveness of selection of five types of initial explants from different parts of the Phalaenopsis plant (explants of roots, leaves, flower stalk, columns and anthers) was studied. Determination of the most productive for microclonal propagation explants of Phalaenopsisin vitro culture was performed by the indicators of survival rate, proliferation and callus formation. Flower stalks and anthers have been shown to be the best explants for in vitro culture.*

Key words: *Phalaenopsis, in vitro culture, survival rate, proliferation, callus formation.*

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

Although foreign scientists have accumulated a lot of experience in orchid propagation, today they do not have a single opinion on the method of microclonal propagation of orchids, especially monopodial species (including *Phalaenopsis*) due to the difficulty of choosing effective types of explants for for the initial stages of *in vitro* culture and high percentages of survival rate, proliferation and callus formation [Шемедюк, 2014].

Theaim of this work was to study the optimal use of different parts of the *Phalaenopsis* orchid as explants in the initial stages of introduction into *in vitro* culture.

The tasks of the study included:

1. To determine which types of explants give the highest survival rate in the initial stages of *in vitro* culture.
2. To investigate the optimal use of different parts of the *Phalaenopsis* orchid as initial explants to increase the percentage of proliferation.
3. To determine which types of explants give the highest percentage of callus formation.

Materials and methods

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

As experimental plants young orchids of the *Phalaenopsis amabilis* species in the stage of intensive growth with healthy, strong flower stalks, free from fungal, bacterial and viral infections were used. Flower stalk sections with dormant buds, aerial roots, leaf explants, as well as anthers and columns obtained from the flower of an intact plant were used as initial explants for the experiments (Fig. 1).

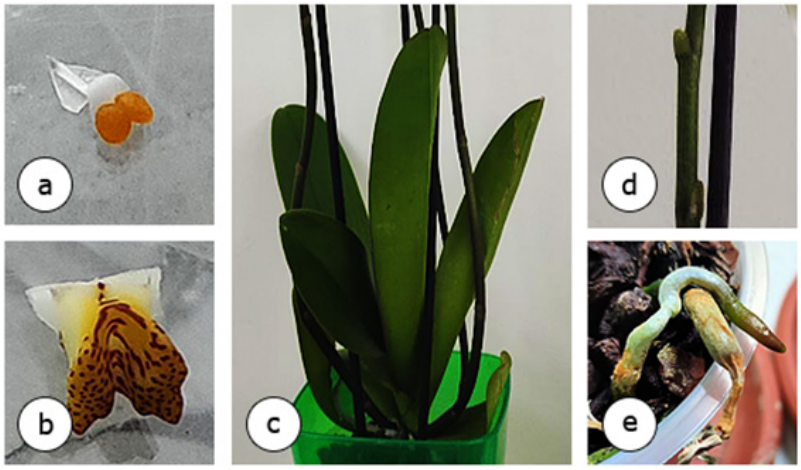

Fig. 1. Sources of explants for introduction into *in vitro* culture (*original*)
a. Anthers explant. Б. Column explant. В. The source of leaves explants. Г. The source of flower bud explants. Д. The source of aerial roots explants.

For sterilization explants were wiped with 95% ethanol, and immersed for about 15 min in a 10% solution of sodium hypochlorite.Tthen explants were re-immersed in a 5% sodium hypochlorite solution with three drops of Tween 20 and shaken occasionally for 10 min. After this period the cylinder were filled again with autoclaved distilled water for rinsing. Explants were then placed on 30 ml of solid medium [Tesliuk et. al., 2021].

For the introduction of *Phalaenopsis* explants *in vitro* culture a modified [Теслюк, 2009] nutrient medium Murashige - Skuga (MC) was used [Murashige, 1962]. This media was supplemented with 2 mg 1^{-1} IAA, 10 mg 1-1 BAP and 20 g 1^{-1} sucrose and solidified with 8 g 1^{-1} agar. Explants were cultivated at 24 °C under 16 h photoperiods. Survival percentage of explants, the beginning of proliferation and starting of callus formation were recorded for 14 days [Tesliuk et. al., 2021].

Results

Determination of the most productive explants for microclonal propagation of *Phalaenopsisin vitro* culture was performed by studying survival rates of explants, their proliferation and starting of callus formation. The analysis of the obtained results is shown in Fig. 2.

Based on data analysis, survival rate of *Phalaenopsis* leaf explants was only 40%, 10% of which were able to form callus tissue and had swelling in the area of the leaf cut. Most authors [Paek et al., 2010; Sinha et al., 2011; Niknejad et al., 2013; Balilashaki et al., 2016] recommend orchid leaves as a reliable source of explants due to their ease of cultivation and high survival rate forobtaining protocorm-like bodies.

However, our research has shown that anther explants are more suitable for callus formation. Thus, the survival of anthers in our studies is 35% higher than that of leaf explants, and the percentage of explants capable of callus formation is 50%

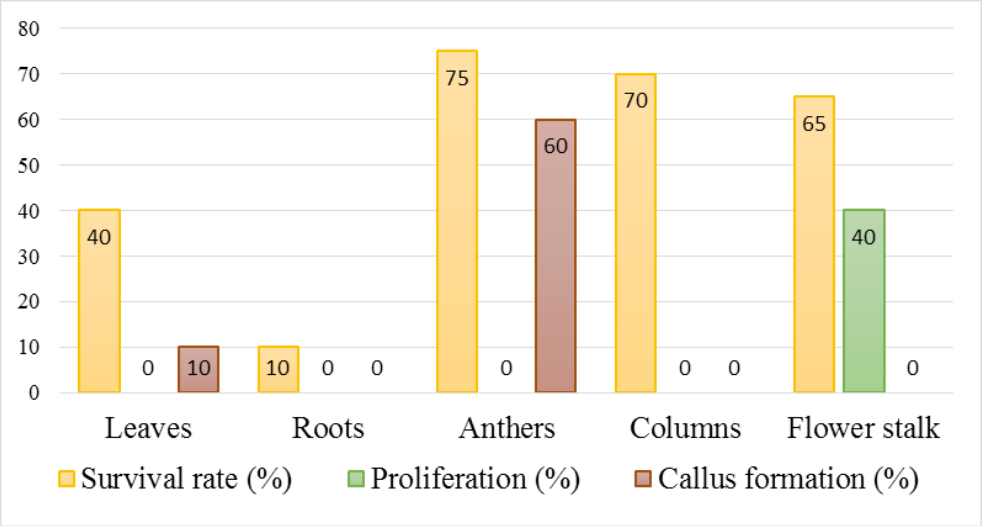

Fig. 2.Indicators of survival rate, proliferation and callus formation of different types of *Phalaenopsis* explants on the MS nutrient medium

higher than the percentage of leaf explants capable of callus formation. Probably, such discrepancies of our results with results of researches of other scientists are connected with the fact that as intact plants for reception of leaves explants we have taken two-year old blossoming orchids which were grown in the *in vivo* conditions. According to Cherevchenko T. M. [1986], obtaining leaf explants from a young plant (seedling or regenerate plant) grown *in vitro* culture is more effective for microclonal propagation of *Phalaenopsis* species.

It was found that the root explants on the second day of observation revealed the presence of infection of exogenous origin. In this experiment, survival on the second day was 40%. On day 6, 90% of aerial root explants were infected with the microorganisms. Probably, such a high percentage of contamination is associated with the presence of mycorrhiza, or inappropriately selected sterilization method for this type of plant material.

The explants of the columns, which were taken from the *Phalaenopsis* flower, had a high survival rate, but did not show the ability to proliferate or form a callus tissue (Fig. 2). Most column explants remained unchanged for up to 14 days of cultivation.

In our study, explants that were obtained from the *Phalaenopsis* flower stalk showed the best results – their survival rate was 65%. On the 10th day of observation, swelling of the axillary bud was detected and the beginning of proliferation was noted in 40% of explants.

Thus, we found that the most effective explants of *Phalaenopsis* for the initial stages of *in vitro* culture are the tissues of the flower stalk and anthers.

Conclusions

1. The highest survival rates were observed in explants of anthers (75%), columns (70%) and flower stalks cuttings (65%).

2. To obtain a high level of proliferation, it is optimal to use as explants flower stalks cuttings of *Phalaenopsis*, which showed a high percentage of proliferation (40%).
3. To obtain callus tissue, it is recommended to use anthers because of their high percentage of callus formation (60%).

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MORPHOLOGY CHARACTERISTICS OF ACTINOBACTERIA ISOLATED FROM THE BLACK SEA MUSSELS

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Abstract. *Until recently, the most promising source of producers of new antibiotics have traditionally been soil microorganisms, whose biotechnological potential is considered exhausted, so microbiologists have turned their attention to marine sources of a search for producers. It is important to search among the biological diversity of marine actinobacteria for producers of new biologically active metabolites, to study their biological characteristics and to determine their biotechnological potential.*

The study aimed *to isolate actinobacteria from mussels of the Black Sea, to study their basic morphological properties and to carry out primary identification by fatty acid composition.* **Methods**. I*solation was performed on media oat agar with sea salt, Gauze №1 and Gauze №2. The morphology of colonies and cells was studied according to generally accepted methods. Identification of strains by fatty acid composition was performed by gas chromatography using an automatic system for the identification of microorganisms MIDI Sherlock.* **Results.** *It was isolated 12 strains. All strains belonged to the genus Streptomyces.*

Keywords: *actinomycetes, bioactive compounds, isolation, cultivation*

Introduction

The world's oceans cover about seventy percent of the earth's surface, serving as a habitat for a wide variety of microorganisms. Marine microorganisms, primarily actinobacteria, produce a large number of secondary metabolites, the action of which had been actively studied. Actinobacteria are present in a variety of marine habitats. These habitats had influenced by numerous geographical and physical parameters, such as temperature, salinity, ocean currents. Ecosystems such as salt marshes, wetlands, estuaries, continental shelves, as well as the open ocean and the deep sea are habitats for specific marine actinobacteria [1, 2].

Actinomycetes have been received much attention, as these bacteria produce a variety of natural drugs and other bioactive metabolites, including antibiotics, enzyme inhibitors, and enzymes. More than 22,000 bioactive secondary metabolites (including antibiotics) from microorganisms had been identified and published in the scientific and patent literature, and about half of these compounds had been produced by actinomycetes. Currently, approximately 160 antibiotics had used in human therapy and agriculture, and 100–120 of these compounds, including streptomycin, erythromycin, gentamicin, vancomycin, vermectin, etc., are produced by *actinomycetes* [3, 4].

However, the use of general approaches to developing new drugs from *actinomycetes* is more and more difficult. Although a large number of microorganisms

had been identified, described, screened, and used, more than 90% of all microorganisms remain uncultivable [9, 10].

These uncultivable microbes might offer new hope for the development of new drugs. Novel microbes should contain new gene clusters synthesizing novel secondary metabolites. Many laboratories and companies have focused on new *actinomycetes* sources from new habitats, such as oceans, extreme environments, plants, faeces of animals and lichens, for the development of new drugs [11, 16].

Materials and methods:

Deep-water samples had taken from mussels in the area of the Biological Station of Odessa Mechnikov University:

Samples were taken in June - July 2020. Sampling was performed in sterile containers. Sowing on nutrient media was carried out no later than 2 hours after sampling. Previously, samples of mussels had placed in sterile seawater in flasks and shaken on a shaker (150 rpm, 30° C for 30 min). To isolate actinobacteria, the method of sowing from dilutions preheated at a temperature of 50° C for 15 minutes suspensions on dense nutrient media had been used. This approach, in addition to isolating thermophilic actinobacteria, aims to prevent the growth of unwanted microbiota [7].

The following medium had used nutrient agar, Gauze №2, Czapek agar, ISP №4, soil agar. To isolate the activated material from marine objects, the nutrient medium was prepared on seawater. The antibiotic nalidixic acid had added to the medium at a concentration of 0.01 g/l to prevent the growth of most foreign microorganisms. Sowings on the whole medium were carried out in three repetitions. Incubation had carried out at a temperature of 28° C for 2 - 3 weeks [12].

In the presence of characteristic colonies, actinobacteria were isolated in pure cultures on Oat agar with sea salt, Gauze №1 and Gauze №2. When assessing growth on these media, the following phenotypic traits had taken into account: morphology of colonies, pigmentation of the environment and pigmentation of colonies, the formation of a substrate and aerial mycelium [7].

Cell morphology and spores had studied by microscopy of Pfeiffer's magenta-stained preparations. Used a microscope Axio Scope. A1 (Zeiss), at magnification 100*10.

Twelve strains of actinobacteria were isolated from mussel samples, isolation and determination of fatty acid composition had performed. Identification of strains by fatty acid composition was performed by gas chromatography using an automatic identification system MIDI Sherlock and gas chromatograph Agilent 7890 equipped with the flame-ionization detector, with ULTRA 2 column. Fatty acid methyl esters were isolated according to the standard protocol [12].

Results and Discussion

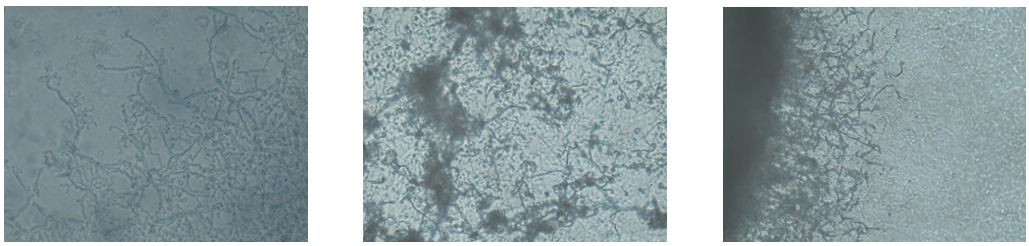
As a result, we isolated twelve strains. Isolated strains had characterized by various morphological properties, which had explained by the uniqueness, including morphological, species of actinobacteria, and heterogeneity within one species. Given this fact, further cultivation had carried out on media: oat agar with sea salt, Gauze №1 and Gauze №2 (Table 1).

Table 1

Morphological properties of strains on different nutrient media

Strain	Oat agar with sea salt	Gauze №1	Gauze №2
Myt 1	Rounded, yellow colonies, which formed a white mycelium	Rounded, yellow colonies, which formed a white mycelium	Rounded, yellow colonies, which formed a gray mycelium
Myt 2	Rounded, colorless colonies, which formed a gray mycelium	Rounded, white colonies, which formed a white mycelium	Rounded, colonies of golden color, which formed a gray mycelium
Myt 3a	Rounded, colorless colonies, which formed a white mycelium	Rounded, colonies are yellow, which formed a gray mycelium	Rounded, colonies are yellow, which formed a gray mycelium
Myt 3b	Rounded, colonies of golden color, which formed a white mycelium	Rounded, colonies of golden color, which formed a white-graymycelium	Rounded, colonies of golden color, which formed a white-graymycelium
Myt 4b	Rounded, colorless colonies, which formed a mycelium of light graycolor	Rounded, colonies of golden color, which formed a white-gray mycelium	Rounded, colonies of golden color, which formed a white-gray mycelium
Myt 5	Rounded, yellow colonies, which formed a white mycelium	Rounded, golden colonies, which formed a white mycelium	Rounded, colonies of yellow color, which formed a light gray mycelium
Myt 6	Rounded, colorless colonies, which formed a mycelium of light graycolor	Rounded, colonies of graycolor, which formed a light gray mycelium	Rounded, colorlesscolonies
Myt 7	Rounded, colorless colonies, which formed a white mycelium	Rounded, yellow colonies, which formed a white-gray mycelium	Rounded, yellow colonies, which formed a dark gray mycelium
Myt 8	Rounded, colorless colonies, which formed a light pink mycelium	Rounded, yellow colonies, which formed a light pink mycelium	Rounded, golden colonies, which formed a light pink mycelium
Myt 10	Rounded, yellow colonies, which formed a white mycelium	Rounded, yellow colonies which formed a white mycelium	Rounded, golden colonies which formed a white mycelium
Myt 11	Rounded, colorless colonies, which formed a light gray mycelium	Rounded, yellow colonies, which formed a gray mycelium	Rounded, white colonies, which formed a gray mycelium
Myt 12	Rounded, colorless colonies, which formed a white mycelium	No growth	Rounded, golden colonies, which formed a white mycelium

Microscopy of colonies on nutrient media made it possible to establish the structure of the air mycelium, the presence and location of actinospores in the hyphae of the air mycelium (Fig. 1).

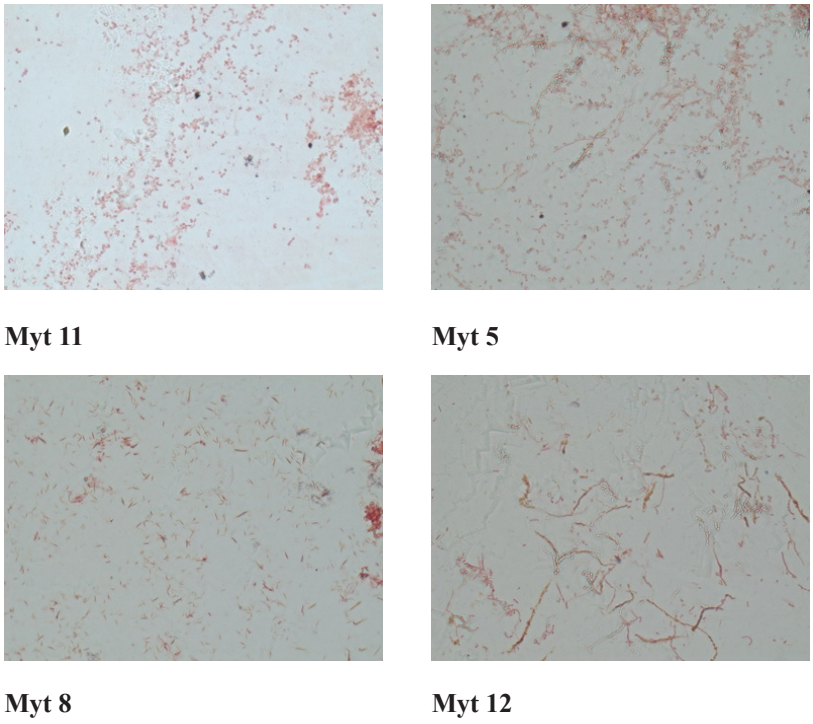


a) **b)** **c)**

Fig. 1. Morphology of the air mycelium of actinobacteria strain Myt 8 on the media: a) Oat agar with sea salt; b) Gauze №1; c) Gauze №2

Interestingly, the colonies of some strains, forming the substrate mycelium, had easily removed from the nutrient media, others for example Myt 8; - grow strongly into the nutrient media.

The morphology of cells of isolated actinobacteria had determined by microscopy of fixed preparations stained with Pfeiffer's magenta (Fig. 2).



Myt 11 **Myt 5**

Myt 8 **Myt 12**

Fig. 2. Morphology of actinobacteria cells isolated from mussels

We could observe that the cells of most actinobacteria are represented by short rods of small size, located alone, in pairs in chains, V-shaped, chaotic. In addition, filamentous cells had found in the preparations along with short rods. The cells of some of the studied strains were in the form of coccal forms. Different forms of cells had observed in the preparations at the same time: from cocci to filamentous.

We also carried out the primary identification of strains by the fatty acid profile using an automatic identification system MIDI Sherlock (Table 2).

Table 2

Primary identification of strains by the fatty acid profile

Strain	Speciesname
Myt 1	*Streptomyces-halstedii-scabies*
Myt 2	*Streptomyces-halstedii-scabies*
Myt 3a	*Streptomyces-halstedii-scabies*
Myt 3b	*Streptomyces-halstedii-scabies*
Myt 4b	*Streptomyces-violaceusniger-violaceusniger*
Myt 5	*Streptomyces-halstedii- scabies*
Myt 6	*Streptomyces-rochei-rochei*
Myt 7	*Streptomyces- halstedii-olivaceus*
Myt 8	*Streptomyces-halstedii-scabies*
Myt 10	*Streptomyces-halstedii-scabies*
Myt 11	*Streptomyces-halstedii-scabies*
Myt 12	*Streptomyces-violaceusniger-violaceusniger*

Conclusions

Isolated strains, according to the primary identification of the fatty acid profile, belong to the genus *Streptomyces*. The genus *Streptomyces* alone produces a large number of bioactive molecules. A large number of *Streptomyces spp*. have been isolated and screened from the soil in the past several decades. Consequently, the chances of isolating a novel *Streptomyces* strain from terrestrial habitats have diminished [2].

Secondary metabolites from marine actinomycetes may form the basis for the synthesis of novel therapeutic drugs, which may be efficient to combat a range of resistant microbes. The existence of cousins of terrestrial actinomycetes had been reported in the relatively untapped marine ecosystem. The immense diversity of this habitat along with it is under exploitation is the fundamental reason for attracting researchers towards it for discovering novel metabolite producers. Actinomycetescomprise about 10% of the bacteria colonizing marine aggregates and can be isolated from marine sediments [5, 6].

Many actinomycetes isolates from deep oceans contain non-ribosomal polyketide synthetase (NRPS) and polyketide synthetase (PKS) pathways, the

hallmarks of secondary metabolite production. There is an occurrence of distinct rare genera in the marine ecosystem as evidenced by the taxonomic description of the first marine actinomycete *Rhodococcusmarinonascens* [13, 14].

Thus, we can say that the isolation of actinobacteria from marine representatives and their study is promising for the subsequent search for new antimicrobial secondary metabolites [8].

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UDC 575.87:577

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COMPARISON OF *BACILLUS VELEZENSIS* ONU 553 AND OTHER STRAINS OF OPERATIONAL GROUP *BACILLUS AMYLOLIQUEFACIENS* (OGBA) GENOME SEQUENCES

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Abstract. *A comparative analysis of the Bacillus velezensisONU 553 strain's genome, which was isolated from deep-water deposits of the Black Sea, was conducted via pangenomic analysis methods. A high degree of relativity was shown between the analyzed strain and other members of the operational group B. amyloliquefaciens (OGBa), which were retrieved from the NCBI database using BLAST+ (ver. 2.8.1+). It is proven, that species Bacillus velezensis has an open pangenome. Results from Roary and Anvio programs have helped to reveal 68 unique genes in the examined strain, which can be connected with the location of its isolation.*

Keywords: *pangenomic analysis, Bacillus velezensis*

Introduction.

It is known that the 16S rRNA gene sequence identity between *Bacillus velezensis*and *Bacillus amyloliquefaciens*exceeds 99% [Roberts et al., 1994; Wang et al., 2014; Dunlap et al., 2016], because these strains were attributed to the operational group *B. amyloliquefaciens*(OG*Ba*) [Priest etal., 1987; Ruiz-García etal., 2005; Dunlap etal., 2016].

It was reported that the members of the operational group *B. amyloliquefaciens*(OG*Ba*) are able to increase their stimulating activity towards plants on account of increasing resistance to stress or inhibiting growth of the phytopathogenic bacteria [Boriss et al., 2011; Qiao et al., 2014]. These species produce such secondary metabolites as surfactin, fengycin, baccilomycin D, macrolactin, bacillaene, difficidin, oxydifficidin, plantazolicin, amylocyclicin, bacillysin, bacteriocins as well as siderophores [Baruzzi et al., 2011;Sumi et al., 2015]. Additional advantages of strains of this group are resistance to UV light, thermostable spore formation, extended period of storage [McSpadden, 2014], which makes them an ecologically clean alternative to agrochemicals. Nowadays some strains of *B. amyloliquefaciens*are available as commercial agents of biological control and general plant growth stimulants [Chen et al., 2007; Choi et al., 2014].

However despite their general likelihood, due to adaptation towards different ecological niches and enviroments*Bacillus velezensis*have their specific genomic and physiological characteristics [Borriss et al., 2011;Chowdhury et al., 2015].

Therefore the aim of this paper was to study the pangenome of the *Bacillus velezensis*ONU 553 and other closely related strains, which were selected from the NCBI database.

Materials and methods

Pangenome is a complex of protein coding genes, which are present in a number of genomes of one species or genus of bacteria. Traditionally pangenome is divided into the core genome, dispensable or accessory genome and strain-specific or unique genome [Medini et al.2005; Tettelin et al., 2005; Lapierre and Gogarten, 2009] and flexible regions [Rodriguez-Valera and Ussery, 2012].

Pangenomic analysis was carried out via two programs – Roary [Andrew et al., 2015] and Anvio [Eren et al., 2021]. Input files of Roary are .gff format files, which are markups of genes and other elemetns of DNA, RNA and protein sequences. In order to obtain such files Prokka program was used [Seemann, 2014]. Input files of Prokka are .fasta format files, which are text files with nucleotide sequences, output files are .gff format files.

After obtaining .gff format files of all examined genomes they were processed through Roary. Coding regions were extracted, translated and filtered in order to delete partial sequences and preclustered via CD-HIT [Fu et al., 2012], after which a whole comparative analysis among acquired sequences with a set percent of sequence identity (usually 95%) was carried out via BLASTP.

Later clustering of sequences is conducted via Markov clustering algorithm (MCL) and finally clusters, which were obtained from CD-HIT are combined with this data. Utilizing information from the neighboring conserved genes, homologous groups, which include paralogs, are split into groups of true orthologues. The next step is the construction of a cluster graph according to their placement in sequences in a way that provides context to each of the gene, and after clustering is performed in relation to the presence of the genes in the accessory genome.

Anvio has a similar pipeline, however input files are .fasta format files. Firstly, these files are transformed into contigs databases, after which these databases are analyzed, using BLAST, MCL and others.

Results

After the pangenomic analysis in Anvio a diagram was retrieved (fig. 1) in which 11 clustered analyzed genomes (starting from the central sector: *B. Velezensis* QST713, *B. Amyloliquefaciens* XJ5, *B. Velezensis* DSYZ, *B. velezensis* 10075, *B. Amyloliquefaciens* ZJU1, *B. Velezensis* W1, *B. Amyloliquefaciens* SRCM10126, *B. Amyloliquefaciens* KC41, *B. Amyloliquefaciens* WF02, *B. Velezensis* CBMB205, B. *Velezensis* ONU 553); the next 3 sectors representing the presence of COG annotations of categories (3612 are known, 2104 are unknown), functions (3612 are known, 2104 are unknown) and pathways (850 are known, 4866 are unknown), the next sector representing the presence of «core» clusters, next three sectors depicting the degree of homogeneity, the last three sectors depicting the number of paralogs, genes and genomes, which are in a cluster, are shown.

Figure 1 shows distinct differentiation between core gene, unique and accessory genes clusters (genome genes, which are included into the cluster have a much

vivid color in contrast to the excluded ones). In total the pangenome is represented by 5716 clusters of genes, out of which 3329 comprise the core genome, 1352 – the accessory one, and other 1035 are unique genes of each of the strain.

In the right hand corner of figure 1 one can notice a heatmap of full identity of genomes. Results suggest that *B. velezensis* ONU 553 is mostly related to *B. velezensis* CBMB205.

Acquired Roary results are shown in figure 2.

As it can be seen (fig. 2) with the increase of genome quantity the quantity of new genes decreases, meanwhile the quantity of unique genes also increases, from which a conclusion can be made, that *Bacillusvelezensis*have an open pangenome and later in the evolutional process members of this species will be able to acquire new genes from the pangenomic pool, which characterizes them as qualitative potential biotechnological producents.

Pangenomic analysis in Roary states that there are 7887 gene clusters, out of which 2509 are related to core genome, 2297 – to accessory and 3081 – to unique (fig. 3).

Fig. 1. Comparison results of selected genomes in Anvio program, depicted in a circle diagram

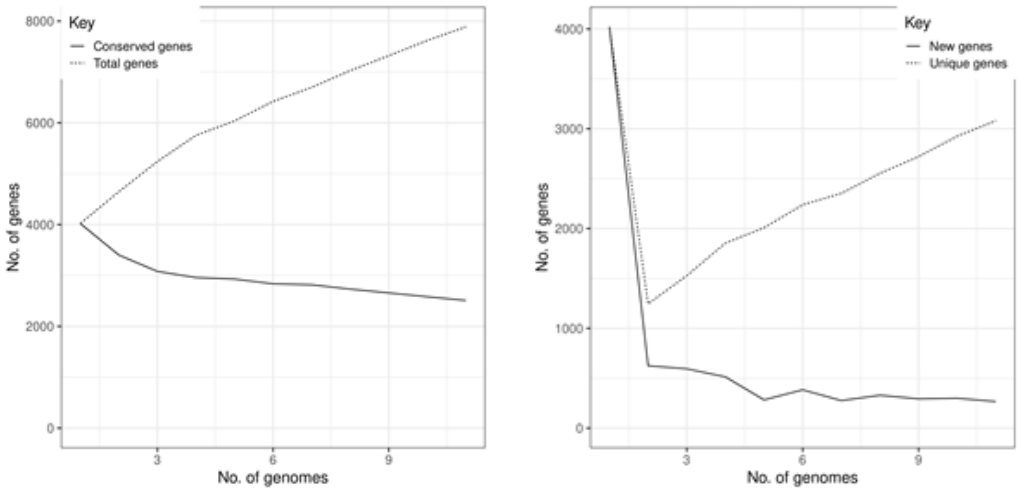

Fig. 2. Plots of distribution of conserved, new and unique genes in analyzed genomes (according to the results from Roary)

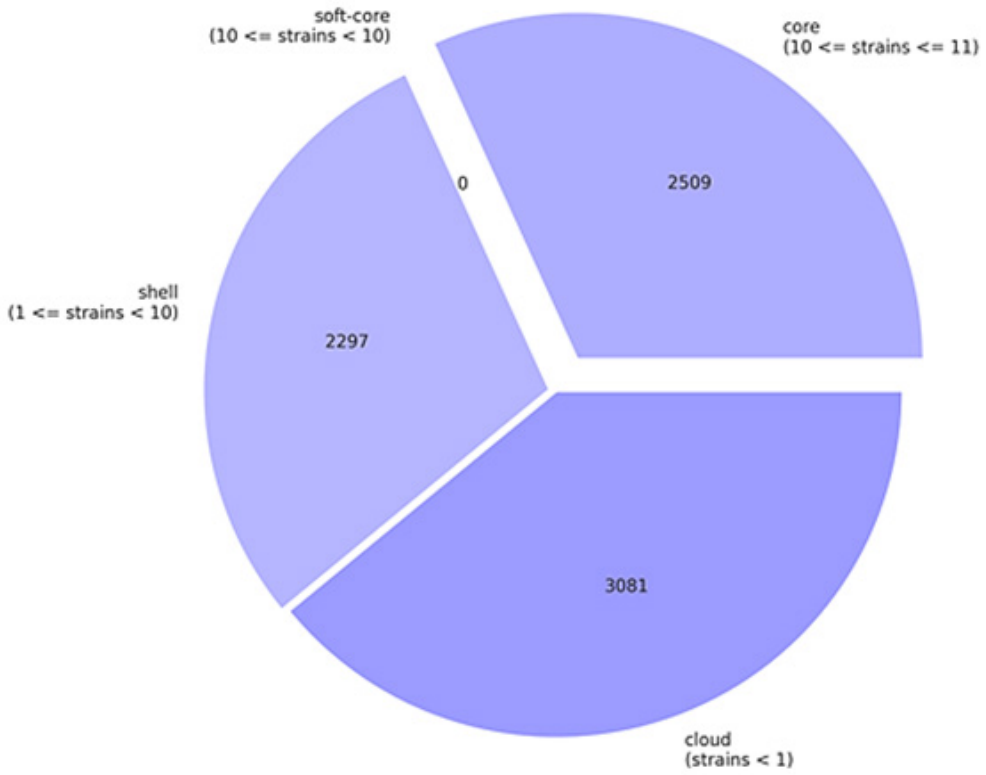

Fig. 3. Pangenome pie-chart of analyzed genomes, acquired via Roary

Figure 4 depicts the result of gene clustering according to their uniqueness.

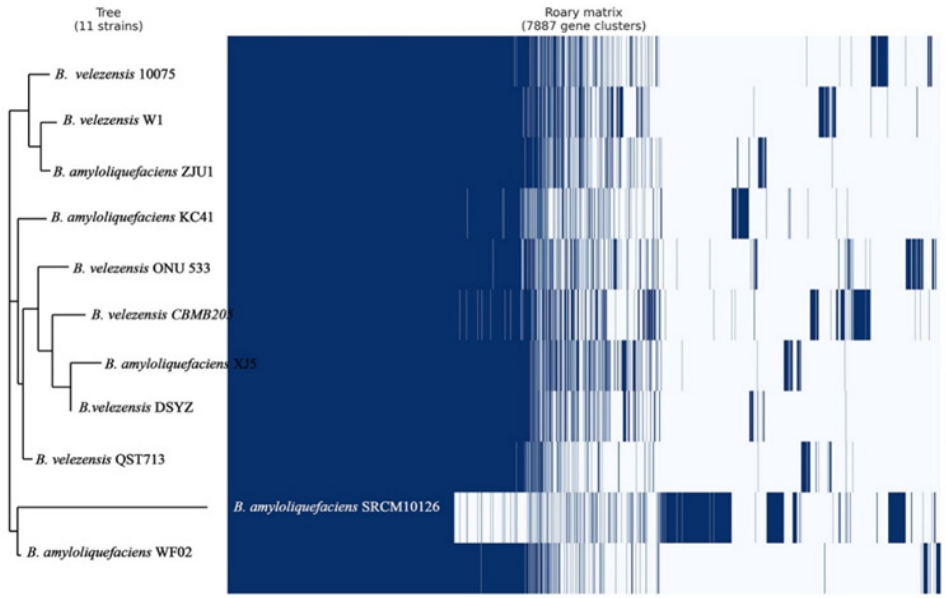

Fig. 4. Pangenomic analysis of *Bacillus* strains based on the Roary matrix. The generated tree based on the core genome was compared with the matrix, in which core and accessory genes were either present (blue) or absent (white)

Analysis of unique genes from *B. velezensis*ONU 553 has helped to note 2 regions. The first region had 9 genes and was located from the 720638 to 726010 nucleotide (tab. 1).

Table 1

Regions which are related to the first unique cluster of strain *B. Velezensis* ONU 553 and their annotation via UniProt database

Region	Annotation (UniProt)
642947…645952	Superfamily II DNA or RNA helicase (*Sporanaerobacteracetigenes*)
720638…721930	SIR2_2 domain-containingprotein (*Alkalihalobacilluskrulwichiae*)
721927…722397	Signaltransductionproteincontainingdiguanylatecycl ase/phosphodiesterasedomain (GGDEF) anddomain (EAL) (*Clostridiumacetobutylicum*)
722401…724308	Uncharacterizedprotein (*Fictibacillusarsenicus*)
724497…724623	Syntaxin-bindingprotein 3 (*Nosemaapis* BRL 01)
724697…724799	Conjugaltransferprotein (*Virgibacilluspantothenticus*)
724698…725011	Conjugaltransferprotein (Virgibacilluspantothenticus)
725024...725407	YdcPfamilyprotein
725404…726010	ATP-bindingprotein (*Paludifilumhalophilum*)

The second region is comprised of 3 genes and is located from the 3835503 to 3839758 nucleotide (tab. 2).

Table 2

Regions which are related to the second unique cluster of strain *B. velezensis* ONU 553 and their annotation via UniProt database

Region	Annotation (UniProt)
3835503..3836951	Abortive phage infection protein (*Paenibacillussambharensis*)
3836935..3837771	Restrictionsystemprotein (*Flavobacteriumcroceum DSM 1*)
complement (3837908..3839758)	Putative ATP-dependent endonuclease of OLD family (*Aureibacillushalotolerans*)

Conclusion

Recently conducted genomic and pangenomic research of the operational group *B. amyloliquefaciens*(OGBa) members have shown a presence of a large quantity of gene clusters (up to 10% of the whole genome) connected with secondary metabolites production with known or unknown antimicrobial activity, which generates interest of biologists towards this group of microorganisms [Robertsetal., 1994; Wangetal., 2014; Dunlap etal., 2016]. Foremost it is due to new approaches of secondary metabolites cluster search in potential producent's genome, search of new ecological niches, which might be inhabited by strains with new and previously unknown properties [Fanetal., 2018]. In this paper *Bacillusvelezensis*ONU 553 strain's genome was compared to other members of this group. All genomes of examined members of *B. amyloliquefaciens*group were retrieved from the NCBI database and their phylogenetic relativity was later confirmed in other research. The results have shown, that the analyzed strains demonstrate strain specific features, even while sharing a common core genome. The pangenomic analysis has demonstrated that *B. velezensis*have an open pangenome, including *B. velezensis*ONU 553 and and later in the evolutional process it will be able to acquire new genes from the pangenomic pool, which characterizes it as a qualitative and prospective producent of active secondary metabolites.

Also 68 unique genes of the *Bacillus velezensis*ONU 553 strain were identified, using Roary and Anvio programs.

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UDC 575.87:577

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RESULTS OF THE PHYLOGENETIC ANALYSIS OF STRAIN *BACILLUS VELEZENSIS* ONU 553

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Abstract. *A phylogenetic analysis of Bacillus velezensisstrain ONU 553, which was isolated from deep-water deposits of the Black Sea, was conducted with closely related to it members of the operational group B. amyloliquefaciens(OG-Ba), which were retrieved from NCBI database via BLAST+ (ver. 2.8.1+). It was confirmed that all analyzed strains make up a monophyletic clade on the phylogenetic tree.*

Keywords: *phylogenetic analysis, Bacillus velezensis*

Introduction

Bacillus velezensis – is a new species of *Bacillus*, which belongs to the operational group *B. amyloliquefaciens* (OGBa) and is widely studied and used for its direct or indirect growth stimulating activity towards a wide range of plants and antimicrobial activity towards opportunistic pathogens [Fanetal., 2018].

Due to emerging taxonomic problems from close relations of strains connected with species *B. velezensis, B. amyloliquefaciens*subsp. *plantarum, B. methylotrophicus*and *B. oryzicola* [Priest et al., 1987; Fritze, 2004; Ruiz-García et al., 2005; Dunlap et al., 2016] it was suggested that species *B. amyloliquefaciens, B. velezensis and B. siamensis*are to be kept as individual species in their respective clades, however on the grounds of their close relations these species must be included into an «operational group *B. amyloliquefaciens* » inside the *B. subtilis* group [Fan et al., 2018].

The research aim was to confirm the affiliation of the *Bacillus velezensis* ONU 553 strain with the operational group *B. amyloliquefaciens* (OGBa) on the basis of phylogenetic analysis

Materials and methods

For the search of closely related genomes amongst known ones local BLAST+ (ver. 2.8.1+) was used and a corresponding database was installed – ref_prok_rep_ genomes (15 bases were used in total from the NCBI server).

In order to conduct the phylogenetic analysis a RAxML (Randomized Axelerated Maximum Likelihood) algorithm was used for consecutive and parallel output of large phylogenetic trees based on Maximum Likelihood method. The algorithm is available through https://realphy.unibas.ch/realphy/. Visualization of the acquired tree was completed using T-REX server (http://www.trex.uqam.ca/index.php?action=home) and an R package (ver. 4.5.1).

For the research of identity value between strains an OrthoANI (Orthologous Average Nucleotide Identity Tool) algorithm was used.

Results

According to the results of conducted BLAST analysis it was shown, that amongst the diverse collection of strains, stored in the ref_prok_rep_genomes database, only two whole genome sequences were retrieved, which had a high identity value regarding the query strain (tab. 1). Other sequences were provided in the form of contigs, i.e. not whole genomes. Thus a restriction on strains only related to *Bacillus amyloliquefaciens*and *Bacillus velezensis* was set for conducting phylogenetic analysis.

Table 1

Local BLAST+ results with the installed ref_prok_rep_genomes database

Sequencesproducingsignificantalignments:	Score (Bits)	E Value
NZ_CP053376.1 *Bacillus amyloliquefaciens* strain WF02 chromosom complete..	4.114e+05	0,0
NZ_CP011937.1 *Bacillus velezensis* strain CBMB205 chromosome, complete	3.880e+05	0,0
NZ_AJVF01000013.1 *Bacillus siamensis* KCTC 13613 strain KCTC 13613	2.350e+05	0,0
NZ_AJVF01000041.1 *Bacillus siamensis* KCTC 13613 strain KCTC 1361	1.912e+05	0,0
Z_AJVF01000041.1 *Bacillus siamensis* KCTC 13613 strain KCTC 13613	1.463e+0	0,0
Z_AJVF01000041.1 *Bacillus siamensis* KCTC 13613 strain KCTC 13613	1.346e+05	0,0

During the retrieval of genomes from NCBI database the genome size, organism's origin and its affiliation with the operational group *B. amyloliquefaciens*(OG-*Ba*) were taken into account (tab. 2). For further analysis strains *Bacillus amyloliquefaciens* strain KC41, *Bacillus amyloliquefaciens* strain SRCM10126, *Bacillus amyloliquefaciens* strain WF02, *Bacillus amyloliquefaciens* strain XJ5, *Bacillus amyloliquefaciens* strain ZJU1, *Bacillus velezensis strain* 10075, *Bacillus velezensis*strain CBMB205, *Bacillus velezensis*strain DSYZ, *Bacillus velezensis*strain QST713, *Bacillus velezensis*strainW1 retrieved from the NCBI database were used.

The phylogenetic tree, which was constructed using whole genome sequences of the 11 selected strains, is depicted in figures 1-3. Obtained results confirmed that the analyzed strain *Bacillus velezensis*ONU 553 is affiliated with the operational group *B. amyloliquefaciens*(OG*Ba*).

The alignment of the assigned genomes via RAxML program resulted in a .newick format file.

Table 2

Information regarding selected genomes from the NCBI database Genome Assembly and Annotation report page

[https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/848/]

Strain	RefSeq	Size (Mb)	GC%	Source of isolation	Geographic location
***Bacillusamyloliquefaciens* strain WF02**	NZ_CP053376.1	4.03	46.50	soil	Taiwan
Bacillusamyloliquefaciens KC41	NZ_CP044444.1	4,12	46.00	soil	South Korea
Bacillusamyloliquefaciens SRCM10126	NZ_CP021505.1	4.09	45.89	food	South Korea
Bacillusamyloliquefaciens XJ5	NZ_CP071970.1	4.44	45.60	Catalpa	China
Bacillusamyloliquefaciens ZJU1	NZ_CP041691.1	4.06	46.40	Morusalba L.	China
***Bacillusvelezensis* CBMB205**	NZ_CP011937.1	3.93	46.50	rice rhizosphere soil	South Korea
Bacillusvelezensis 10075	NZ_CP025939.1	4.34	46.09	chinese food lobster sauce	China
Bacillusvelezensis DSYZ	NZ_CP030150.1	4.32	45.72	rhizosphere soil	China
Bacillusvelezensis QST713	NZ_CP025079.1	4.23	45.90	commercial product Serenade	France
Bacillusvelezensis strain W1	NZ_CP028375.1	4.24	45.80		China
Bacillusvelezensis strain ONU 553	NZ_CP043416	3.93	46,69	bottomsedimen	Ukraine

Note: reference genomes are marked in bold

Visualization of the acquired tree was conducted via T-REX server (http://www.trex.uqam.ca/index.php?action=home), which reconstructs phylogenetic trees using Maximum Likelihood method as well.

As it can be seen in figure 1 all of the analyzed genomes can be split into two clusters. The first was comprised of *Bacillus velezensi*s strain W1, *Bacillus amyloliquefaciens* ZJU1, *Bacillus velezensis* 10075, *Bacillus amyloliquefaciens*KC41, *Bacillus amyloliquefaciens* strain WF02**,** *Bacillus amyloliquefaciens* SRCM10126 genomes.

The second one included strains *Bacillus velezensis* DSYZ, *Bacillus amyloliquefaciens* XJ5, *Bacillus velezensis* QST713, *Bacillus velezensis* ONU 553. The most related strain to *Bacillus velezensis*ONU 553 was *Bacillus velezensis* CBMB205.

A quite peculiar fact came to light, which is that reference genomes, i.e. *Bacillus velezensis* CBMB205 and *Bacillus amyloliquefaciens* strain WF02 belonged to separate clusters (fig. 1). On the whole the resulting phylogenetic tree can be characterized as monophyletic, however such separation of reference genomes into clusters emphasizes the identity degree, which is connected with species affiliation.

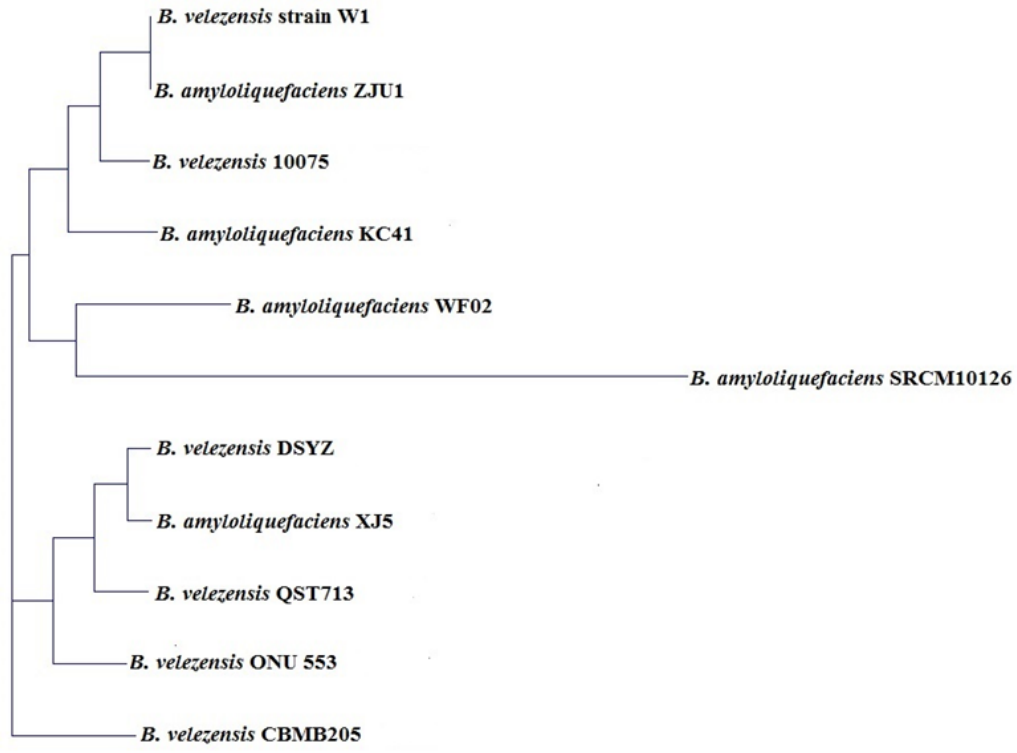

Fig. 1. Phylogenetic tree of analyzed genomes (Maximum Likelihood method)

It should also be noted, that the majority of *Bacillus velezensis*members belongs to the cluster with *Bacillus velezensis* strain ONU 553, while *B. amyloliquefaciens*are grouped in the other with one exception – *Bacillus velezensis* 10075. However these results do not contradict the concept of operational group *B. amyloliquefaciens*(OGBa).

Using an R package (ver. 4.5.1) other variants of visualized trees were acquired, which are based on the .newick formatted file. Figure 2 includes a variant with an unrooted phylogram, which was produced using ape, phangorn and phytools packages.

Figure 3 depicts a variant with a circular unrooted tree, which was produced using ape, phangorn and phytools packages.

According to the results of the OrthoANI algorithm acquired data (shown as a heatmap), which give evidence regarding high identity degree of nucleotide sequences of *Bacillus velezensis* ONU 553 genome and its closely related members of *Bacillus velezensis.* OrthoANI values between *Bacillus velezensis* ONU 553 and other *Bacillus velezensis*genomes were as follows: *Bacillus velezensis*10075 (97,93%), *Bacillus velezensis* CBMB205 (98,27%), *Bacillus velezensis* QST713 (97,99%), *Bacillus velezensis* DSYZ (98,58%), *Bacillus velezensis*strain W1 (98.16%). Values higher than 97% indicate the affiliation of strains to one species (fig. 4).

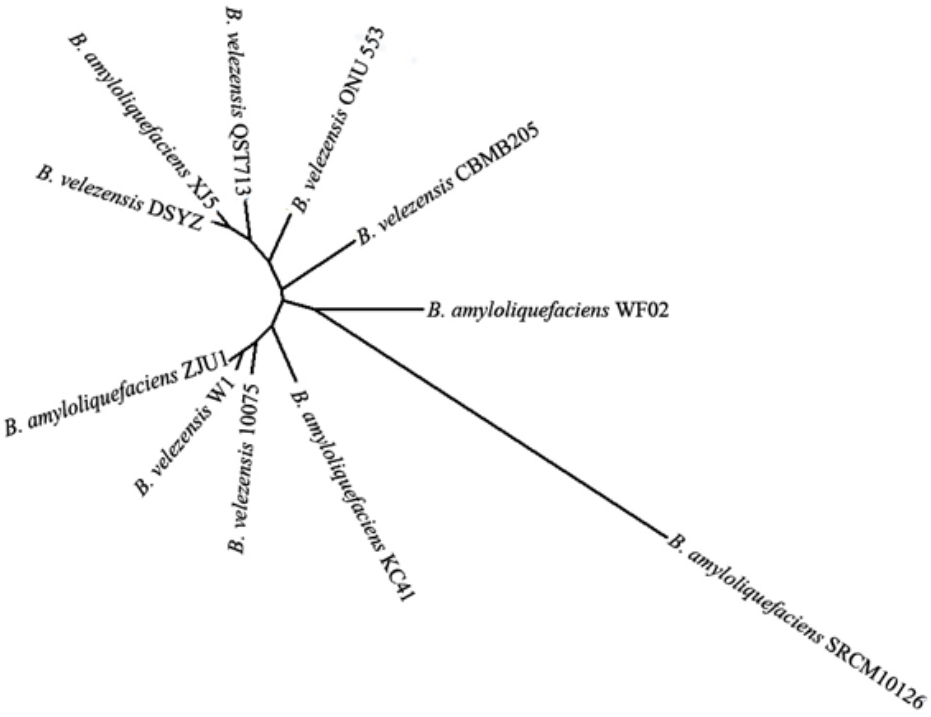

Fig. 2. Unrooted phylogram of the analyzed genomes

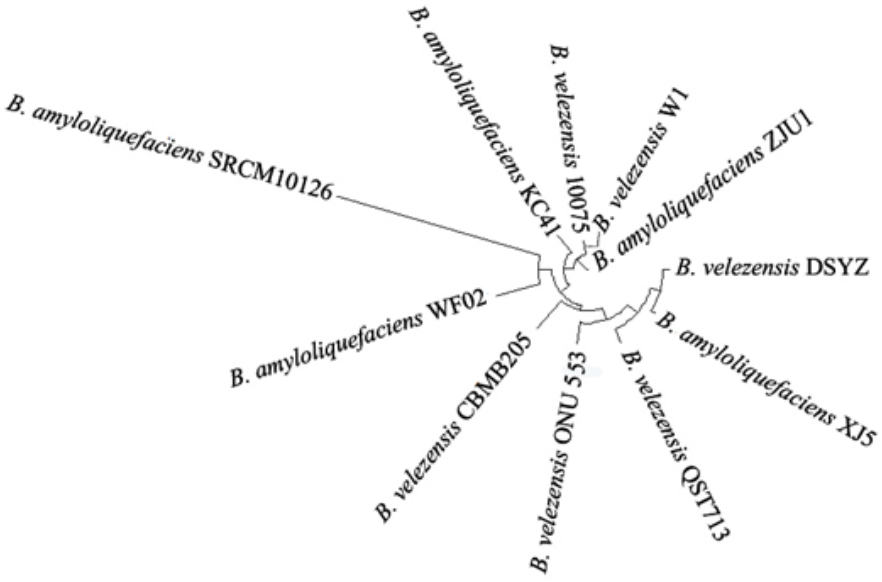

Fig. 3. Circular unrooted tree of the analyzed genomes

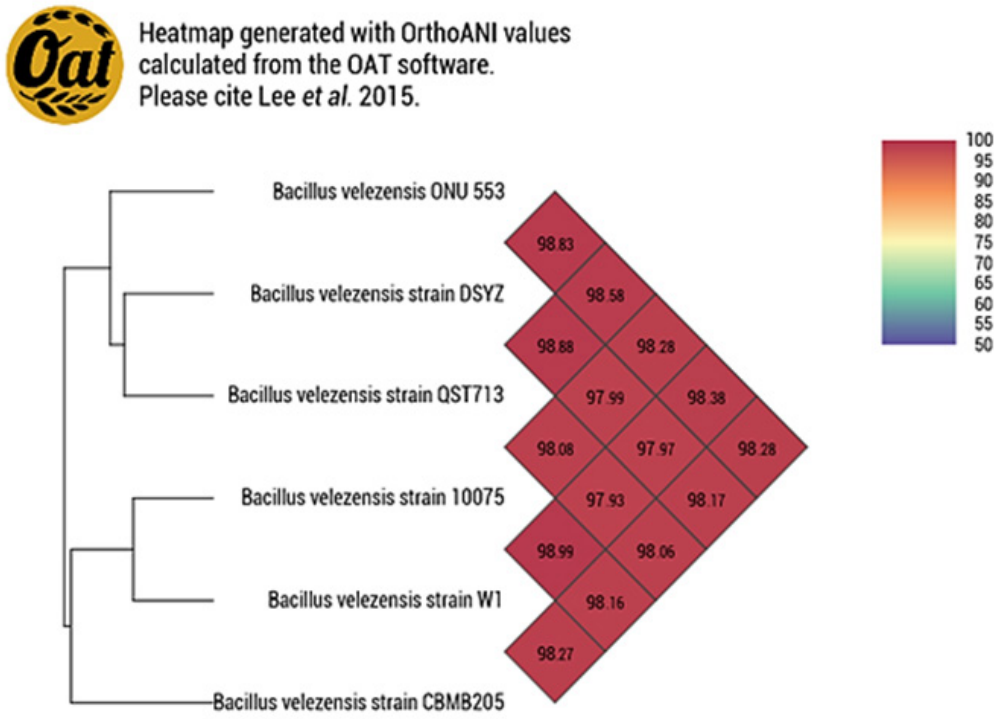

Fig. 4. Phylogenetic analysis of Bacillus velezensisONU 553 based on the average nucleotide identity

Thus conducted research has established that strain *Bacillus velezensis*ONU 553 has high values of average nucleotide identity towards other *Bacillus velezensis*strains. Phylogenetic analysis confirmed that all of the analyzed strains are a part of operational group *B. amyloliquefaciens*(OG*Ba*) and have a high relation degree amongst each other.

Conclusion

Recent taxonomic studies have revealed that *B. subtilis* is heterogeneous and should be considered as a complex of closely related species [Nakamura et al., 1999; Fritze, 2004; Dunlap et al., 2016; Fan et al., 2018]. The issue of phylogenetic analysis is especially important for the representatives of *B. subtilis, B. amyloliquefaciens,* and *Bacillus velezensis*, which are phenotypically similar species, which makes them easy to confuse. Therefore, it is not surprising that in most large-scale studies the isolates are attributed to *B. subtilis* without a deeper analysis of their taxonomy. Comparative analysis of *Bacillus velezensis*ONU 553 with other representatives selected for the analysis of *Bacillus* species showed the close relativity of these species. A high degree of average nucleotide identity was shown among the strains. Phylogenetic trees confirmed monophyletic nature of the clade, which included all of the analyzed strains

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UDC 579.222

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BUTANOL ACCUMULATION BY BUTANOL STRAINS PRODUCERS USING APPLE POMACE

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The processes of microbiological synthesis of butanol significantly depend on the properties of strains producers, cultivation conditions and degree of substrate assimilation.The butanol yield can be increased by metabolic pathways optimizing of synthesis.The paper presents results of butanol accumulation study during cultivation on the food enterprises waste - apple pomace.We usedfor research: butanol strains producer Clostridiumsp. IMB B-7570, IFBG C7P from "SE "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine" Collection's of microorganisms strains and plant lines for agricultural and industrial biotechnology", apple pomace (total humidity 4%) after getting juice. It was shown the possibility of using apple pomace for butanol accumulation. Butanol 6g/l accumulation was determined using pomace concentration of 20 g/l.

***Key words**: butanol, apple pomace, cultivation, Clostridiumsp*

Introduction.

Wastes of plant origin and processing of agricultural raw materials are generated annually in significant volumes and leads to negative consequenceson the environment. Problem of their further use or disposal is reflected in the National Waste Management Strategy in Ukraine until 2030 [1, 2]. Processing of secondary raw materials from plant origin and way to solve the environmental problem associated with environmental pollution in Ukraine is relevant [3]. Vegetable waste occupies a significant place in the total amount of waste from processing and food industries [4].The main wastes in the production of juices, canned fruits and vegetables are squeezes and residues after passing through a sieve of tomatoes, apples, carrots, beets, tomato pulp and waste from cleaning various fruits and vegetable.In the range of fruit waste, about 70.0% is accounted apples, the pomace of which is 35.0–40.0% and part of it can be used in vinegar, alcohol and production of pectin preparations [5].

Study of butanol accumulation by strains producers using apple pomace as a substrate was the aim of this work.

Materials and methods

Butanol strains producer *Clostridiumsp.*IMB B-7570, IFBG C7P from the SE "Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine Collection's of microorganisms strains and plant lines for agri-

cultural and industrial biotechnology" and pomace (total humidity 4%) after juice squeezing were used for the research. Apple pomace of various quantities and tap water were used as a medium for cultivation. Medium was sterilized by autoclaving at 0.5 atm. for 30 minutes. Cultivation using hydroacid gates at 35^0C for 72 hours was performed. Biomass was determined by weight method. Ethanol and butanol in the culture fluid presence was determined using a gas chromatograph with flame ionization detector [6]. The acidity was determined by amount of 0.1 N NaOH spent on adjusting pH to original.

Results and discussion

The first step in the process of using any plant material as a substrate is to determineits macro-component composition. The macrocomponent composition of apple pomace was analyzed and their quantitative (%) and qualitative composition (Fig. 1) were determined: sugars 39.2; cellulose 16.6; lignin 12.8; ash 2.6; humidity 4.2; pectin 11.9; protein 4.5; wax 1.7; lignocellulose 6.5. The results show that apple pomace contains a high percentage of sugars and a small percentage of lignin (which is not fermented) and can be a promising substrate for alcohol production. Cultivation using (as a carbon source) apple pomace at a concentration of 50 g/l was performed (Fig. 2).

It was determined that culture fluid accumulated 1g/l of butanol (using *Clostridium sp.* IMB B-7570) and 3g/l of ethanol (using *Clostridium sp.* IFBG C7P) after fermentation other alcohols were present in trace amounts. *Clostridium sp.* IFBG C7P primarily accumulated ethanol using apple pomace. Cultivation was also carried out using pre-prepared substrate (grinding apple pomace). It is shown that in the case of crushed apple pomace use in the culture fluid accumulated 2g/l of butanol using the strain *Clostridium sp.* IMB B-7570 and 5g/l ethanol in the case of *Clostridium sp.* IFBG C7P, other alcohols were present in trace amounts (Fig. 2).

Thus, the increase in the accumulation of alcohols was due to use of preliminary substrate preparation – grinding of pomace. Technological indicators of pomace fermentation are given in Table 1.

The results show that culture fluid accumulated butanol (2 g/l) after fermentation by *Clostridium sp.* IMB B-7570 and ethanol (5 g/l) by *Clostridium sp*. IFBG C7P. For both strains, the dry substrate residue was 6g/l (of which lignin was 4g), bacterial biomass 0.8 and 0.9 g, respectively, amount of hydrogen and carbon dioxide released mixture was almost the same (3.5 and 3.2 g, respectively).

Medium (culture fluid) was acidified in the process of cultivation. The main butyric and acetic acids were determined, which correlates with results of [1,6].

Clostridium sp. IMB B-7570 at different concentrations of grinding apple pomace was cultivated. Results of the study are presented in Fig.3.

Proportional increase in butanol accumulation with concentration of apple pomace increase to 20 g/l was shown. Further increase of substrate concentration led to decrease in the target product yield.

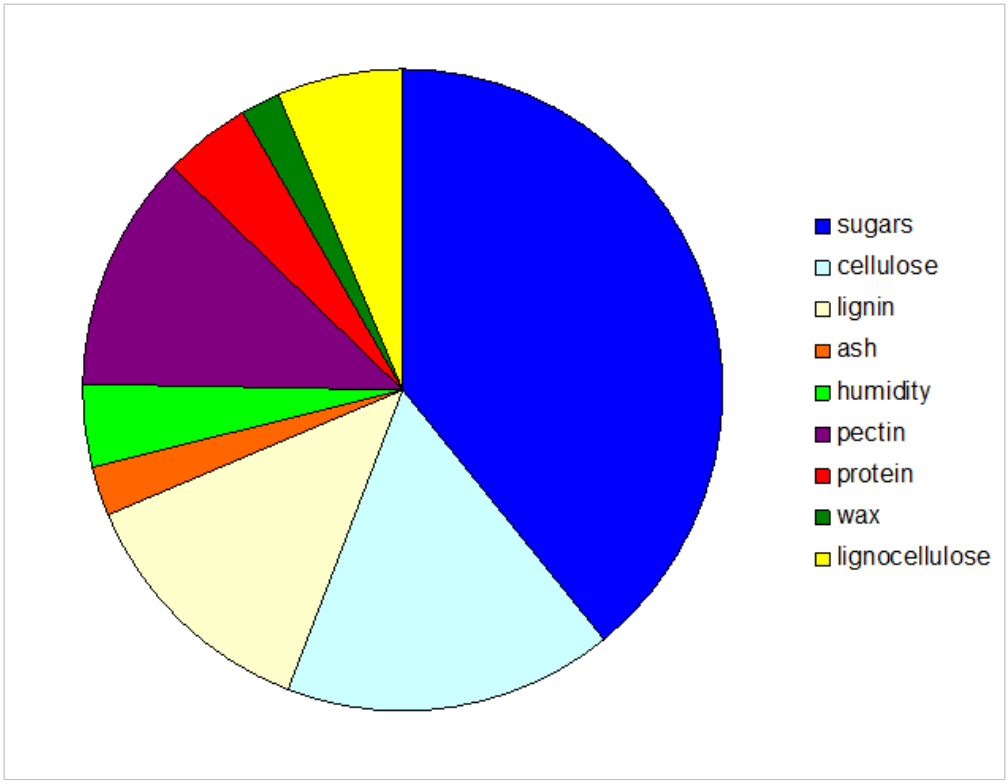

Fig. 1.Macrocomponent composition of apple pomace

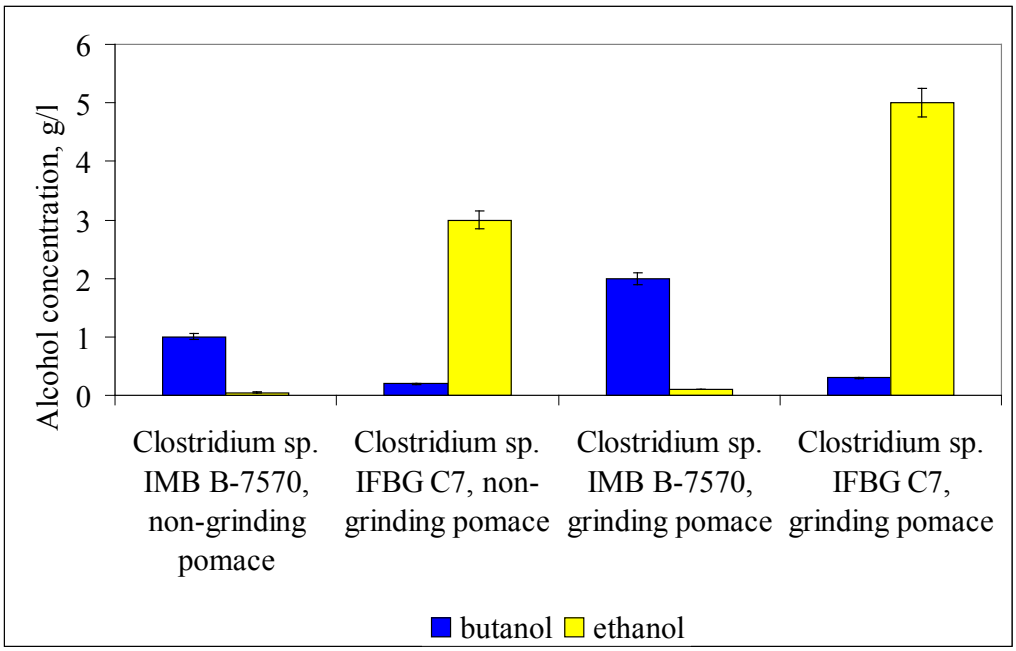

Fig. 2. Accumulation of butanol in apple pomace

Table 1

Technological indicators of fermentation

Strains	CO_2g/100 ml	pH	Acidity	Drymatter, (%)	Biomassg/l	Alcoholcontentg/l
***Clostridiumsp.* IMB B-7570**	0,35	3,8	7,7	7,20	0,8	2
Clostridiumsp. IFBG C7P	0,32	3,6	7,4	5,90	0,9	5

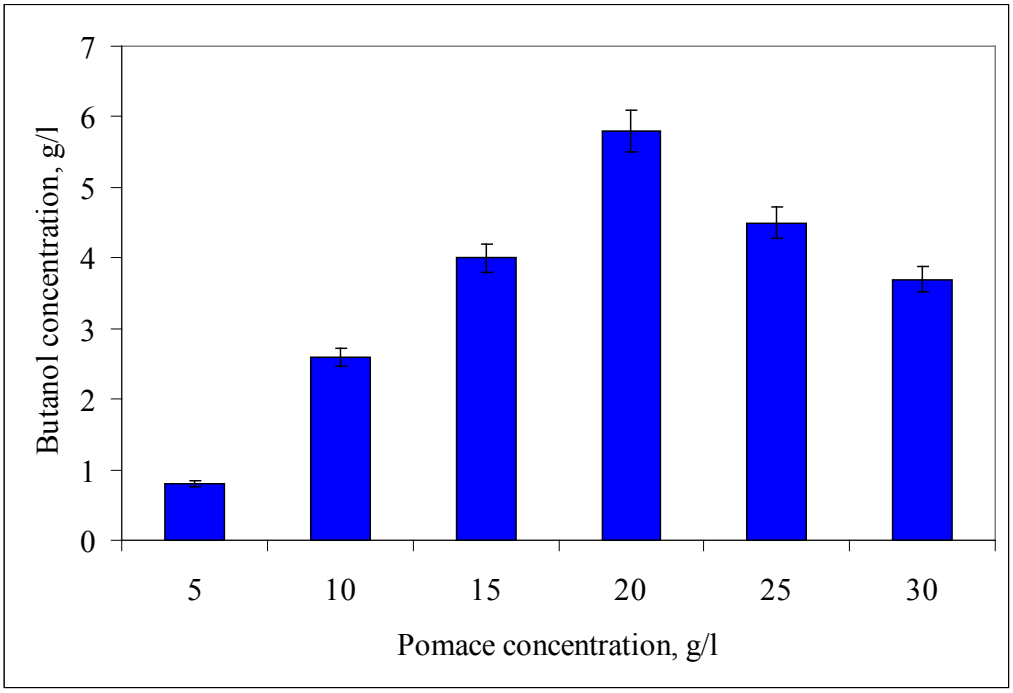

Fig 3. Accumulation of butanol by strain *Clostridium sp.*IMB B-7570 at different concentrations of apple pomace

Conclusions

Accumulation of butanol using apple pomace as a substrate with and without prior preparation pretreatment (grinding) was shown. It was shown that substrate grinding increased butanol accumulation up to 6 g/l using a pomace concentration of 20g/l. Further increase of substrate concentration led to decrease of butanol accumulation. The results of study showed that waste of apple processing (pomace) in juice obtaining can be an effective substrate for butanol production.

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БІОЛОГІЯ, БІОТЕХНОЛОГІЯ, БІОМЕДИЦИНА

XVI Міжнародна конференція літньої школи

Матеріали конференції

22 червня – 5 липня 2021 року, Одеса

За редакцією авторів

Підписано до друку 00.00.2021 р. Формат 70x100/16.
Ум.-друк. арк. 5,53. Тираж 100 пр.
Зам. № 000.

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