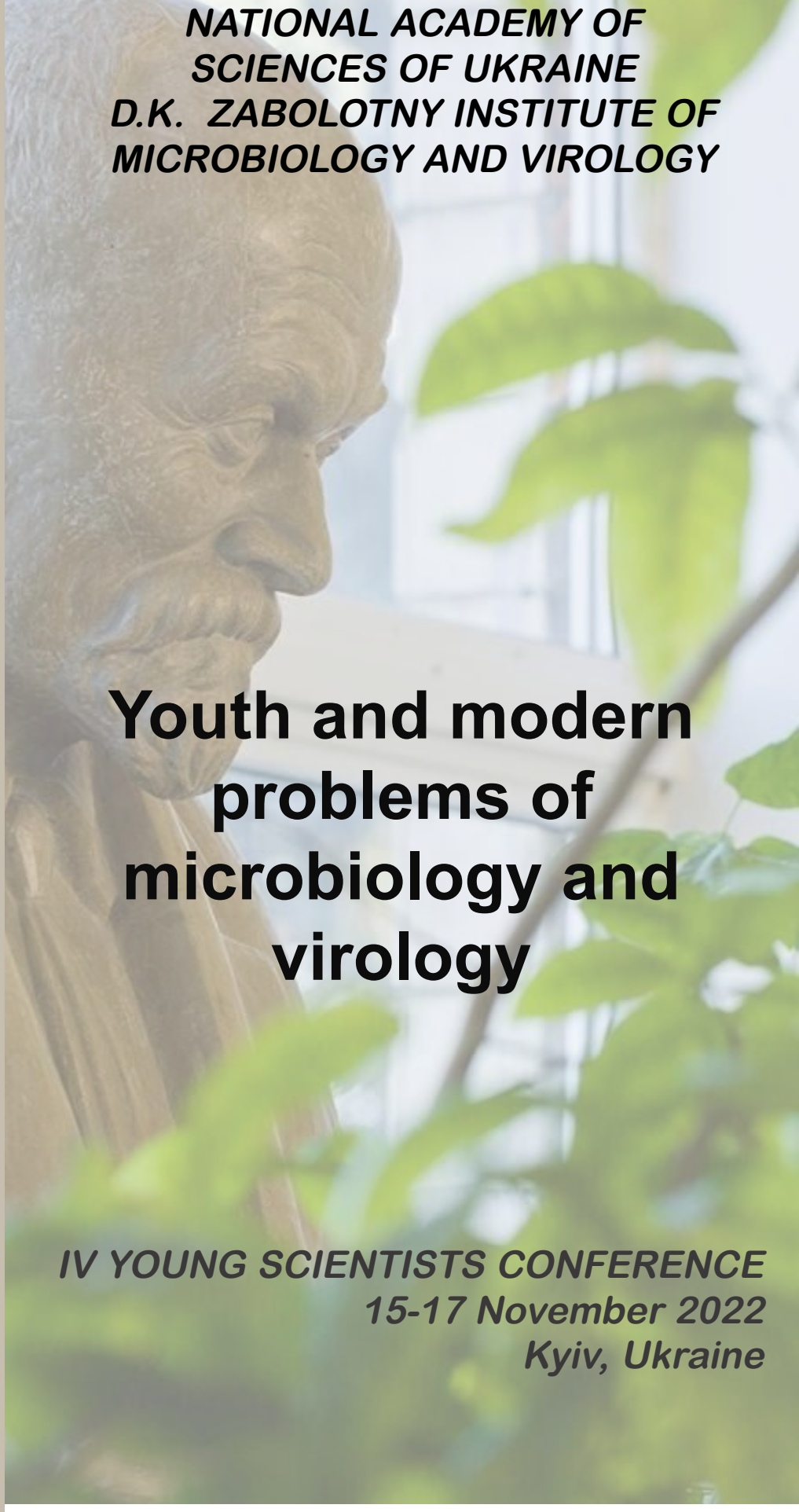

NATIONAL ACADEMY OF SCIENCES OF UKRAINE
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Youth and modern problems of microbiology and virology

IV YOUNG SCIENTISTS CONFERENCE
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"YOUTH AND MODERN PROBLEMS OF MICROBIOLOGY AND VIROLOGY"

IV YOUNG SCIENTISTS CONFERENCE

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DYNAMICS OF BIOMASS AND EXOPOLYSACCHARIDES PRODUCTION BY *XYLARIA POLYMORPHA* IN SUBMERGED CULTURE

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Xylaria polymorpha is one of the most widespread representatives of the largest genus in *Xylariaceae* family, which is known for the production of a wide range of bioactive compounds by its representatives. Latest studies have shown that due to the abundant production of a large variety of secondary metabolites, *Xylaria* fungi have potent pharmacological properties with antioxidant, antimicrobial, anti-inflammatory, antiviral, and other effects (Wangsawat et al., 2021; Jayasekara et al., 2022). Some of these activities, like antioxidant and antitumor, are connected with polysaccharides. The aim of our study was to investigate biomass accumulation and exopolysaccharide synthesis in submerged culture by two strains of *X. polymorpha* from the Mushroom Culture Collection (*IBK*) of the M.G. Kholodny Institute of Botany of the NAS of Ukraine.

Mycelia of studied strains were cultivated under submerged conditions in flasks containing 100 ml of glucose-yeast-peptone medium (g/L: glucose – 25, yeast extract – 3, peptone – 3, $MgSO_4$ – 0.25; KH_2PO_4 – 1; K_2HPO_4). The culture liquid was separated from mycelium by filtration on the 3rd, 5th, 7th, and 9th day of cultivation and evaporated using a vacuum evaporator at 40 ± 0.1 °C in 2-3 times, after which it was precipitated with 96% ethanol in the ratio 1:2 to volume for 24 h at 4 ± 0.1°C. The obtained fraction of exopolysaccharides was dried to constant weight at 60 ± 0.1 °C. The total amount of exopolysaccharides was determined gravimetrically.

The results demonstrated similar dynamics of biomass production for two *X. polymorpha* strains. For strain 2720 biomass accumulation grew from 2.9 to 9.74 g/L on the 5th and 7th days of cultivation, respectively. The maximum was recorded on the 9th day and amounted to 10.69 g/L. For strain 2736 analogical values were 4.72 and 8.7 g/L, respectively, with the slightly higher maximum of biomass production on the 9th day – 11.07 g/L. Almost no difference in the dynamics of exopolysaccharide synthesis was observed between two studied strains, and for both the maximum value 1 g/L was obtained on the 9th day of cultivation. The increase in the amount of exopolysaccharides was observed in the exponential phase of growth from 0.62 g/L on the 5th to 0.97 g/L on the 7th day for *X. polymorpha* 2720 and for *X. polymorpha* 2736 from 0.66 to 0.93 g/L, respectively. Meanwhile, it was noted that the pH value during cultivation dropped by 0.52 for strain 2736 and 0.36 for strain 2720.

Thus, the production of biomass and exopolysaccharides in submerged culture were determined for *X. polymorpha* strains as potential producers of important for biotechnological application compounds.

THE PRECIPITATION OF BIVALENT TOXIC METALS VIA THE DISSIMILATORY SULFATE REDUCTION

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Metal mining is the base for the economic development of any country, but it is hazardous for ecosystems. Heavy metals are released into the environment as a result of metal ore mining. It has caused significant environmental damage to soil and water resources. We offer a biological method based on dissimilatory sulfate reduction to solve the problem of metals precipitation. Such metabolic pathway as the dissimilatory sulfate reduction is promising to solve the problem of treatment of metal-contaminated ecosystems. In the process of sulfate reduction, microorganisms use sulfates as terminal electron acceptors. In this way, sulfates are reduced to sulfides, which precipitate a wide range of bivalent cations (Co^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Fe^{2+}) in the form of insoluble metal sulfides ($CoS\downarrow$, $NiS\downarrow$, $ZnS\downarrow$, $HgS\downarrow$, etc.).

The aim of the work was to investigate the patterns of Co^{2+} and Ni^{2+} precipitation by sulfate-reducing microorganisms during anaerobic fermentation of model protein waste. For this purpose, slightly soluble gypsum $CaSO_4$ (Klebrig, Czech Republic) was studied as an electron acceptor. Protein polymers (meat) and alanine (amino acid) served as electron donors. The meat was pre-cleaned and cut into 5 mm cubes. The methane tank sludge was sampled at the Bortnychi aeration station of Kyiv and used as a source of sulfate-reducing microorganisms (inoculum). The study of the bivalent cations precipitation via sulfate reduction was tested by the insertion of Co^{2+} and Ni^{2+} solutions into the culture medium to a final concentration of 100 mg/L. Cultivation was carried out in hermetic flasks (250 mL) at 32 ^{o}C. The metals concentration was determined by the colorimetric method with 4-(2-pyridylazo)resorcinol (PAR) (0,1%).

As a result of the study, the process of precipitation of bivalent metal cations by sulfate-reducing microorganisms was highly effective. The duration of complete Ni^{2+} precipitation in the medium with both alanine and meat was 25 days. The duration of Co^{2+} precipitation in the medium with alanine and meat was 23 and 20 days, respectively. Hydrogen was synthesized during the fermentation of model protein waste. The high concentration of H_2 in the gas phase (20-30%) indicates the possibility of using sulfate-reducing microorganisms for both the precipitation of toxic metals and for producing a high-energy carrier.

Thus, the high effectiveness of bivalent metals precipitation via dissimilatory sulfate reduction was experimentally confirmed. This approach can be used as a basis for the development of new biotechnologies for the treatment of soils and water reservoirs polluted by heavy metals with the simultaneous utilization of protein waste and biogas production.

ANALYSIS OF THE VARIABILITY OF GENES FOR HEMAGGLUTININ, NEUROMINIDASE AND NUCLEOPROTEIN OF AVIAN INFLUENZA STRAINS *h1n1* AND *h7n9*. IDENTIFICATION METHOD PCR COMBINED WITH RFLP

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The active evolutionary process in some groups of infectious agents, combined with the activation of migration flows in human populations, creates the prerequisites for the emergence and global spread of infectious diseases. At present, the spread of epizootics of highly pathogenic avian influenza in the world is of considerable concern. Multiple outbreaks caused by the *H5, H7,* and *H9* virus subtypes in wild birds have given rise to observations of avian influenza viruses in various regions around the world.

The aim of the research was to create a system for epidemiological monitoring of the circulation of a highly pathogenic avian influenza virus of the "wild" strain *h7n9* and *h1n1* based on the test systems developed by us for molecular diagnostics and genotyping.

Based on the analysis of hemagglutinin, neuraminidase, and nucleoprotein gene polymorphisms, variable pairs of oligonucleotides specific for the *H1N1* and *H7N9* viral subtypes were obtained. More than 8,000 influenza A, *NA*, and *NP* gene sequences of the *H1N1* and *H7N9* subtypes, allotted by 2017, were used for primer selection. The sequences were analyzed using Alignment Service and Lasergene *(version 6.0)*. The homology level of the selected primers is not less than 95%. The method involves conducting a polymerase chain reaction with RNA virus combined with the amplification reaction for three genes, analysis of the reaction mixture by agarose gel electrophoresis and detection of RNA strains of influenza A viruses by analysis. The first is the PCR with primers specific to the site of the influenza A hemagglutinin gene. The second reaction is RFLP with endonucleases specific for the hemagglutinin gene, neuraminidase and nucleoprotein regions. Identification of the strain *H1N1*, the samples of which form in RFLP analysis using good restriction enzymes to the gene *NP* unique products sizes 49-50, 348-350, 592-599 BP, others – fragment amplification sizes 21, 39, 201-203, 471-480 BP, identical to the products of RFLP analysis using good restriction enzyme strain *H7N9*. The *in silico* analysis of the *HA, NA* and *NP* gene amplicons allowed us to obtain theoretical PCR-RFLP electrophoregrams of the analysis, to calculate the reaction conditions, to determine restriction sites in matched restriction enzymes.

The developed method of express identification based on PCR combined with RFLP analysis makes it possible to significantly simplify the identification method due to the specific amplification of the RNA region with a polymorphic restriction site. The state testing of this locus is possible by preliminary PCR and restriction of the amplified fragment. It was established that the PCR-RFLP express diagnostic method was capable of detecting the RNA of influenza A virus of highly pathogenic *H1N1* and *H7N9* strains with high sensitivity indicators (100% sensitivity).

PHYSIOLOGICAL REACTION OF *COMAMONAS TESTOSTERONI* TO HEXACHLOROBENZENE

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The species of *Comamonas testosteroni* is widely spread in soils, activated sludge and seabed sediments. *C. testosteroni* can degrade xenobiotic compounds, such as chlorobenzenes, pentachlorophenol, using that chemical compounds as a carbon source. Hexachlorobenzene (HCB) was actively used in the 20th century as a fungicide to protect plants from phytopathogens. Also, HCB is a waste of textile, dyeing, rubber and other industries that accumulates in the soil. Due to its high persistence, it remains in the soil for years. The search for biologically active destructors of HCB is promising. The HCB effect on the cellular lipids remains almost unexplored. We isolated and identified *C. testosteroni* UCM B-400 and B-401 strains from the HCB landfill. Therefore, **the main goal of the study** was to determine the lipids peroxidative products in *C. testosteroni* UCM B-400 and B-401 strains in response to HCB toxicity, as well as the catalase and peroxidase activity.

Methods. *C. testosteroni* B-400 and B-401 strains were grown in a mineral LB medium (pure control) and with the addition of 10 mg/L and 20 mg/L of HCB. The lipid peroxidation products and the enzyme activities of antioxidant system were determined spectrophotometrically.

Results. Primary peroxidation products – diene conjugates under 20 mg/L HCB were higher up to 2 times for *C. testosteroni* UCM B-400 strain and up to 8 times for *C. testosteroni* UCM B-401 strain, compared to pure control. Malondialdehyde in B-400 strain cells decreased up to 5 times, but increased up to 2 times in B-401 strain, compared to pure control. Schiff's bases amount in B-400 strain cells was 2–3 times lower than in pure control. However, Schiff's bases amount in B-401 strain cells under higher HCB dose was at the same level as in pure control. Catalase activity was 1.5 times higher in all experimental variants, compared to the pure control (in B-401 strain cells), but in was 2 times lower in B-400 strain cells, compared to the pure control. The response of the two strains to HCB was similar only in peroxidase activity terms, which was slightly higher, compared to the pure control.

Conclusions. The reaction of both strains to the HCB presence differed in the content of diene and triene conjugates, malondialdehyde, as well as different catalase and peroxidase activity levels. Catalase activity is one of the key of adaptation processes to toxic conditions, which was more pronounced for *C. testosteroni* UCM B-401 strain. The level of physiological response of *C. testosteroni* UCM B-400 and B-401 strains confirms their tolerance to HCB, and indirectly – the ability to destroy the specified toxic compound.

INFLUENCE OF ADAMANTANE DERIVATIVE ON THE FORMATION OF *PSEUDOMONAS AERUGINOSA* PERSISTER CELLS

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Introduction. Today, the problem of treating patients with chronic infections caused by biofilms, the structured microbial communities, remains relevant. One of the reasons for the ineffectiveness of antimicrobial chemotherapy in patients with biofilm infections is the presence of metabolically inactive persister cells in the biofilm, which are characterized by resistance to antimicrobial drugs. Most often, persister cells are formed under the action of antibiotics that affect the metabolic processes of bacteria, including fluoroquinolones, aminoglycosides, etc. Our previous studies have shown that compounds with adamantyl radical exhibit antibiofilm activity against *P. aeruginosa* [Vrynchanu, N., 2007].

Objectives. The objective is to evaluate the effect of a compound with an adamantyl radical on the formation of *P. aeruginosa* persister cells.

Materials and Methods. The 4-(1-adamantyl)-(1-aminobutyl)-benzene (code AM-166) compound and ciprofloxacin as a reference preparation was used in the study. Studies were performed on a clinical isolate of *P. aeruginosa* 449 that was resistant to cefepime and cefotaxime, moderately susceptible to ceftazidime and aztreonam, susceptible to meropenem, ciprofloxacin, gentamicin, and amikacin. The activity of the compound was investigated at concentrations of 15 μg/mL and 250 μg/mL (minimum inhibitory concentration (MIC) was 100 μg/mL). The presence of persister cells in the population of *P. aeruginosa* under the action of the adamantane-containing compound was determined according to [Marques C.N.H., 2015, Chen C.Y.A., 2003]. Statistical analysis of the data was performed using the ANOVA method.

Results and Discussion. In previous experiments, it was found that the compound AM-166 exhibits antibiofilm activity: at a concentration of 250 μg/mL the biomass of the biofilm is 23.4 % as compared to the control, which may be due to the formation of metabolically inactive persister cells. The obtained data on the formation of persister cells under the influence of AM-166 showed that at the concentration of 250 μg/mL, the proportion of the formed subpopulation of persister cells was 0.016 %, which is probably lower compared to ciprofloxacin (0.9 %, $p<0.05$). At the concentration of 15 μg/mL, all cells were in the persister state.

Conclusions. Thus, the 4-(1-adamantyl)-(1-aminobutyl)-benzene compound (250 g/mL) does not prevent the formation of *P. aeruginosa* persister cells, but their number is less than under the action of ciprofloxacin.

SIMPLE EDUCATIONAL MODEL OF MICROBIAL FUEL CELL

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Microbial electrogenesis is considered to be one of the alternative methods of energy obtaining in bioenergetics. This process is well studied in scientific communities, but it is limited in educational field. Commercial devices, called microbial fuel cells (MFCs), were created to raise attention to this topic. However, it is essential to create a simple educational model of the microbial fuel cell for the widespread popularization of this technology.

The aim of this work was to create several examples of microbial fuel cells for educational purposes. We propose to combine microbial electrogenesis approach with the demonstration of such crucial microbiological processes as alcoholic fermentation, lactic acid fermentation, and microbial diversity of lake mud.

Each MFC model consists of two chambers made of plastic pumps – cathode (aerobic) and anode (anaerobic), with immersed electrodes (nails) and cooper wires fixed on them inside. Sets of chambers were connected by electron-permeable bridge. All cathodic chambers were filled with 140 ml of boiled water, previously cooled to room temperature. Contents of anodic chambers are the following:

- Alcoholic fermentation: 140 ml of boiled water, 5 tsp. of sugar and 1 tsp. of dried yeast;
- Lactic acid fermentation: 140 ml of yogurt with 1.6% of fat without fillers and sugar;
- Lake mud microbial consortium: 140 ml of mud taken from the local lake.

Electron-permeable bridge was filled with agar gel (10% of agar and 10% of sodium chloride in water). To create anaerobic conditions, anodes must be sealed with electrical tape before connecting them with cathodes. The value of the voltage on each MFC was measured every 35 minutes using a multimeter. Results were different for each model. In the first, the voltage initially decreased due to aerobic chamber leakage, but after refilling, the voltage increased due to active electrogenesis. In the second, the voltage decreased at the beginning, but as yogurt warmed, its value started to increase. In the third, voltage decreased due to the adaptation of consortium to new conditions, but under light exposure, bacteria started to divide, increasing voltage.

Three ideas for educational MFCs were proposed. Positive results of their application were obtained. Several advantages of these models can be identified as the low cost of materials needed for their construction and the serial connection of all chambers may produce more voltage.

METABOLISM OF *LACTOBACILLUS*, *BIFIDOBACTERIUM* AND *BACILLUS* PROBIOTIC STRAINS IN THE PRESENCE OF CeO_2 NANOPARTICLES IN THE CULTIVATION MEDIUM

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The use of nanotechnology products, in particular nanocrystalline cerium dioxide, in the creation of probiotic preparations could increase their effectiveness by changing the metabolic activity of probiotic microorganisms. Understanding the mechanisms of the stimulating effect of nanoparticles (NP) on microorganisms is possible only by studying their metabolic profiles. Therefore, the purpose of our work was to study the metabolic profiles of probiotic strains of microorganisms in the presence of CeO_2 nanoparticles in the culture medium.

The objects of the study were 8 strains of probiotic bacteria of *Lactobacillus*, *Bifidobacterium,* and *Bacillus* genera. Cultivation of microorganisms was carried out with the addition of different concentrations (2.5 μg/L; 25 μg/L; 250 μg/L) of CeO_2 NP. Metabolites from cell biomass were extracted with a mixture of methanol/chloroform. The qualitative composition of trimethylsilyl derivative metabolites was studied by gas chromatography-mass spectrometry on an Agilent 6890N/5973inert device ("Agilent Technologies", USA) using an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) ("J&W Scientific", USA).

It was shown that CeO_2 NPs significantly influenced the metabolic profiles of the studied probiotic strains, changing the quantitative content of 48-82 % of the total amount of all endometabolites of the strains. A significant effect of CeO_2 NP on the synthesis of organic acids, in particular lactic, and a negative effect on the synthesis of a number of amino acids – asparagine, alanine, phenylalanine, and tyrosine – was noted. It was established that the content of lactic acid is positively correlated with the content of isoleucine, citric, and phenyllactic acids, and negatively – with valine. According to the nature of the correlation patterns, the investigated strains were divided into two groups: the first group included *L. plantarum, L. acidophilus*; *L. rhamnosus*, *L. casei* strains, to the second – *L. delbrueckii*, *B. animalis* strains. A feature of the second group of strains is the predominance of positive dependencies in correlation patterns. This phenomenon is probably related to the features of the primary metabolism of strains of the second group.

So, we have shown that in the presence of CeO_2 nanoparticles, the studied strains have their original patterns of metabolites. Patterns of correlation of lactic acid content with other endometabolites of probiotic strains were revealed.

BIOFILM FORMATION AS A KEY FACTOR OF ANTIBIOTIC RESISTANCE IN *MYCOBACTERIUM TUBERCULOSIS*

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Relevance: Biofilm formation is identified as a key factor of antibiotic resistance (ABR) in *Mycobacterium tuberculosis* due to slow or incomplete penetration of antibiotics into the biofilm. Current treatment for tuberculosis (TB) entails the use of isoniazid, rifampicin, pyrazinamide, streptomycin, and ethambutol, for a minimum of 6 to 9 months, leading to multidrug-resistant TB (MDR-TB). Ukraine is a country with a high burden of MDR-TB and war only aggravates the world's most serious MDR-TB epidemic.

Objective: Comparative analysis of the incidence of TB in Ukraine, the EU/EEA, and the US for 2010-2020 and the search for scientific articles on M. tuberculosis biofilm-specific genes concerning ABR.

Methods: Statistics on TB incidence, mortality, and MDR-TB from 2010 to 2020 in Ukraine, the EU/EEA, and the US were obtained from official reports and open sources. Statistical processing of information was carried out using the Microsoft Office Excel 2010 program. Polynomial regression (PR) was used to forecast trends in the incidence of MDR-TB in Ukraine for the years 2021-2023.

Results: A decrease in the incidence of TB was established in Ukraine, the EU/EEA, and the USA, respectively – in 2020, 73/9.2/2.7 new cases per 100,000 population were registered, against 110/15/3.6 in 2010. But the rate of MDR-TB in Ukraine increased – in 2020, 23.8 cases per 100,000 population were registered, against 18.3 in 2010. PR showed that MDR-TB in Ukraine may exceed 23.8 and approach 24.7, 25.8, and 27.0 within the years 2021-2023. The incidence rate of MDR-TB in the EU/EEA and the US remained stable.

Biofilm-specific genes, in particular *Ppib* (codes for Ppib), *Rv1284, Rv3588c,* and *Rv3273* (code for carbonic anhydrase (CA) β-1, β-2, and β-3), are important for drug repurposing. Ppib inhibitors, including cyclosporine-A, acarbose, and gallium nanoparticle, inhibited biofilm formation and caused a 2-4 fold decrease in the dosage of isoniazid and ethambutol. CA inhibitors include sulfonamides, mono- and dithiocarbamates, phenolic natural products, and phenolic and carboxylic acids.

Conclusions: The incidence of MDR-TB in Ukraine is increasing, which prompts doctors and pharmacists to look for new or alternative drugs. Biofilm-specific protein inhibitors, namely CA inhibitors and Ppib inhibitors, are a valuable source of drug repurposing targets.

IRON IMMOBILIZATION VIA METHANE FERMENTATION OF *SOLIDAGO CANADENSIS* INVASIVE PLANT

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The accumulation of toxic metals in the environment, particularly iron, is a significant environmental problem. The spread of harmful weeds, in particular *Solidago canadensis* (Canadian goldenrod), in bio- and agrocenoses is another ecological problem. We consider *Solidago* plants to be a promising and free renewable substrate for the production of methane gas and iron detoxification. In this way, we solve three problems of a global level, the lack of energy carriers, metals detoxification as well as the utilization of ecologically hazardous *S. canadensis* plant.

In this regard, the aim of the work was to study the microbial methods of iron immobilization by methanogenic microorganisms and methane production.

The optimal pathways of iron detoxification by methanogenic microbiome were substantiated by the thermodynamic prognosis method. Dried plant material of *S. canadensis* weed was used as a substrate for the methane fermentation and iron immobilization. The methane tank sludge was used as the inoculum to ferment. Fermentation was carried out for 3,5 months at 30 °C. The Fe(III) solution was added to the bioreactor during the fermentation process to final concentrations of 100 and 200 mg/L. The concentration of Fe(II) and Fe(III) was determined spectrophotometrically via the reaction with *o*-phenanthroline for Fe(II) and potassium rhodanide under acidic conditions for Fe(III).

According to the thermodynamic prognosis, anaerobic methanogenic microorganisms are able to immobilize toxic iron compounds with high effectiveness due to the precipitation of Fe(III) as a result of increasing the pH of the medium during methanogenesis as well as due to the precipitation of iron ions by S^{2-} formed during microbial sulphate reduction that accompanies methanogenesis.

The high efficiency of iron immobilization was substantiated theoretically and confirmed experimentally. The effectiveness of iron immobilization at the concentration of 100 mg/L was 100 % within 7 days. An increase of Fe(III) concentration to 200 mg/L inhibited the metabolic activity of microorganisms. Despite this, the effectiveness of Fe(III) immobilization was also 100% with the duration of 50 days. Microorganisms adapted to such extreme conditions, completely immobilized soluble iron compounds and synthesized methane, the concentration of which was 50-60% in the gas phase.

Thus, the high efficiency of toxic iron immobilization and methane production by methanogenic microorganisms during the fermentation of *S. canadensis* plant was experimentally confirmed. The obtained results are promising for the development of environmental and energy biotechnologies.

EVALUATING THE IMPACT OF TOXICITIES IN HEMICELLULOSIC HYDROLYSATES DURING BIOTECHNOLOGICAL VALORISATION PROCESSES

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Transition of economy towards sustainability requires efficient use of available resources. Hemicellulose hydrolysate is an example of such substrate as it is byproduct of many agro-industrial sectors as ethanol, pulp and paper, beverages production and, until this day, no efficient utilization has been established. One of the promising approaches is to use hemicellulose hydrolysates as a main carbon source for microbiological production to obtain high-value products. However, hemicellulosic hydrolysates contain inhibitory compounds formed during the hydrolysis processes, namely, 5-hydroxy methylfurfural (HMF), acetic acid, phenols. We have identified a non-conventional yeast *Rhodotorula toruloides* as a potential cell fatory to valorise hemicelluosic hydrolysates while naturally producing significant amount of microbial lipids and carotenoids.

Our aim was to investigate the ability of *R. toruloides* to grow in media comprising hemicellulosic hydrolysates of birch wood as a main source of carbon with different concentrations of total sugars and inhibitors. Hence, 5 batches of industrially produced hemicellulosic hydrolysates (Fibenol OÜ, Estonia) by different evaporation levels were used as a carbon sourceand diluted to the final total concentration of sugars of 70, 85, 105, and 120 g/L. Optical density and CO_2 release were monitored together with sugar consumption and level of acetic acid.

The growth and sugar consumption of *R. toruloides* showed strong reverse correlation with the concentration of undissociated form of acetic acid (HAc) present in hemocellulosic hydrolysate ($R^2 = 0.8$). Additionally, HMF showed a significant, although not as strong inhibiting effect on the growth of *R. toruloides*. Increase of total amount of sugars in media did not show inhibitory effect in our studied concentration range.

Obtained results demonstrated an inhibitory effect of acetic acid on growth of *R. toruloides* with a threshold around 1 - 1.1 g/L of undissociated form of the acid. To avoid inhibitory effect of acetic acid, starting pH should be increased to 6.0 which will decrease concentration of undissociated acetic acid, hence, remove an inhibitory effect. The results obtained with this work established guidelines for the process development of both the hydrolysate production and the microbial cultivation steps.

THE RESISTANCE OF TOMATO PLANTS MICROCLONES *LYCOPERSICON ESCULENTUM* MILL. OBTAINED BY USING OF METABOLIC BIOFORMULATION OF STREPTOMYCES ORIGIN AGAINST PARASITIC NEMATODES

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Tomato is a commercially useful agricultural plant. Its low tolerance against pathogens and parasites is an obstacle in obtaining of high-quality harvest, and parasitic nematodes are one of the widespread harmful reasons of it. Therefore, a complex approach of obtaining of tomato plants microclones resistant against them is relevant. The widespread use of streptomycetes in agriculture is connected with their ability to synthesize a complex of biologically active substances (BASs) which suppress phytopathogens and parasites, increase plants resistance against stresses. **The aim** of the research was to prove the effectiveness of the use of Phytovit bioformulation based on streptomycete metabolites to obtain tomato plants microclones *Lycopersicon esculentum* mill., resistant against nematodes.

Phytovit is a complex metabolic bioformulation which consists of ethanolic extract of *Streptomyces netropsis* IMV As-5025 biomass and culture liquid. It inhibits phytopathogenic fungi and bacteria and provides a priming effect for plats indirectly through other BASs. Averkom is based on *Streptomyces avermitilis* IMV Ac-2179 biomass extract and culture liquid and was used as a positive control. It contains Avermectin with antiparasitic properties, and other BASs. They were constructed at the D.K. Zabolotny Institute of Microbiology and Virology, NASU. Microclonal reproduction was carried out at Institute of Food Biotechnology and Genomics, NASU. Bioformulations were added in nutrient medium and thent plants were grown in vegetative conditions without further treatment by them.

The decrease of the amount of parasitic nematodes in the roots and rhizosphere of tomato plants of the 1st generation under the influence of Phytovit and Averkom was shown by nematological analysis. Bioformulations showed high biological efficiency against *Ditylenchus dipsaci*, *Pratylenchus pratensis*, *Helicotylenchus dihystera*, *Paratylenchus nanus*) in the roots, which was 84.92%, 40.87%, and 57.14%. Their effectiveness against *Paratylenchus nanus* was 100%. An increase of stem length by 29% was observed. The amount of tomato fruits was 2-fold increased, and the weight of them from one plant – 3.3-fold under the action of 75 µl/mL of Phytovit. This resistance was also preserved for the 2nd generation of plants, which indicates the prolonged effect of bioformulations.

Summary. The efficacy of soil streptomycete metabolites for *in vitro* obtaining tomato plants microclones *L. esculentum* Mill resistant against parasitic nematodes was shown for the first time. It was associated with the induction of plant systemic resistance by streptomycetes secondary metabolites.

Key words: *Streptomyces netropsis*, Phytovit, bioformulation, biologically active substances, microclones, nematodes.

THE SELECTION OF RESISTANCE TO ERYTHROMYCIN IN CLINICAL STRAINS OF *STAPHYLOCOCCUS EPIDERMIDIS* AND THE INFLUENCE OF 50% ETHANOLIC RUTA GRAVEOLENS L. EXTRACT ON THE RATE OF ITS DEVELOPMENT

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Skin pathogens are characterized by a high level of antibiotic resistance, especially to antimicrobials of MLS-group. Usually, it happens due to the frequent use of erythromycin (ERY) as a treatment for skin diseases.

In our study, six clinical isolates of *S. epidermidis* with different susceptibility to ERY were used: 3 strains with intermediate susceptibility (MIC 62,5-125 µg/mL) and 3 susceptible strains (MIC 0,5-1 µg/mL). The study of the rate of ERY-resistance development for staphylococcal strains alone and in combination with 1/4 MIC of 50% ruta herb extract was performed by serial dilutions in MH broth. Selection of resistance was performed in two rows of test tubes: the first row included tubes with 3 double dilutions below and 3 double dilutions above the MIC of ERY, to the second row - 1/4 MIC of the investigated extract was added. After incubation at 37^{o}C for 24 hours, the aliquots from the last tubes with the highest antibiotic concentration, where visible bacterial growth was observed, were taken, diluted 1:100, and inoculated into the second set of serial dilutions. Such procedure was repeated for 30 days. After every fifth passage test strains were identified and the MIC of ERY was determined.

Susceptible strains of staphylococci quickly developed resistance to ERY and its MICs after the last passage were 4000-8000 µg/mL. Investigated extract slowed rates of antibiotic resistance development in all susceptible strains and after the last passage MICs of ERY were 250-500 µg/mL. MICs of ERY after the last passage for strains with intermediate susceptibility were 2000 µg/mL and MICs of ERY from tubes with extract were 1000-2000 µg/mL.

All *S. epidermidis* isolates have developed resistance to ERY after 30 passages. The rate of antibiotic resistance differed between investigated strains. In susceptible strains, MICs of ERY were increased 31-62 times after 10 passages, 2000-4000 times after 20 passages, and 4000-8000 times after 30 passages. To compare, MICs of ERY for the same strains incubated with 50% of ruta herb extract were increased 31, 125-250, and 250 times after 10, 20, and 30 passages, respectively. For strains with moderate susceptibility to ERY, drug MICs increased 8-16 times after 10 passages and stopped at that rate until the end of the experiment. Besides, there was no difference between the results for samples with investigated extract and without.

There is a dependence between the initial susceptibility of tested microbial isolates to ERY and the rate of resistance development to it. Ethanolic ruta extract decreased the rate of resistance development only in strains that were susceptible to ERY.

ANTAGONISTIC ACTIVITY OF LACTIC ACID BACTERIA AGAINST YEAST ISOLATED FROM FLOUR

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Baking is a significant part of the food industry. The quality of produced food products depends strongly on the leavening agents added to the dough, as well as on the microorganisms of the flour itself. The microbiota of flour is mainly represented by lactic acid bacteria (LAB), which provide organoleptic properties, and yeast, which give structure to bread. Modern trends towards a healthy lifestyle have led to a demand for bread made using sourdough starters comprising LAB. The interaction of bread sourdough cultures and wild microbiota of flour determines the degree of control over the technological process.

The aim of the work was to investigate the interaction between strains of lactic acid bacteria - candidates for sourdough production, and strains of wild yeast isolated from rye flour.

Materials and methods. In this work, we used 56 strains of LAB of the genera *Leuconostoc* (19 strains), *Lactobacillus* (36 strains), and *Enterococcus* (1 strain). The antagonistic activity of LAB was investigated using well method; growth inhibition zones were measured after 24 hours of cultivation at 30°C. Test cultures were represented by two strains of wild yeast that were previously isolated from rye flour. The role of organic acids was evaluated by neutralizing culture fluid. The chemical nature of substances with antagonistic action was investigated by treating the culture fluid with catalase, lipase, α-amylase, and proteolytic enzymes. The protein fraction of the culture liquid was obtained by salting out with ammonium sulfate (60% saturation).

Results. As a result of the screening, 17 strains of LAB were selected, which are producers of metabolites with antagonistic action against two strains of wild yeast isolated from rye flour. The antagonistic activity was preserved in the neutralized culture liquid, therefore, it is not caused by the action of organic acids. Enzymatic processing revealed the protein nature of the antagonistically active metabolites of the culture liquid of LAB strains. This was confirmed by the study of the antagonistic activity of the protein fractions of the culture liquid The mean growth inhibition zone on the yeast test cultures was 14.1 ± 1.6 mm. The protein fraction of the *L. fermentum* c215 strain had the smallest zone of growth inhibition (11 mm), *Leuconostoc mesenteroides* 23ap strain had the largest (16.5 mm).

Conclusions. We have been identified LAB strains producing protein metabolites, possibly bacteriocins or bacteriocin-like substances that inhibit the growth of wild yeast strains. The use of selected cultures of the LAB has a positive effect on the controllability of the bread-making process by suppressing the development of unwanted wild flour microbiota.

SCREENING OF PHYTOPATHOGENIC BACTERIAL STRAINS CAUSING BACTERIAL DISEASES OF LENTIL IN UKRAINE

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Bacterial diseases of lentil have not been studied much in Ukraine. This plant is quite widespread and used in our country, but the study of the causative agents of bacterial diseases of this plant began relatively recently. Lentil is the most protein-rich crop among legumes. It is on the second place after peas and soybeans in terms of consumption volumes. Lentil is quite actively grown in Ukraine because of favorable climate and competitive advantage, but the number of lentil diseases caused by plant pathogenic bacteria is increasing. That can lead to serious losses of crop yield and financial loss.

The aim of the work was to isolate and identify phytopathogenic bacteria that cause lentil diseases among different varieties. In this work, the following subjects were used: seeds from three lentil varieties "Darinka", "Linza" and "Serpanok", pathogenic isolates from lentil seeds and reference strains of phytopathogenic bacteria for comparison *Pseudomonas syringae* pv. *syringae* УКМ В-1027т= IMB B-8511, *P. syringae* pv. *syringae* УКМ В-1027т = В-8414, *P. savastanoi* pv. *phaseolicola* IMB B-9066, *P. savastanoi* pv. *glycinea* IMB B-9190 and *Pectobacterium carotovorum* УКМВ-1075т =IMB-B-8982.

The biochemical and microbiological methods were used in this research. In the course of the experiment, we isolated five main isolates that had pathogenic properties which were tested by artificial infection under greenhouse conditions. Strains 1 and 9 were the most aggressive and caused similar symptoms which included chlorosis, yellow and brown splotches in injection marks and twisted leaf.

Strains 1 and 9 were isolated from the lentil cultivar "Linza". It should also be noted that the majority of pathogenic isolates which were morphologically similar to the studied strains were also isolated from this cultivar. It means that this cultivar has low resistance to bacteriological diseases.

Since one of the strains was similar to the genus *Pseudomonas* according to biochemical tests, additional serological tests were performed. As a result, it showed the greatest similarity with the reference strain *P. savastanoi* pv. *glycinea*.

Thus, according to the results of morpho- cultural, physiologo-biochemical and serological tests, two isolates were assigned to the genus *Pseudomonas sp*. (strain 9) and *Pectobacterium sp*. (strain 1).

THE STUDY OF YEAST-LIKE FUNGI ON THE ORAL MUCOSA OF PATIENTS WITH SJÖGREN'S SYNDROME

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Topicality. The frequency of yeast-like fungus colonization of oral mucosa (OM) in patients with Sjögren's syndrome constitutes 40.0 ± 1.63%. Fungi are detected on the mucous membrane of the tongue more often (36.7 ± 1.61%) than on the mucous membrane of the gums (23.3 ± 1.41%).

The research objective was to study the level of candida colonization of various OM areas in patients with Sjögren's syndrome.

Materials and methods of the research. 30 patients with Sjögren's syndrome associated with rheumatoid arthritis underwent our observation. The comparison group included 15 apparently healthy individuals. The microscopic method was applied to search for pseudomycelium and some candida cells in impression smears from different OM areas, namely gums, tongue, and cheeks. Impression smears were stained according to the Romanovsky method. The samples were collected with a sterile cotton ball from tongue and gums mucosa with an area of 1 cm^2 separated by a special stencil. The cotton ball was placed in a test tube with 1.0 ml of a sterile normal saline solution where the selected mucus was carefully resuspended. Fungus cultures were separated on chromogenic medium ChromID Candida (bioMerieux, France). The final identification of yeast-like fungus cultures was conducted on the basis of 40 biochemical tests with the use of VITEK 2 system using VITEK 2 YST ID card (bioMerieux, France).

Results of the research and their discussion. In oral mucosa of the patients with Sjögren's syndrome, we identified 14 strains (56.0 ± 1.98%) of *Candida albicans (10 patients)*. Three strains of *C. kefyr (Kluyveromyces marxianus)* were isolated from 2 patients (12.0 ± 1.30%), two strains of *C. lipolytica (Yarrowia lipolytica)* were isolated from 2 patients (8.0±1.09%). One strain of *C. tropicalis* (4.0 ± 0.78%) and two strains of *Cryptococcus laurentii* were isolated from different biotopes of the same patient (8.0 ± 1.09%). Four strains of *C. lusitaniae* were isolated from 3 patients (16.0±1.47%). It should be noted that we detected certain species of yeast-like fungi (in particular, *C. lusitaniae, C. lipolytica, C. laurentii*) on OM of the patients with Sjögren's syndrome for the first time.

Conclusions. 1. The frequency of yeast-like fungus colonization of oral mucosa (OM) in patients with Sjögren's syndrome constitutes 40.0±1.63%.
2. Some species of yeast-like fungi (*C. lusitaniae, C. lipolytica, C. laurentii*) were detected on OM of the patients with Sjögren's syndrome for the first time.

INFLUENCE OF METABOLIC PRODUCTS OF *LACTOBACILLI* PROBIOTIC STRAINS ON CELL CYCLE OF EUKARYOTIC CELLS

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The normal human intestinal microflora performs a number of important functions. One of the fundamental ones is the creation of colonization resistance, a kind of barrier on the mucous membrane of the digestive tract. With the emergence and development of dysbiotic disorders in the intestines, there is a decrease in colonization resistance, which leads to an increase in susceptibility to infectious diseases. The leading approach correct these disorders is the use of probiotics on the basis of bacterial strains - characteristic representatives of normal intestinal microflora, which have the ability to positively influence the human body, restoring and stabilizing the balanced composition of the microbiocenosis of the gastrointestinal tract. The purpose of the study was to clarify the influence of metabolites of probiotic strains of lactobacilli on the cell cycle of eukaryotic cells.

The following biological objects and reagents were used in the study: surface-dependent cell line Hep-2; exogenous metabolites of lactobacilli strain *Lactobacillus delbrueckii subsp. lactis*; tumor necrosis factor TNF-α. Cell cycle parameters were assessed in dynamics of cultivation using Annexin V-FITC and flow cytometry method.

Results showed that metabolites of lactobacilli do not affect the cell cycle parameters of cell culture. It was shown that, compared to the control culture, the number of proliferating cells under the effect of TNF-α decreased, and with the combined effect of TNF-α and the preparation of lactobacilli metabolites, the number of proliferating cells normalized regardless of the age of the culture. Metabolites stimulate the proliferative activity of young, mature and old cells, both individually and in combination with TNF-α. It was found that the number of cells in the late stage of apoptosis in a young cell culture was reduced under the influence of TNF-α or lactobacillus metabolites. At the same time, as the degree of culture maturity increased, the number of late-apoptotic cells increased, that is, apoptotic changes became irreversible. With the simultaneous effect of TNF-α and the preparation of metabolites of lactobacilli, the number of cells at the stage of irreversible apoptosis is significantly higher than the control indicators.

In general, it allowed to visualize the model of changes in the life cycle of epithelial cells when using preparations based on known probiotic strains of lactobacilli and can be used in further studies.

RETROVIRAL FACTOR (HIV) IN THE PATHOGENESIS OF KAPOSI'S ANGIOSARCOMA

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Relevance. Viruses are one of the factors that interact with the cell of a living object, turning it into a tumor. This type of virus is called oncogenic. Representatives of this type of pathogens are present in almost every family of viruses. The largest list of oncogenic viruses consists of the following families: hepadnaviruses, herpesviruses, retroviruses, papillomaviruses, and others. Any of the listed types of viral pathogens provoke the development of dangerous oncological pathologies that can cover the cytological and histological structures of a wide variety of organs, blood cells, and tissue groups. Cancer cells are divided into several types: carcinoma, leukemia, sarcoma, lymphoma, glioma. Kaposi's sarcoma is one of the most dangerous and understudied oncological pathologies caused by an oncogenic virus.

Aim. To determine the role of the retroviral factor on the example of HIV in the progression of Kaposi's angiosarcoma in the body of people infected with herpes virus type 8.

Methods: Analytical and statistical information processing methods, system-structured and comparative methods were used.

Results. Kaposi's sarcoma is a malignant tumor that covers the walls of blood vessels. It has a pathophysiological characteristic in the form of painless points on the surface of the integument of red, purple, or brown color. Also, the disease can cover mucous membranes and individual components of internal organs. According to research, Kaposi's sarcoma occurs 300 times more often in HIV/AIDS patients than in surgical interventions and transplant procedures. Patients who had HIV and HHV-8 at the same time developed this pathology for 10 years. The percentage of people who simultaneously had two viruses in their composition, as a result of which this pathology progressed, was 45%. The main pathogenic agent of Kaposi's sarcoma is oncogenic herpes virus type 8 (HHV-8). It is a DNA-containing pathogen from the herpesvirus family. Its negative impact in the form of oncopathology is manifested as a result of a persistent decrease in the functioning of the immune system or possible cases during treatment measures that lead to its gradual decrease (organ transplantation, chemical or radiotherapy measures). One of the main causes in pathogenesis Kaposi's sarcoma is the human immunodeficiency virus (HIV). The role of retroviral infection in the pathogenesis of Kaposi's sarcoma is determined by the neutralization of immune T cells, thereby minimizing their concentration in the patient's leukocyte formula. As a result, the herpes virus is activated and begins to manifest itself. It also has the ability to remain in a latent state in lymphocytes throughout the entire period of post-embryonic ontogenesis of a person. It is also noted that Kaposi's sarcoma was a rare disease before the large-scale HIV pandemic and occurred in 6% of cases per 100,000 infected with HHV-8. With the gradual geographical spread of HIV, the frequency of recording a dynamic outbreak of Kaposi's sarcoma in patients with herpes virus type 8 has increased by 20-25%.

Conclusions. The retroviral factor in the oncogenesis of Kaposi's sarcoma is its main cause. HIV provokes a decrease in the T-cell link of immunity, as a result of which the accompanying virus HHV-8 gets the opportunity to produce a dangerous oncological pathology.

STUDY OF SYNERGISM OF FLUCONAZOLE WITH THIAZOLIDINE DERIVATIVES

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Nowadays, fungal diseases are the 5th most common among all human infectious diseases. Etiologically, fungal diseases are most often represented by such species as *C. albicans* and *C. tropicalis*. Antifungal drugs are used to treat this type of infection, but more and more information is being received about the resistance of fungi to traditional drugs. To solve this problem, research is being conducted on new alternative compounds and their combination with common drugs.

The aim of our study was to reveal the antifungal properties of new synthetic compounds - thiazolidine derivatives and their effectiveness in combination with fluconazole.

A total screening for the antifungal activity of 330 compounds - thiazolidine derivatives was carried out using the agar diffusion method. The screening results revealed 3 active compounds - 6-oxo-5,6-dihydro [1,3]thiazolo [2,3-b][1,2,4]triazol-6-one derivatives - L95, L1369, and L1558, which then were used to study synergism with fluconazole by the method of serial dilutions in 96-well microplates. Clinical strains *C. albicans* and *C. tropicalis* were used as test strains. The results were evaluated by determining the minimum inhibitory concentration (MIC) of substances.

For *C. albicans*, MIC of L95 was 50 μg/ml, MIC of L1369 - 100 μg/ml and MIC L1558 - >100 μg/ml. For the *C. tropicalis*, MIC of L95 was 25 μg/ml, MIC of L1369 - 25 μg/ml and MIC of L1558 - 25 μg/ml. MIC of fluconazole for *C. albicans* was 1000 μg/ml and for *C. tropicalis* was 4000 μg/ml.

While in combination with fluconazole (1/8 of MIC), MIC of L95 was 25 μg/ml, MIC of L1369 - 100 μg/ml and MIC L1558 - 50 μg/ml for *C. albicans*. For *C. tropicalis* - in combination with 1/8 MIC of fluconazole, MIC L95 is 3.125 μg/ml, MIC L1369 - 6.25 μg/ml and MIC L1558 - 12.5 μg/ml.

Even better results were shown in the combination of MIC of fluconazole with 1/8 MIC of substances. For *C. albicans*, MIC of fluconazole in combination with 1/8 MIC of L95 was 16 μg/ml, with 1/8 MIC of L1369 - 125 μg/ml, with 1/8 MIC of L1558 - 125 μg/ml. For *C. tropicalis*, MIC of fluconazole in combination with 1/8 MIC of L95 was 16 μg/ml, with 1/8 MIC of L1369 - 16 μg /ml, with 1/8 of MIC L1558 - 16 μg/ml.

The obtained results indicate that the synergism of fluconazole with thiazolidine derivatives increases the sensitivity of *Candida* to these compounds by 4-6 times. So, we can talk about the discovery of new, potentially promising compounds with antifungal activity, the effect of which also increases in combination with known antifungal drugs.

PSEUDOMONAS SYRINGAE – THE AGENT OF SORIZ BACTERIAL SPOTS

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Due to climate change, in particular, an increase in average temperature and a decrease in rainfall, drought-resistant crops such as sorghum family are promising for cultivation. Varieties and technologies for growing a new cereal crop from the sorghum family, Soriz, are being improved in Ukraine. At the same time, it is necessary to perfect the identification of pathogens of the sorghum family since the spectrum of phytopathogens changes with the introduction of new crops and changes in their growing conditions.

The aim of the work was to monitor sorghum crops in the Cherkasy region of Ukraine for the detection of bacterial diseases; isolation of pathogens and the study of their main biological properties. For this purpose, generally accepted phytopathological, microbiological and biochemical methods were used. *Pseudomonas syringae (P. holci)* 8300 and a type strain of *P. syringae* UKM B-1027^T were used as control strains from the collection of the Department of Phytopathogenic Bacteria of IMV NASU.

It was found that the main bacterial disease of soriz is bacterial spotting. 7 isolated bacterial isolates were selected for research of biological properties and identification. When artificially infected with isolates from soriz and sorghum crops, it was found that all of them showed high aggressiveness on soriz, grain, and sugar sorghum, as well as Sudan grass, field horsetail, and trailing bindweed.

Isolates from soriz are Gram-negative motile rods, do not form spores, have no oxidase, show no protopectinase activity, do not reduce nitrate, do not form hydrogen sulfide and indole, do not use glucose anaerobically, and lactose, rhamnose, maltose, dulcitol, inulin and salicin, yes as well as *P. syringae* 8300 and *P. syringae* UKM B-1027^T. All strains produced a fluorescent pigment, caused a hypersensitivity reaction on tobacco and used glucose, galactose, fructose, arabinose, mannitol, glycerol and citrate, variable – sucrose, sorbitol, inositol. However, isolates from Soriz and *P. syringae* 8300 did not use raffinose, unlike the type strain. Most of the isolates from Soriz ferment trehalose, only two isolates had gelatinase, as *P. syringae* 8300 and *P. syringae* UKM B-1027^T. According to the data of NEFERMtest 24 (MikroLaTEST, ErbaLachema), isolates from Soriz, like collection strains, did not produce urease, ornithine decarboxylase, lysine decarboxylase, acetamide, N-acetyl-β-D-glucosaminidase, γ-glutamyltransferase, but had phosphatase and β-glucosidase, and hydrolyzed esculin.

So, in terms of morphological and biochemical properties, the isolates from Soriz were identified as *P. syringae* van Hall 1902. The isolates differed from each other in some characteristics, but most were similar to the type strain *P. syringae* UKM B-1027^T. The above differences do not go beyond the biological properties of the *P. syringae* group, for which heterogeneity in the use of some sources of carbon nutrition was noted.

VACCINE-DERIVED POLIOVIRUSES IN UKRAINE

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According to The World Health Organization (WHO), since the withdrawal of type 2 oral poliovirus vaccine (OPV2) from routine immunization in 2016, there has been an increase in vaccine-derived poliovirus type 2 (VDPV2), related to a fall in population intestinal mucosal immunity to type 2 polioviruses in children born after April 2016 globally. This pronounced the polio Public Health Emergency to the International Concerns.

The risks of vaccine-derived polioviruses (VDPV) spreading in Ukraine has been the central aim of the study.

An analysis of the incidence of poliomyelitis in Ukraine and the level of vaccination of children over the past 10 years was carried out according to the data of the Public Health Center of Ukraine.

In October 2013, the WHO Independent Monitoring Board and the Global Polio Eradication Initiative included Ukraine in the "red" list of countries with the highest risk of poliomyelitis outbreaks.

In 2015, two cases of VDPV were confirmed in the regional reference laboratory of WHO, where feces of two children from the Zakarpattia region of Ukraine were analyzed. The children (aged 4 years and 10 months) were not vaccinated against polio and got infected by a paralytic form of poliomyelitis.

In 2021, a 17-months-old toddler was detected with acute paralysis caused by VDPV2 in the Rivne region along with 18 people who were in contact. All the positive isolates were similar to isolates circulating during the 2020-2021 cVDPV outbreak in Tajikistan.

In December 2021, the last case of paralysis caused by VDPV2 appeared, represented by a 2-years-old unvaccinated child from the Zakarpattia region.

An outbreak of polio in any country can only be prevented if at least 95 % of children are vaccinated. In Ukraine, the current level of vaccination is the lowest among the last 20 years. As a reference, in 2021 the vaccination against polio for children aged less than 1 years was only 80.1 % (68.5 % in the Zakarpattia region). Hence, the minimum requirements for prevention of polio are not achieved.

VDPV circulating occurs when scheduled or additional immunizations are not performed competently, rendering the population vulnerable to infection by both VDPV and wild strains. Consequently, the problem is not in the vaccine itself but in the inadequate vaccination. Thus, a comprehensively vaccinated population is protected against both the wild virus and VDPV.

THEORETICAL AND EXPERIMENTAL EVALUATION OF THE ANTIVIRAL ACTIVITY OF ORGANIC ACIDS SPECTRUM AGAINST INFECTIOUS BRONCHITIS VIRUS

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The new betacoronavirus SARS-CoV-2 caused COVID-19 and the fifth pandemic of respiratory disease after the 1918 flu pandemic. There is an urgent need to find new antiviral drugs against respiratory coronaviruses but also to study the antiviral effect of well-known substances to identify new therapeutic approaches for people with COVID-19. An expanded search for new, effective antiviral drugs against coronaviruses is needed using both *in silico* and subsequent *in vitro* studies. The aim of this study was theoretical *in silico* and *in vitro* evaluation of the antiviral activity of several well-known organic acids against infectious bronchitis virus (IBV), the prototype strain of the *Coronaviridae* family.

The cytotoxic effect of organic acids was evaluated on a monolayer of cell culture BHK-21. Cultivation, accumulation, and determination of the infectious titer of IBV by cytopathic action were performed using cell cultures of chicken embryo fibroblasts and BHK-21. The antiviral effect of a substance was assessed by measuring its chemotherapeutic index. Possible mechanism of antiviral activity of a number of aminocarboxylic acids was studied with the use of molecular docking of the "protein-ligand" interaction with the "key-lock" modeling type. The spike protein and main protease of IBV were chosen as targets for modeling in .pdb format (Protein Data Bank). The structures of the ligands: 4-aminobutyl, 5-aminovaleric, 6-aminocaproic, 7-aminoheptanoic, 8-aminooctanoic acids, as well as methyl-6-aminohexonate were taken in .sdf format from Pubchem, and subsequently converted into 3d structures in .pdb using Avogadro® and Discovery Studio 3.5®. Results were visualized using AutoDock 4.2.6, PyMol® and Protein-Ligand Interaction Profiler®.

This study’s results, performed *in vitro*, showed that effective inhibition of virus reproduction in preventive and treatment regimens indicated that 4-aminobutyric acid and 6-aminocaproic acid affect the early stages of coronavirus reproduction. The results of molecular docking showed that the best binding of the amino acid to the spike protein was shown for 4-aminobutylic acid; however, 6-aminocaproic acid showed good binding energy with the main protease. In general, the binding energy of amino acids with the main protease was higher than with the spike protein, which can be explained by the complex structure of the spike protein and, probably, by another mechanism of its inhibition and should be explored in the future work.

INHIBITORY ACTIVITY OF CELL-FREE SUPERNATANT OF MARINE MICROBIOTA ISOLATED FROM MUSSELS IN BLACK SEA

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Introduction. Antibiotics resistance has become a challenge for scientists in recent decades. Therefore, the development of new effective medicine against bacterial infections is needed. Researchers have already used living things from the environment in the medical field, but there are still many things to be discovered, and the sea is an excellent place to start with. Marine microbiota produces various chemical substances, and it became the main interest of study upon the presence of antibiotic activity of secondary metabolites that will lead to the production of new effective antibiotic drugs. In our research, bacteria from the Black Sea were used to study their metabolites for antibiotics activity.

Materials & methods. Samples were isolated from the surface of mussels in the harbour of Odesa Bay in the Black Sea. Marine microbiota has been identified using MIDI Sherlock™ fatty acid analysis as *Pseudomonas aeruginosa* (M1, M3, M4) — group 1, *Bacillus subtilis* (MC3), *B. atrophoeus* (MH4) and *Alcaligenes faecalis* (AF) — group 2. Liquid media Iso-sensitest broth Oxoid™ and Lennox broth (LB) were used. Gram(-) bacteria (*Pseudomonas aeruginosa* PA01 and *Escherichia coli* K12) and gram(+) (both clinical strains of *Staphylococcus aureus* and *Mycobacterium smegmatis*) were used as test strains. Cultivation was performed separately and in pairs (group 1 with group 2) in plastic tubes with 2 ml of ISO:LB 1:1 (v/v) with 1% sea salts for 20 hours at +37C with shaking at 200 rpm. The supernatant was filtered using 0.2 µm filters and stored at -20C. Inhibition activity was studied by dropping 30 µL of cell-free supernatant on the paper disks that were placed on the bacterial lawn of test strains. The inhibition zone was identified after incubation.

Results & conclusions. Bacterial inhibition was observed from the MH4 strain and its pair with M3 on *S. aureus* and *M. smegmatis*. No inhibition was shown on PA01 and K12 indicating that active substance in supernatant had action towards Gram(+) bacteria. The results from the cultivation of MH4 with other strains from group 2 showed no inhibition, indicating that MH4 could produce metabolite with antibiotic activity just with some bacterial strains.

In conclusion, the MH4 strain of marine microbiota of the Black Sea produces substances that demonstrate the inhibitory effect against Gram(+) bacteria. The inhibitory effect was observed by mono-culture and in pair with M3, and this effect mainly occurred by MH4, and perhaps this strain plays the main role in antibiotis production.

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EFFECT OF CERIUM DIOXIDE NANOPARTICLES ON BIOFILM FORMATION AND AUTOAGGREGATION OF *BIFIDOBACTERIUM ANIMALIS* STRAINS

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Bacteria of the *Bifidobacterium* genus and their metabolites play an important role in the microbiome of macroorganism under normal conditions and during infectious and inflammatory pathologies.

Autoaggregation is a process by which bacterial cells can physically interact with each other. It plays an important role in biofilm formation. In most cases, the aggregation ability is related to the adhesive properties of the cells, which also includes their ability to survive and persist in the gastrointestinal tract. Moreover, aggregation can suppress the growth of pathogenic microorganisms.

The aim of the work was to investigate the influence of cerium dioxide nanoparticles on the process of autoaggregation and biofilm formation of *Bifidobacterium animalis* VKB and *B. animalis* VKL strains.

Materials and Methods. The subjects of the study were two probiotic strains *B. animalis* VKB and *B. animalis* VKL deposited in the Ukrainian Collection of Microorganisms (IMV of the NAS of Ukraine). Autoaggregation studies of test strains were performed according to the Balakrishna method [Balakrishna A., 2013]. The ability to form biofilms was studied by determining adhesion to polystyrene microplates according to the Rode method [Rode et. al. 2007.]. Cerium dioxide nanoparticle concentrations from 2.5 μL to 250 μL per liter of Bifidum medium (Merck, Germany) were used. Cultivation of bifidobacteria was carried out in an anaerostat using GasPack (bioMerieux, France) at a temperature of 37 ^{0}C for 24-48 hours.

Results. 27 % of autoaggregation by *Bifidobacterium* genus bacteria was detected after 1-hour of cultivation, it increased each hour and reached the highest value at the fifth hour of the study. *B. animalis* VKL strain had a higher degree of autoaggregation compared to *B. animalis* VKB strain (57.1 ± 1.13 % and 54.75 ± 1.21 %, respectively).

The relationship between the increase of CeO_2 concentration and the potential of biofilm formation by *B. animalis* VKB strain was established; with an increase in the concentration of nanoparticles, the ability to form a biofilm decreases. When studying the *B. animalis* VKL strain, the opposite effect was established. Cerium nanoparticles at a concentration of 250.0 μg/L have no significant difference in effect on biofilm formation potential, while the use of 2.5 μg/L has a strong inhibitory effect on biofilm formation by this strain. Cerium dioxide nanoparticles at a concentration of 250 μl/L of culture medium had the highest effect on the process of biofilm formation.

Conclusions. The high percentage of aggregation of *B. animalis* VKL (57.1 ± 1.13 %) and *B. animalis* VKB (54.75 ± 1.21 %) strains will increase their adhesive activity and contribute to their survival in the gastrointestinal tract. Cerium dioxide nanoparticles at a concentration of 250 μl/L of culture medium had the highest effect on the process of biofilm formation by *B. animalis* strains.

THE SEARCH AND *DE NOVO* DESIGN OF COMPETITIVE LOW-MOLECULAR INHIBITORS OF SARS-COV-2 3-CHYMOTRYPSIN-LIKE PROTEASE WITH ACCEPTABLE PHARMACOKINETIC PROPERTIES

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Introduction: SARS-CoV-2 is a new coronavirus that is believed to have originated at the end of 2019 in the city of Wuhan (the People's Republic of China). As of now, millions of people have died due to pathological disorders caused by this pathogen. 3CLpro is the main protease of SARS-CoV-2, which ensures the processing of two polyproteins (pp1a and pp1ab), which are directly formed as a result of the translation of the viral genome. Accordingly, inhibition of the proteolytic activity of this enzyme is obviously a promising way of countering the reproduction and spread of the new coronavirus.

The aim of this work is the search and *de novo* design of a pool of competitive low-molecular-weight inhibitors of 3CLpro enzymatic activity with pharmacokinetic properties that are likely to be acceptable for systematic use.

Methodology: Initial data were obtained from the RCSB PDB (3CLpro, PDB ID: 6w79) and DrugBank (library of FDA-approved compounds). Their preparation included the removal of all but the target molecules and the generation of possible isomeric forms. Ranking of all FDA-approved medicinal compounds according to their affinity for 3CLpro was performed by virtual screening. One selected compound was analyzed using ADMETlab 2.0. An iterative approach based on molecular dynamics simulation was used to improve its ADMET and affinity. Each of its iterations consisted of three successive stages - the molecular dynamics simulation of the ligand-receptor complex, analysis of the obtained results, and modification of the ligand. Acceptable ligand configurations were selected based on their ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) and affinity for the 3CLpro active center (AC).

Results: Among more than 2500 FDA-approved drugs, only Anlotinib showed acceptable affinity parameters for 3CLpro (RMSD: 1.7-2 Å). At the same time, its calculated logP, logD, logS, QED and Tox21 parameters exceeded the values acceptable for medicinal compounds. Further research made it possible to design 15 Anlotinib derivatives: md_a34, md_a49, md_a86, md_a273, md_a388, md_a447, md_a452, md_a508, md_a562, md_a597, md_a603, md_a675, md_a682, md_a690 and md_a711, which are targeted to 3CLpro by two alternative forms of protonation of histidines 41, 163, 164, and 172. All of them have better predicted ADMET parameters than Anlotinib. At the same time, the degree of their affinity to the 3CLpro AC is the same or higher than that of Anlotinib.

Conclusion: Virtual screening identified Anlotinib as the only potential inhibitor among the entire library of FDA-approved medicinal compounds. Due to the unacceptability of its calculated pharmacokinetics, 15 of its structural analogues were designed, which demonstrated a much better calculated ADMET profile. At the same time, the affinity of these substances to the 3CLpro AC was preserved or improved in comparison with Anlotinib.

THE INFLUENCE OF SILVER NANOPARTICLES ON DIFFERENT STAGES OF REPRODUCTION OF INFLUENZA A VIRUS (H1N1)

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Since the 19th century, the bactericidal properties of silver have been widely used in medicine, and in recent years the nanotechnology industry increasingly pays attention to them. Studies of the effect of silver nanoparticles (AgNPs) on various microorganisms, including viruses, are constantly being conducted. After all, the problem of the lack of antiviral drugs remains urgent, especially for RNA-containing viruses, which very quickly acquire resistance. Therefore, the aim of this work was to study the effect of AgNPs mixtures on different stages of reproduction of influenza A virus (H1N1). The following methods were used – the MTT-test and determination of antiviral activity according to three schemes: prophylactic (before infection), effect on adsorption (during infection), and post-exposure (effect on infection). Experiments were performed using MDCK cell culture and influenza virus strain A/FM/1/47. The studied AgNP were represented by three samples: Ag60C (a mixture of nanoparticles from 4 to 30 nm in diameter, mostly 8-10 nm), Ag40C (a mixture of 5 to 45 nm, mostly 10-20 nm), and Ag25C (a mixture of 5 to 100 nm, mostly 40-50 nm). Oseltamivir phosphate was used as a reference drug.

To assess the cytotoxicity, the CC50 index was calculated based on the results of the MTT-test. In the concentration range from $1x10^{-5}$ M to $1x10^{-9}$ M, nanoparticles did not have a significant effect on cell viability, so it was impossible to calculate the CC50 index for them. Oseltamivir phosphate demonstrated a classical dose-dependent effect, therefore, its CC50 was $2.6x10^{-3}\pm0.1x10^{-3}$ M.

According to the prophylactic scheme of the determination of antiviral activity, Ag60C showed the highest efficiency among the three types of AgNPs - from 74.53±3.72% to 92.37±4.61% inhibition of virus reproduction. Ag40C also demonstrated relatively high efficiency - from 64.67±3.23% to 75.36±3.76% inhibition of virus reproduction. Ag25C nanoparticles, the largest in diameter, inhibited virus reproduction only at the highest tested concentration of $1x10^{-5}$ M by 4.34±0.08%, which is lower than that of the reference drug (7.18±0.35%). These results well illustrate the dependence of the size of AgNPs on their activity since small nanoparticles quickly release Ag^+ ions, which are the active form of silver.

The next scheme of determination - the influence on the adsorption of virions - demonstrated the absence of an effect of AgNPs on this process: the highest percentage of inhibition of virus reproduction did not exceed 6% for Ag25C, oseltamivir phosphate also showed no activity. In the post-exposure scheme, the reference drug best inhibited the reproduction of the virus - by 27.19±1.35%. For the studied AgNPs, the inhibition efficiency of virus reproduction ranged from 7 to 11%, with the highest rate of 11.03±0.55% for Ag25C.

Thus, taking into account the high prophylactic antiviral effect of AgNPs mixtures and their low cytotoxicity for MDCK cell culture, the studied nanoparticles can be considered potential candidates for further experiments as antiviral agents.

COMPLETE GENOME SEQUENCING OF TWO *ERWINIA* STRAINS

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Advances in genome sequencing have produced hundreds of thousands of bacterial genome sequences, many of which have integrated prophages derived from temperate bacteriophages. These prophages play critical roles by influencing bacterial metabolism, pathogenicity, antibiotic resistance, and defense against viral attacks (Gauthier et al., 2022). Here we aimed to provide the complete genome sequences and *in silico* prophage content analysis of two *Erwinia horticola* strains, the causative agent of beech black bacteriosis in Ukraine.

The genomic DNA of *E. horticola* 60-2n and 43II was isolated using the DNeasy UltraClean microbial kit (Qiagen). The short reads library was prepared using the Nextera Flex DNA Library Kit (Illumina). The library quality was verified using the Bioanalyzer 2100 (Agilent) (High-sensitivity DNA kit). Both library preps were sequenced using the MiniSeq Mid Output flowcell (300 cycles; 2*150 bp reads). Basecalling and demultiplextion were done using the Illumina Miniseq system. In parallel, long sequencing reads were obtained by means of the MinION sequencer from Oxford Nanopore Technologies (Flowcell R9.4.1) using the Rapid Sequencing Barcoding Kit. Guppy (v3.1.5) was used for the basecalling. The genomes were assembled by Unicycler and annotated by the RAST (Wick et al., 2017, Brettin et al., 2015). Preliminary genome analysis was performed using the Comprehensive Genome Analysis Service (https://www.bv-brc.org/). The assembled genomes were considered for prophage prediction using PHASTER (Arndt et al., 2016).

The genome of *E. horticola* 60-2n consists of a circular chromosome of 5,001,269 bp with an average GC content of 55.07 %. This genome has 4,729 protein-coding sequences (CDS), 79 transfer RNA (tRNA) genes, and 22 ribosomal RNA (rRNA) genes. The annotation included 769 hypothetical proteins and 3,960 proteins with functional assignments. A total of four prophages were identified. Out of those, three prophage elements (11,732 bp, 31,389 bp and 35,799 bp) were incomplete and one prophage sequence (43,298 bp) was intact. The genome of *E. horticola* 43II has a chromosome of 5,061,335 bp (55.03% GC content) and one plasmid (183,568 bp). Annotation predicted 4,707 CDS, 78 tRNA genes, and 22 rRNA genes. Annotated genes encode 893 hypothetical proteins and 4,026 proteins with predicted functions. One intact and one incomplete prophages were identified (53,313 bp and 15,227 bp, respectively). Phylogenetic core genome analysis revealed that both *Erwinia* strains were close to *E. billingiae.* The Average Nucleotide Identity between the studied strains and *E. billingiae Eb661* was 97.6 %, suggesting that these genomes belong to the same species.

As a result, whole genome sequencing of two *Erwinia* strains was obtained. The general characteristics of genomes and mobile genetic elements were identified. Preliminary phylogenetic analysis indicated that the studied strains should be assigned to *E. billingiae* species.

THE GROWTH OF *TRAMETES VERSICOLOR* ON WOOD HYDROLYZATE IN SUBMERGED CULTURE

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Basidiomycetes of *Trametes* genus produce a number of biologically active compounds with antibacterial, antiviral, antitumor, hepatoprotective, antioxidant, hypolipidemic, hypocholesterolemic, and other properties. *Trametes* sp. is undemanding to the composition of nutrient media. Therefore, waste from various industries, in particular woodworking, can be used to grow mushrooms. Representatives of this genus are characterized by a high level of biomass accumulation in submerged culture, which makes them promising objects of biotechnology.

The aim of the study was to investigate the biomass production by basidiomycete *Trametes versicolor* in submerged culture on wood hydrolyzate.

Materials and methods. Cultivation of three strains of *T. versicolor* was carried out for 14 days in conical flasks (250 cm^3) on the shaker (120 rpm) at +28 °C in 50 cm^3 of liquid medium (in g/L): glucose – 10; NH_4NO_3 - 1; KH_2PO_4 – 1; $MgSO_4 \cdot 7H_2O$ – 0.5; $FeSO_4 \cdot 7H_2O$ – 0.005; $ZnSO_4 \cdot 7H_2O$ – 0.0044; $CaCl_2$ – 0.0055; pH 6.8. In the experimental medium, oak hydrolyzate was used instead of water. For its preparation, previously chopped and dried pieces of sawdust were placed in a round flat-bottomed flask and filled with 20 times the amount of water. For extraction, the filled flasks were autoclaved at 121°C for 20 minutes. After that, the liquid phase was separated by filtering through FB brand filter paper and used in the preparation of media. At the end of the cultivation, the biomass was separated on a gauze filter and dried at 105 °C to a constant mass.

Results. It was established that the yield of dry biomass did not exceed 2.5 g/L on the control medium. The maximum amount of biomass was accumulated by the *T. versicolor* 353 strain and was 2.31±0.12 g/L. For two other strains, slightly lower levels of synthesis were observed, which did not differ among themselves ($p < 0.05$): *T. versicolor* 5095 produced 1.72±0.20 g/L, *T. versicolor* 5129 – 1.71±0.18 g/L. On the medium with the addition of oak hydrolyzate, the biomass concentration was higher. As on the control medium, *T. versicolor* 353 accumulated the most biomass, namely 2.72±0.24 g/L. However, this characteristic was slightly different for the other two strains. Cultivation on oak hydrolyzate of *T. versicolor* 5095 resulted in obtaining biomass in the amount of 1.93±0.15 g/L, while *T. versicolor* 5129 accumulated 2.18±0.17 g/L. We can see that in the case of *T. versicolor* 353 there was an increase in the level of biomass production by 18.10%; for *T. versicolor* 5095 this indicator was 11.97%, and for *T. versicolor* 5129 – 27.69%.

Conclusions. The application of oak hydrolyzate instead of water in the synthetic medium made it possible to significantly increase the level of biomass accumulation by three *T. versicolor* strains. The most significant effect on production was observed for the strain *T. versicolor* 5129, where the amount of biomass increased by 27.69%.