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BIOTECHNICAL INFORMATION SYSTEMS FOR MONITORING OF CHEMICALS IN ENVIRONMENT: BIOPHYSICAL APPROACH

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The newest biotechnical systems for environment ecological monitoring based on the use of modern information and computer technologies and existing databases of chemical substances have been analyzed. In particular, there were observed such modern biophysical research methods as imitation and program modeling, based on the author results obtained in the experiments with registration of chemo-sensitive transmembrane electric currents in neurons in voltage clamp mode, use of neuronal fluorescent markers and accounting of organisms-bioindicators. The developed systems and methods allow revealing and identification of substances hazardous to living organisms and to make conclusions about their possible biological effects. The functioning of biotechnical information systems for environmental monitoring was analyzed in a wide time ranges, using modern databases, expert subsystems and interfaces capable to identify different types of chemicals. It is shown that for such systematic environmental monitoring it is possible to study and predict the effects of substances influences for a long time from the first moments of their exposure to individual cells of organism to months and years after exposure to the whole organism.

Key words: biotechnical information monitoring system, environmental pollution, bioindicators, databases.

Anthropogenic influence in nature is one of the most important problems of contemporary world. Ecoogical problems linked with environment pollution by chemical substances, hazardous for living organisms, are also important for all contemporary countries. One component of such antropogenic influence is technogenic chemical pollution in industrial regions. It appeared also as a result of accidents, disasters, contemporary military situations on the East of Ukraine, and etc. [1]. People have to direct constant efforts for revealing of such chemical pollution, for studying of its influence on living organisms and for finding of the ways for such pollutants neutralizing or elimination.

For today, solutions to environmental challenges were aided by an arsenal of information and knowledge systems that were unavailable for most of the last 30 years [2]. However after a while biotechnological methods become used more and more for this purpose. Considering that knowledge about the causes of environmental ills had grown, the number of options arise on how to handle them as well as the development of collaborations and partnerships aimed

at harnessing the growing incentive-based approaches to environmental protection. As additional information technologies and knowledge management techniques evolved, environmental considerations will join other areas of strategic importance to industry. Information technologies become unique not just because of their growing use in decision-making and knowledge management systems, important as that is. Their use had also yielded significant improvements in the efficiency of energy and materials use, minimization of environment pollution by harmful organic substances [2]. Advances in information technology are likely to continue the opportunities providing for the development of improved and new versions of information systems in complex with biotechnological, anlytical and other methods and their organization for ecological control [1]. For revealing, studying and neutralizing of pollutants a lot of different technical means, monitoring systems (MS), information systems (IS), and other samples of information and computer technologies (ICT) were invented and constructed during human industrial activity [1–14]. On the other hand people need more and more perfect solutions for these tasks due to appearance of the new ecological challenges as consequences of military actions at the territory of Ukraine today, technogenic disasters like Chornobyl one, other disasters at enterprises of chemical, oil and gas industry, like spread extensive fire of oil tankers in Kyiv region, Ukraine, in June 2015 with massive release of organic matter — pollutants.

Biological information systems (IS) suitable for such purposes, were designed either for academic purposes — to maximize the accumulation of information about the groups of living organisms or for the needs of economy, in particular for biotechnology, for monitoring of polluted areas in industrial centers, and etc. [1–15]. In our previous publications there were written about some mathematic methods used for IS we have constructed for monitoring purposes, about it different parts (databases, expert system, etc.) and other linked solutions [1, 3–12]. The present article unites and summerizes this great volume of published results. Mathematic methods as well as models that we described in our previous articles and published by other authors may be used for ISs functioning or to be simulated in result of their functioning [10–81]. A spectrum of mathematic methods were used for the newest biomedical ISs elaboration [1, 11, 75, 77–80, 82–146]. The databases content described in this article was obtained usually from the results of biological and medical observations and experiments [10, 12–17, 24–44, 47–49, 61, 68, 71–74, 82–90, 94, 104, 106, 109, 111–113, 125–202]. All such technical information systems (tIS) are electronic databases (DB) distributed in networks today [1–11, 25–69, 90–109, 112–120, 159]. Present work was done after the analyzis of more than 300 current publications in fields of biotechnology, other branches of biology and technology, including articles with original authors' works. The newest parts of authors work were defended by patents [172–182]. Prof. Zoya F. Klyuchko and Dr. Elena M. Klyuchko have studied such influences on organisms-bioindicators *Noctuidae* (*Lepidoptera*), and a part of our works under the DBs and ISs construction we did with this biological material [1, 9, 42, 135, 136, 139, 140, 144, 156, 157, 159].

Brief review of some models of technical systems for environmental monitoring. Brief review of some models of technical systems for monitoring of environment using biotechnical means and some biophysical methods for the investigation is suggested below in this sub-chapter. Among great number of prototypes there are technical and laboratory systems, activities of which were directed on environment protection. We picked up them because they have some similar characteristics with our ones in our developed system for environmental monitoring.

1. The authors Nemtsov V. I. and Nemtsov A. V. had suggested a technical network system for monitoring of the state of environment [191]. According to the suggested method they took the probes in environment for further biophysical and analytical studyings. Such a technical network analytical system for the complex analysis and sampling of biophysical aerosols contained an electron microscope, a television microscope, made on the basis of a biological microscope with a fiber optic illuminator of side illumination of large fields of the subject plane for determining the particle size distribution and specific gravity of the particles in the sample. The analytical system had electronic scales and a multichannel sampler with a vertical suction channel. The latter were coupled with a variety of trapping elements with sampler substrate, filter, nutrient media,

and heat-resistant cassette for substrates, impacted and tipped for isokinetic selection. The tips had rotary adapters and were mounted on impactors. Substrates for microbiological analysis were made with recesses and with flat covers, transparent for light and electronic streams of probe radiation. Nutrient medium was enclosed in recesses. Substrates for deposition of physico-chemical aerosols were made in the form of covers similar to that of substrates for microbiological analysis. The method allowed to carry out complex analysis and sampling of biophysical aerosols. The invention increased the study of the nature of mineralogical, physical, bacterial and viral aerosols, allowed also to take measures for environment protection.

2. Another system for monitoring environment based on laboratory studying was described in [192]. It included fixed and mobile monitoring sites equipped with measuring instrumentations. Various environmental parameters were registered and subjected to analysis. More specifically, hydrophysical field signals were registered, the chemiluminescence, chromatographic, ion-selective, spectral and radiometric analysis was performed. Besides, bed acoustic impendance was registered, molecular spin interactions of seawater protons were detected, artifacts resulting from the magnetohydrodynamic, bioelectric and concentration effect were detected, synthetic surfactant content in the aquatic environment, chlorophyll concentrations, microorgasnisms, phytoplankton, zooplankton was determined. The collected data was further transferred to the archivers and modeling was performed. In the course of modeling the industrial facility environment and infrastructure were divided into a number of areas and a material balance model and a forecast model were created for each of them. For the purposes of the method implementation a system comprising a water withdrawal line equipped with hydrophysical field sensors, a filtering plant for chlorophyll concentration, a filtering plant with a Seitz funnel for microorganisms sampling, a Nageotte chamber for counting the phytoplankton content, a Bogorov Counting Chamber for enumerating zooplankton, a centrifugal apparatus for determining chlorophyll content, a geophone, spectral sensor of proton spin echo were proposed. Futhermore, the proposed system comprised the devices for chemiluminescence, chromatographic, ion-selective, spectral and radiometric analysis, a radiation spectrometer, an atomic absorption spectrophotometer, an X-ray fluorometric analyser, TV sensors, infrared sensors, heat sensors, a metrological module, a sidescan sonar, multiple-beam echo sounder, water quality evaluator by TropoSample parameters and bed deposits characteristics, a lidar (a light radar), a penetrometer, methane and hydrogen detection sensors.

3. Other investigators [193] developed an enough perfect method to trace changes in characteristics of biological objects (in organisms-bioindicators) using technical system with “biosensor” for receptors’ antagonists. Their invention related to a method for detection of receptor antagonists comprising the following steps: (I) a sample containing the receptor antagonist fractionated by use of a liquid-based separation means, preferably capillary electrophoresis, (II) a fraction containing the receptor antagonist or modulator that fed directly to a biosensor, which was activated by an appropriate receptor agonist and, as a result of this activation, generated a measurable response. The said agonist being fed to the biosensor through the liquid-based separation means together with the antagonist or modulator. The said activation of the biosensor being pulsed by delivery of the receptor agonist to the biosensor for short period of time. The said periods being separated by other periods when no agonist was delivered to the biosensor. And (III) the change of the response resulting from deactivation of the receptor agonist-activated biosensor by the receptor antagonist or modulator was measured preferably by means of a patch clamp electrode. It was further possible to resensitize the biosensor desensitized as above by use of pulsed superfusion of the biosensor. This invention also related to an apparatus usable for practicing the above mentioned method.

Another patent provided a highly specific modern way of studying changes in biophysical characteristics in bioindicator objects, including responses to external influences using optically active markers [194]. The invention provided polynucleotides and methods for expressing light-activated proteins in animal cells and altering an action potential of the cells by optical stimulation. The invention also provided animal cells and non-human animals comprising cells expressing the light-activated proteins.

The prototype system at the cellular level allowed the following mechanisms to be investigated and used: a) a delivery device comprising a polynucleotide that comprises a nucleotide sequence encoding a light-activated polypeptide, wherein the light-activated polypeptide comprises, from N-terminus to C-terminus; i) a core amino acid sequence that is at least 95% identical to the sequence shown in SEQ ID NO; 3, SEQ ID NO; 1, SEQ ID NO; 2, or SEQ ID NO; 4; ii) an endoplasmic reticulum (ER) export signal; and iii) a membrane trafficking signal; b) a light source; and c) a control device that controls generation of light by the light source.

Ideas about monitoring in few time intervals and about selection for studying of groups of influencing chemical substances. Pollution of environment with chemical substances in industrial and other regions is widespread today. The influence of such substances is long-term usually and starts often from the moment of pollution. That is why we see as crucially important to study the influence of polluted substances at once after the moment of pollution and during long months and even years (if possible) on living organisms and their populations; and this is one stream of our interests in the present study.

The second stream of our interests is the object of studying. There are some effects of organic substances represented by phenol and indole derivatives. Although the wide spectrum of chemical substances was known as components of chemical pollution, the influences on organisms of hazardous and dangerous organic chemicals are less studied because of different reasons. Among them there are their non-stable structures in nature, "masking" of their effects by great number other organic substances in living organisms and so on [190]. From the other side, these chemicals are enough important from the point of view of their "chameleon" effects: hydrophobic circles are able to inquiry cell membranes easily, and their radicals may have hydrophylic nature and to be located outside of the surface cell membranes. Such "anchored" structures are able to occure great effects in living organisms; up to the lethal results sometimes. These substances form the large group of environmental pollutants; but among them also there are powerful toxins from living nature, like Arthropods' or Insects' toxins.

The author of this article studied influence of different organic chemicals, like phenol — and indole — derivatives with polyamine radicals of different length and complexity (PID-PR) during couple of the years [172–183]. Further, the author developed some devices and methods for monitoring the effects of such substances. In general, PID-PR substances play different roles in nature. On the one part, they are incorporated into living organisms by themselves. On the other part, substances with similar structure are components of out fluxes of industrial activity, accidents or disasters that influence hazardously on biological organisms of different hierarchical levels and especially on neurons of organisms [190]. Among ecological toxins there are a lot of substances with such chemical structure (below we call them also ecotoxins).

Novel scheme and technical system for monitoring of some chemicals influence in nature. In the article it is suggested a novel monitoring scheme that included different components: organizational, technical, biotechnical, IS-component, ICT-component, novel invented methods, and etc. (Fig. 1). For this system, its main parts and developed methods of monitoring were defended by patents [173–176, 178, 182, 183].

Monitoring with the use of this developed system could be carried out in three time intervals after beginning of substance action:

1: 0.5 ms — few minutes;

2: 10 min — 4 few hours;

3: during few months and years.

Respectively, there were elaborated different methods and equipment and used technical means for different time intervals. Correlation between them was depicted on Fig. 1. Detailed information about each time interval of monitoring is suggested below.

Purpose and tasks of the work. The work was aimed at a biotechnical information system developing for monitoring in environment the chemical organic substances harmful for living objects with relative methods for diagnostics of such substances in nature for studying of their effects on living organisms and their populations with further making of necessary recommendations for nature protection in different regions.

In the furtherance of this goal it was necessary to solve the following tasks:

1. To analyze contemporary prototypes of different information system for monitoring in environment.

2. To develop new contemporary experimental methodics connected with stated tasks solutions on the base of contemporary biophysical methods.

3. To develop methods for perfection of chemosensitive transmembrane electric currents (CTE — currents) in voltave-clamp conditions by the decrease of the noises' levels during electrophysiological experiments in voltave-clamp conditions; and by perfection of electrical signal revealing on the background of noises.

4. To study the influence of chemical organic substances, harmful for living objects, on chemosensitive transmembrane electric currents (CTE — currents) in voltave-clamp conditions. Mainly to study effects of derivatives of phenol and indole linked with polyamines of different length and complexity.

5. To study the changes in living neurons' optical properties by marking them by fluorochromes using method of retrograd axonal transport.

6. To make mathematical processing of obtained results and to develop relative mathematic and program models for effects studied in (3–5). For different segments of this biotechnical system the software development C++, C#, Java were used.

7. To conduct long-term monitoring of bioindicators populations (*Noctuidae*, *Lepidoptera*) in different regions and conditions with further processing of the obtained results (also in case of environment pollution by studied organic substances).

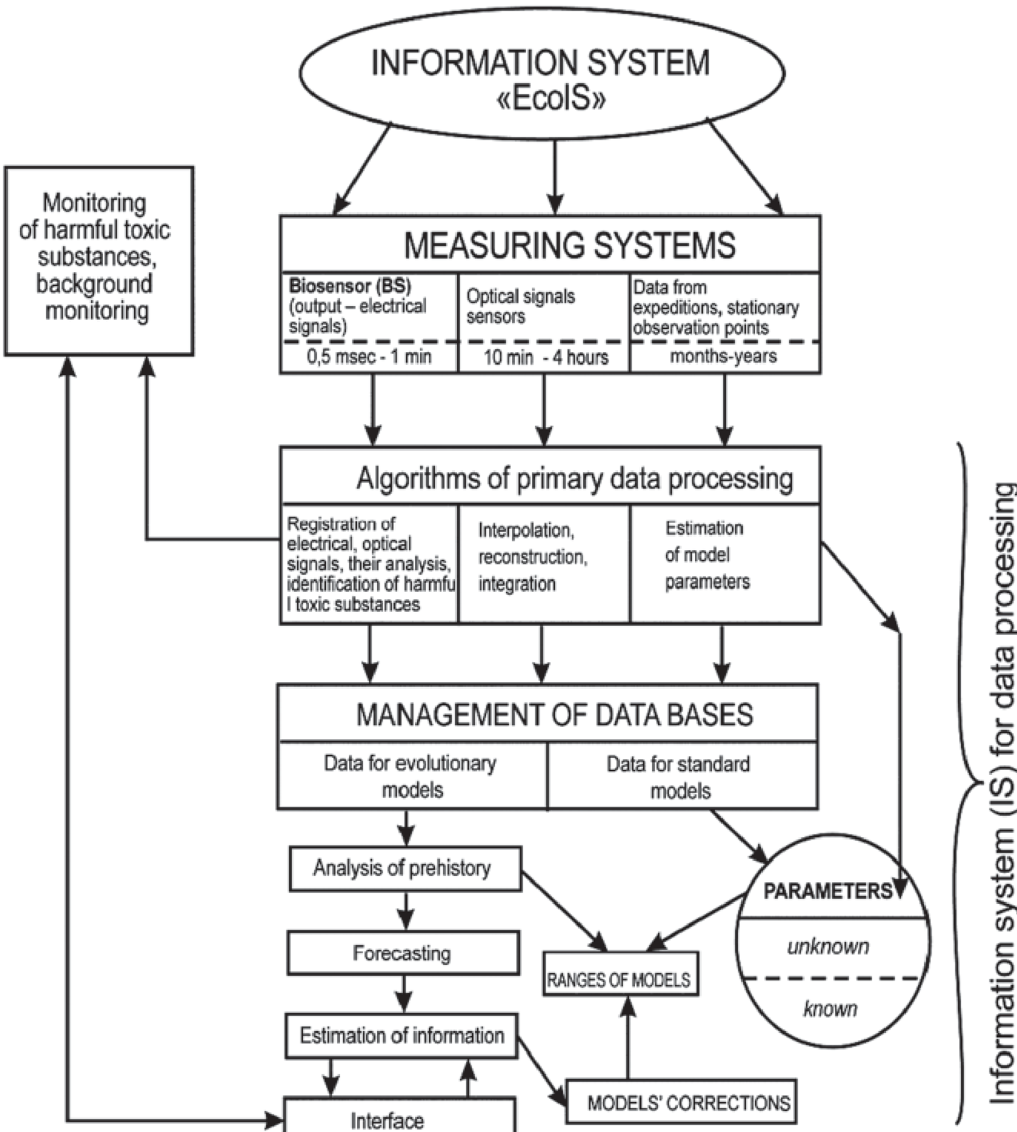

Fig. 1. **Information system for monitoring the influence of some chemical substances on living organisms and their populations "EcoIS" [1, 173–176]**

To develop the new methods of ecological monitoring of different ecosystems state with further analysis of the obtained data and with the recommendations for some regions of Ukraine (Karpathian Mountains, Midde Podniprovie, Donbas — "Striltsivskyi Steppe" Preserve in Luhansk region) and Russia (Caucasus Mountains, Kabardino-Balkar Republic).

8. On the base of (1–7) to develop new contemporary complex biotechnical system for obtaining and processing the data concerning environment with databases (DB), authomated electronic work places (EWP) with interfaces, in which there were united novel measuring devices, biodetectors and bioanalyzers, computer means, algorithms for data processing and methods for monitoring the bioorganisms (bioindicators) and ecosystems state. This biotechnical system was named as "Ecological Information System" — "EcoIS" [176].

So,"EcoIS" was developed to study and to monitor the influence of harmful substances (like some phenol — and indole — derivatives with polyamine radicals of different length and complexity — PID-PR) on living organisms in few time intervals. From the one side, it gives a possibility to study their mechanisms of infuences; from the other side — to trace the influence of PID-PR on organisms during long periods of time (human or non-human organisms). And all of these in complex give more possibilities to prevent and to neutrolize harmful PID-PR (and other) more long-term effects on humans.

Three time intervals were studied, and these time-intervals may be grounded reasonably by biological phenomena in the studied living organisms and the instrumental possibilities (experimental, monitoring, data mining, etc.) So, the time intervals selecting was due to the following:

1) (0.5 ms — few minutes) — in this time interval the changes in neuronal membrana electrical responces under PID-PR influences on CTE — currents have happened; they might be registered in voltage-clamp conditions, patch-clamp, other methods of this pool.

2) (10 min — 4 few hours) — in this time interval biochemical processes in neurons "in response" to chemicals' influences on neuron have happened. They might be revealed by fluorescent markers (the optical studyings were carried out by UV-microscopy method using the "LUMAM" fluorescent microscope producted by "Carl Zeiss" Company in Iena, Germany).

3) (during few months and years) — in this time interval the monitoring of changes in *Noctuidae (Lepidoptera*) organisms and populations was provided (collection of insects was carried out using light traps, field collections, other linked methods).

In such a way it was possible to register different aspects of the studied substances influences: (1) — the quickest electric processes in response to chemicals' influences; (2) — more slow biochemical processes in response; (3) very slow changes in the whole organisms and consequences of chemicals' influences on insects populations.

Brief information concerning the developed biotechnical system for ecomonitoring. The basis for biotechnical system for ecomonitoring elaboration was aimed to develop a method for the use of a network computer biotechnical monitoring system for deep large-scale study of the effect of a large number of types of chemicals on organisms-bioindicators in a wide range of time: from the moment when the chemical substance started to influence to the long-term consequences in a few years (including the effects of pollutants of the environment).

For this problem solution the biotechnical information system were developed called BTSM-3 with databases (DB), in which the subsystems of three types were united. BTSM-3 is a system that unites technical means and methods for monitoring in three time intervals. The system "EcoIS" was based on BTSM-3 but included also other subsystems, services and possibilities as follows:

1. The first subsystem contained at least one sensor (biotechnical system — BTS) with biological fragment (BF — cell, cell membrane, etc.). This sensor was included for the registration of transmembrane electric currents in single cells that might be influenced by different chemical substances. Such sensor might be a part of the whole sensory group with relative methods. It might be called a "sensor" or "detector". Time intervals of registration by the subsystem 2 and subsystem 1 were not always overlapped (Figs. 2–4).

2. The second subsystem was another sensory group — detecting group. It was developed, organized and supplemented with relative methods and serves to perform the optical registration of changes in the internal environment of cells marked *in vivo* by the fluorochromes, the dyes-markers (VDM), such optical changes of the cells' internal environment appeared in response to the action of some chemicals applied to the cells (Fig. 5).

3. The third subsystem was developed and organized to account the biological organisms-indicators (bioindicators) with the purpose to study the results of both qualitative and

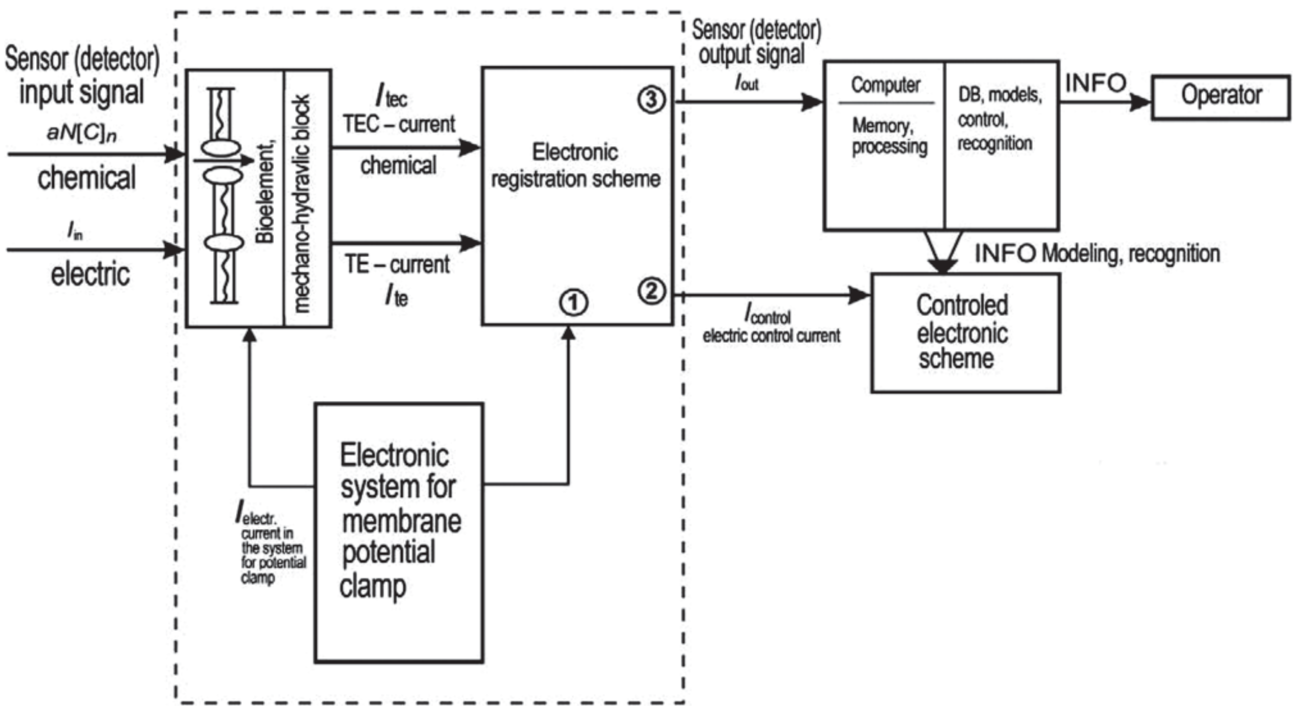

Fig. 2. **Block diagram of the technical sensor system in the "EcoIS" (BTSM-3). This complex may be an element in the block "Measuring system" (Fig. 1):**
at the input of the system information comes in the form of electrical or chemical signals,
at utput — in form of electrical signals

quantitative composition of bioindicators' populations (Fig. 6).

So, we proposed to use the developed biotechnical system BTSM-3 for the large-scale monitoring, using the deep study of the effects of chemicals infuense on the organism in different time intervals, from the moment of the start of their action on the organism. BTSM-3 was constructed as a biotechnical information system on the basis of relevant databases with direct and/or remote access that contain a number of subsystems and sensory groups.

In BTSM-3 there was in-built subsystem-sensor BTS with BF (there may be one or more such sensors, or detectors) characterized by the unity of three parts: mechanical-hydraulic part with BF, electric part and computer part. The last one allowed the registration of new received data, and also makes it possible to record in memory of the computer (PC). The obtained results were possible to record in DB (in local and/or network databases), to visualize them, to perform processing, analysis and data extraction, to make the data transmission using network technologies about the action of various chemicals. The registration process of the BTS occurs in the following sequence: the chemical substances were applied to BFs that were possible to substitute one by another. After respective agonists application there were possible to register the changes of electric transmembrana signals from BF using voltage-clamp, patch-clamp, microelectrodes' techniques or other methods of these types. The effects of applied in BF substances were measurable and able to be recorded.

The developed method and relative biotechnical system BTSM-3 differs from the other because it unites three subsystems that were built into the BTSM-3 for the monitoring of the increased number of chemical substances and for the expansion of the monitoring intervals after the time of start of the substances action.

For the use in sensor group, BF had to undergo preliminary processing according to specially developed procedures [172, 177] including enzyme treatment by proteases of *Aspergillus oryzae* and/or others substances in solutions with a selected composition, which are in contact with the gas environments of special composition, temperature and time modes of treatment. The substances acting on the BF could be obtained using various

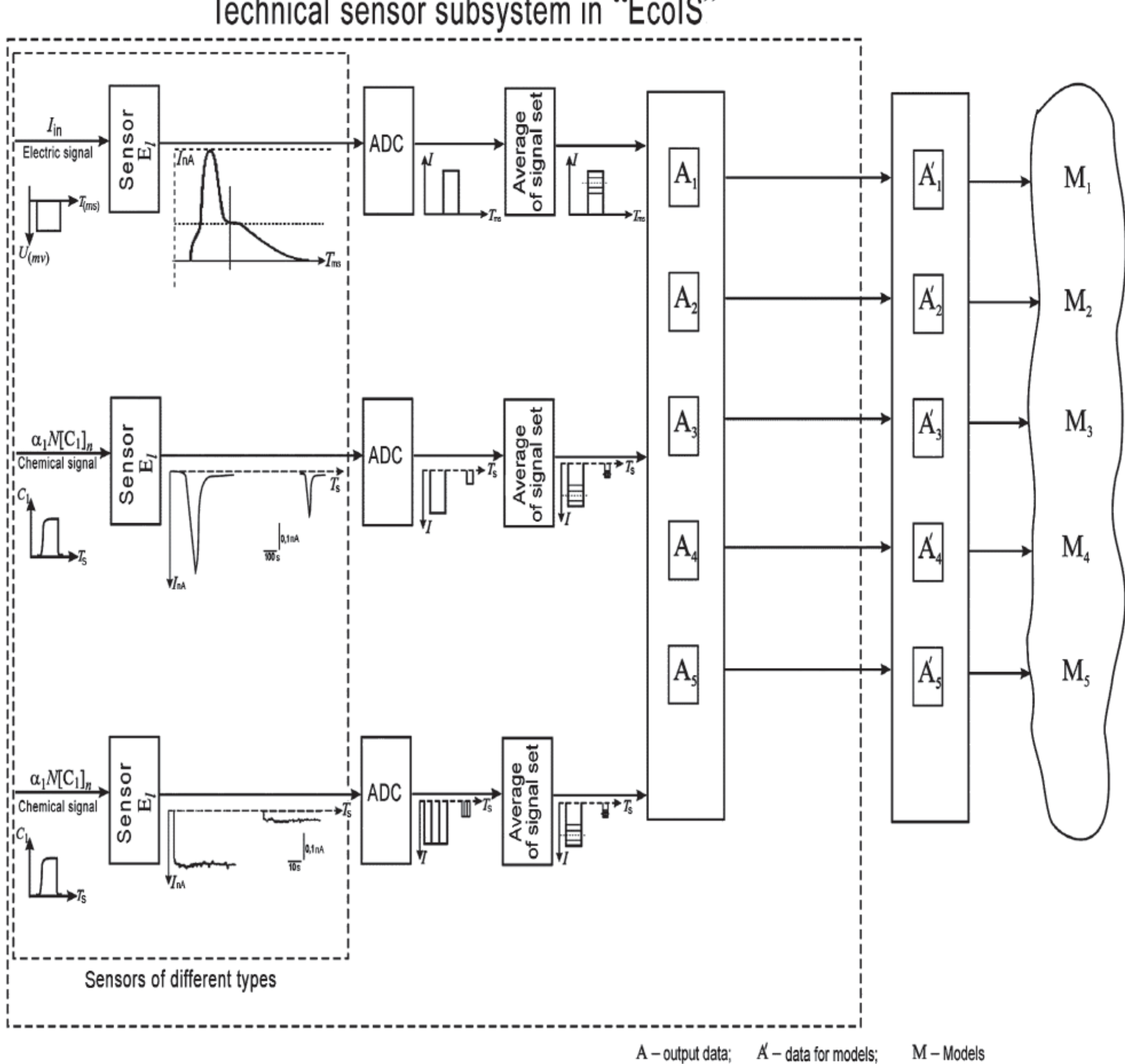

Fig. 3. **From the electrical signals to the mathematical models:**
at the input of the BTS system with BF the information comes in the form of electrical or chemical signals, at utput — in form of electrical signals. M — different mathematic models from the developed models family

chemical and biochemical methods. For the substances application a specially developed concentration-clamp method might be used. It was important as well to improve the registration of the output electric signal, improving its allocation to the background of the noise and significantly reducing the noises by themselves. Also the BF could be replaced depending on the processing of molecules of their surfaces, the type of chemicals that were analyzed. BF acts as the primary link in the sensor — biodetector and/or bioanalyzer of acted substances (including environmental pollutants). The input of computers in the BTSM-3 network received the information from the databases, the data as electrical and optical signals from detector subsystems, and data of bio-indicators' organisms counting.

Let's describe briefly some peculiarities — instrumental, methodics, etc. — for each time period of monitoring in details, as well as some obtained resuts.

Peculiarities of monitoring at the first time interval — at once after the influense of chemical substances. The first time interval of monitoring is between 0.5 ms — few minutes

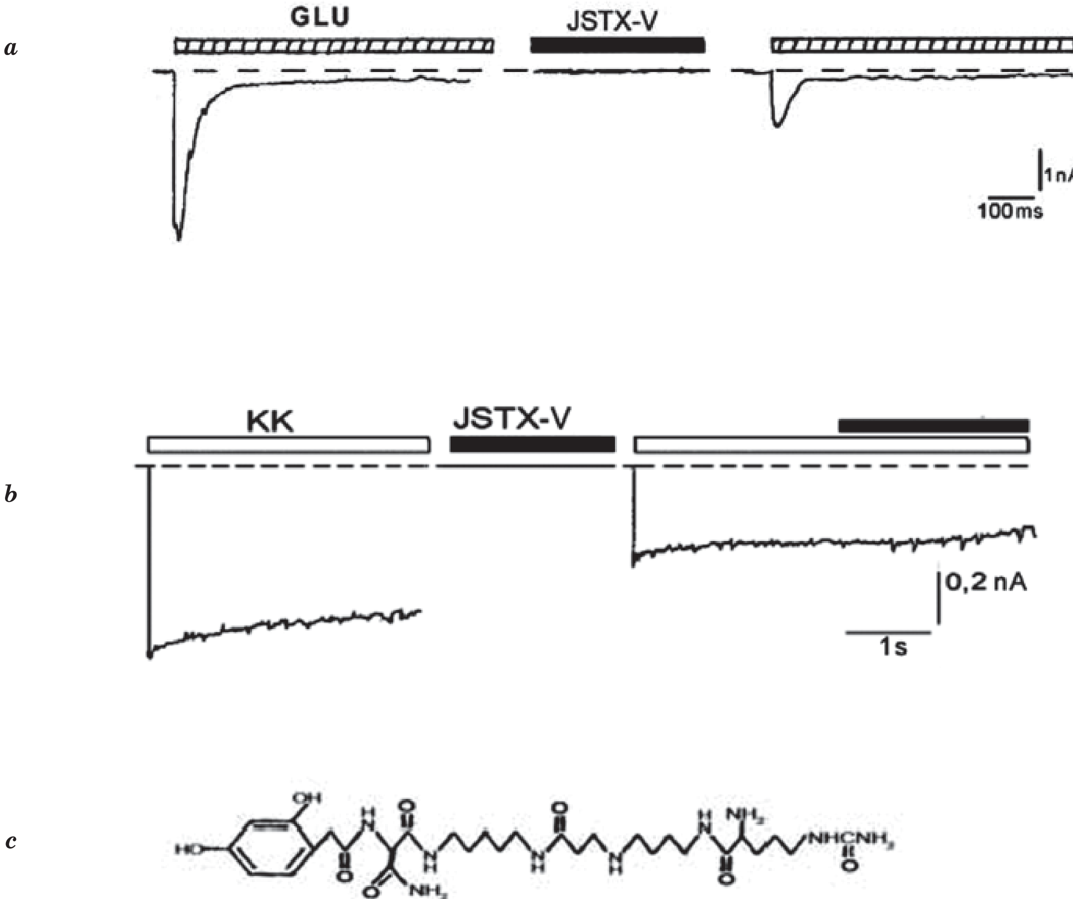

Fig. 4. **Electrical signals at the output of the BTS. Substance JSTX-V blocks chemo-activated transmembrane electrical currents in membrane of rat hippocampal pyramidal neuron:**
a — influence on glutamate-activated (Glu) currents; *b* — influence on kainat-activated (KK) currents. After the registration of the control responses to Glu and KK, the membrane was maintained in Ringer solution with JSTX-V ($2.5 \cdot 10^{-4}$ units/μl) during 3 min. In this experiment the amplitudes of chemo-activated currents decreased after the JSTX-V influence. Experiment with JSTX-V re-application in the same concentration ($2.5 \cdot 10^{-4}$ units/μl) on the background of KK is shown on (*b*). Concentrations of Glu and KK were 1 mmol/l (*c*). Chemical structure of JSTX-3 — active compound of JSTX-V and antagonist of chemo-activated electrical currents V hold — 100 mV [164, 173–178]

after the influence of chemical substances on the cells' surfaces. The most fast processes of electrical nature is possible to record in this time interval. There are changes in neuronal chemosensitive transmembrana electrical currents (CTE-currents) under the influences of different chemical substances (as well as PID-PR influences). CTE-currents' registrations there were possible to do using microelecrode techniques, in voltage-clamp conditions, patch-clamp, and others.

The devices, equipment and methods used there (both standard and newly developed) were elaborated and used in complex. This complex with studied neuronal membrane or membrane of other type of cels (let's call them "biological fragment" — BF) served, in fact, as "biodetector" and "bioanalyzer" for chemicals, applied to BF. To realize this step there were elaborated the new methods: biological cells dissociation, their cultivation, testing of PID-PR influence on trans-membrane currents in cells, some PID-PR diagnostics [172–183]. For these the results of many-year experimental author investigations of electrical signals and processes in natural membranes of neurons (MN) were described. Also there were described experimental data about electrical chemo-activated trans-membrane currents with molecular structures in MN gated them, as

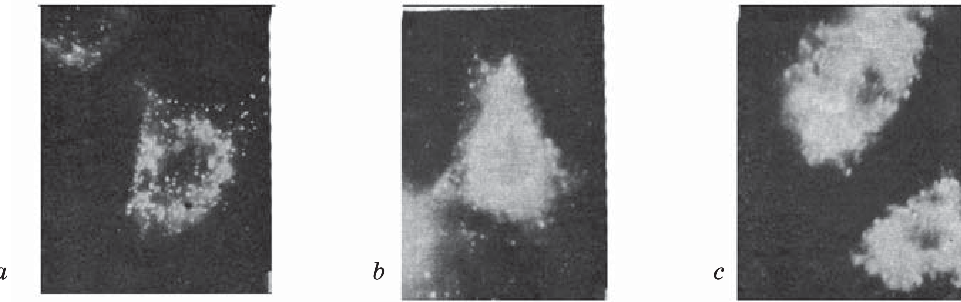

Fig. 5. **Strengthening the brightness of molecular complexes of primulin-protein in neurons after the agonists action:**
fluorescent granules contained complexes of primulin with proteins of the cytoplasm: *a* — control. Weak fluorescence at lack of action of agonists; *b* — enhanced fluorescence of neurons after 20 minutes from the start of action of excitatory agonists (*b* — GABA; *c* — acetylcholine) [1, 17]

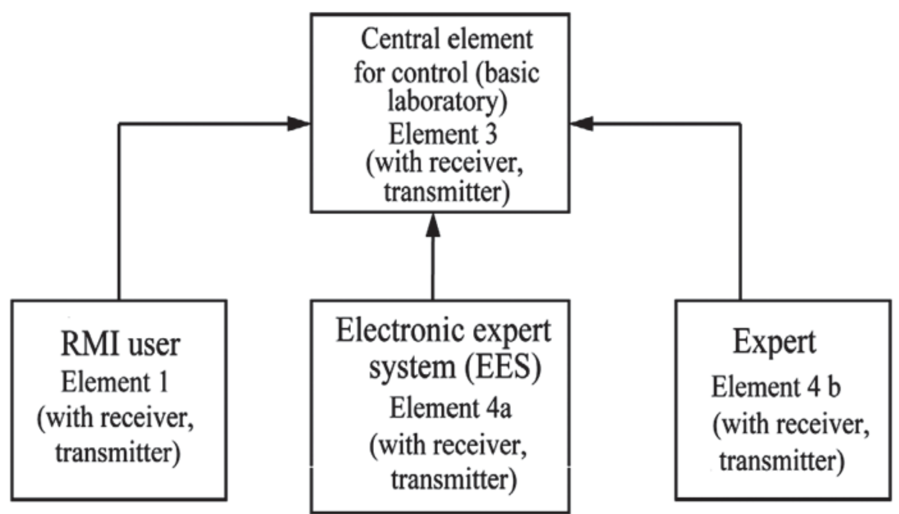

Fig. 6. **Fragment of structural scheme of IS for tracing of insects' population states**

well as methods of such current characteristics control by some organic molecules, like PIDPR. The methods of enhancing of MN chemoactivated currents amplitudes registered in experiments and their perfected revealing among noises were elaborated and patented [172, 177]. Some possible ways of insertion of this complex of devices for electrophysiological experiment into the technical IS were suggested.

The obtained data were important for elaboration of the newest biosensors — electronic elements of technical systems, IS for eco-monitoring, new technical expert systems for detection and diagnosis of ecotoxins in the environment. The appropriate methods and means of the parameters measuring of electrical information signals from neurons were considered. Namely, the experiment examples with voltage-clamp and concentration-clamp on MN were done as well as activation under these conditions of CTE-currents — glutamate-activated (Glu) and kainat-activated (KK-). As it is shown in the work, this control could be achieved, for example, by acting on the MN of the mammal central nervous system by specific toxins — blockers (BTx) of Glu — and KK-activated channel-receptor complexes (or antagonists of Glu — and KK-activated channel-receptor complexes) with the purpose to block CTE-currents. At the same time, under the conditions of the experiments, there were registered the interaction between three types of molecular structures: molecular channel-receptor complex (CRC), agonist molecules A (Glu, KK), and the molecules of CTE-currents' antagonists (BTx).

On Fig. 3 one can see the schematic description of all complex work done in the developed system, from primary registration of bioelectrical signals in the expriment under the influence of different chemical substances on neuronal membranes (left) — to computer

processing of such signals (values averaging and further processing; A — at middle) — and than to the development of mathematical and program models (M — at right).

The main experimental results were described and analyzed in [16, 17, 26, 143, 153, 163, 164, 173-183], the brief list is below.

– The method of amplification of 11.8 times the amplitudes of CTE-currents (electrical signals) at the output of the sensor and the improvement of detection of them at the background of noise during registration in experiments was developed, which is important both for the registration of CTE-currents; the patent was obtained on this method [172, 177];

– algorithms for preparation of BF element of sensor — neuronal membranes (MN) from hippocampus of the rat central nervous system and algorithms for carrying out the experiments, data on the registered physical and chemical properties of experimental objects;

– the experiment results on the registration of CTE-currents — signals at the output of the sensor group, which represent a series of digitized records of CTE-currents in response to the activation of CRC molecule by agonists (A) with known chemical structure of the molecules;

– there were obtained the data on the control of the output signals of BTS by blocking or modifying of the registered CTE-currents after the influence of six different specific toxin (JSTX-V, JSTX-3, AR-V, AR, ARN-1, ARN-2) on the CRC. The molecules chemical structure of four investigated antagonists was established, the other two antagonists were the mixture of the substances.

Data from the series of studied effects of various types of antagonists were summarized in the DB and relative tables. The effects of all studied antagonists' infuences on CTE-currents were similar, but they were characterized also by number of distinct features, which allowed the development of certain approaches for the creation of new methods of qualitative and quantitative analysis of organic substances with toxic effects in the environment.

Further there were done:

– mathematical processing and analysis of the experiments results described above on the registration of CTE-currents at the output of the BTS;

– generalizations and conclusions regarding the correlation of characteristics of the blocking effect of CTE-currents influenced by the toxins with differences in chemical structure, in particular, with different lengths and structure of polyamine;

– elaboration of mathematical models of registered effects under the influence of specific antagonists of Glu — and KK-currents (3 phases of interaction between molecules were considered);

– material on the application of mathematical cluster methods to distinguish similar features in registered effects.

In order to develop fundamentally new methods for qualitative and quantitative analysis of organic pollutants in the environment, the dose-effect dependences for coupling of studied substances were investigated in the experiments. The values of Kd for all possible cases for the action of all antagonists were calculated (for JSTX-3 as well as AR and other antagonists from *Araneidae* venom). The dose-effect dependencies were single-bonded isoterms. They demonstrated the lack of co-operability. It was shown that the magnitude of the amplitudes of currents under the action of AR decreased by 2.7 times, but the nature of dose-effect dependence had not been changed. Consequently, AR did not compete with KK for binding sites on the receptor according to the results of our experiments.

The obtained dependences were proposed to put in base of new methods of detection, quantitative and qualitative analysis of the presence in environment some organic substances-pollutants. Thus, in the samples from industrial territories contaminated with organic harmful, toxic substances [190], on the basis of these dependencies it becomes possible to detect and pre-diagnose the approximate type of chemical pollutant. The regularities of the action of phenol and indole derivatives with polyamine radicals of different lengths and complexity on the CTE-currents were studied [16, 17, 26, 143, 153, 163, 164, 173–183]. The conclusions about the correlation between the chemical structure of various chemical substances (incuding pollutants) with their physiological effect on electric currents both on the molecular level and on the level of organisms were made.

Peculiarities of monitoring at the second time interval (10 min — few hours) after the influense of chemical substances. In this time interval it was possible to register biochemical processes in neurons "in response", after the influense of chemical substances on the cells. These effects were revealed by using the method of neurons retrograde marking by fluorescent markers *in vivo*. Optical studyings

were done using UV-microscopy — "LUMAM" (fluorescent microscope from "Carl Zeiss", Iena, Germany) [1, 17]. For this step realization there were developed the method of neuron state registration using some fluorescent markers that actually gave possibility to visualize the coupling between electrical and chemical changes in neurons with their optical characteristics. Results of these experiments enables to visualize the changes in electrical characteristics of the system as sets of images with their future ordering in databases (DB). Fluorochromes primulin and bis-benzimide were used for these experiments. The signal was received when molecules of agonist (A) were applied to MN at concentrations of approximately 10^{-4} mol/l. Before this, the molecules of fluorescencent marker primulin were introduced inside the neurons using retrograde axon transport; and they formed complexes with proteins in cell soma. The experiments technique at all stages were described [1, 17], ending with observations on changes of optical characteristics of neurons in thin sections of rat brain with the help of luminescent microscope in the mode of incident light (Fig. 5).

From the experiments shown at Fig. 5 it is possibe to see that before the action of the exciting signal (*a*) the brightness of the fluorescent marker primulin was much weaker than after acting on the MN of the agonists (*b*, *c*). After the action of the agonist molecules, the number of light granules in the cytoplasm of the neurons increases tremendously, the brightness of each granule increased (from the photo it is seen that the size of each granule increased), their number increased in the zone of the nucleus with the formation of the ring. Similarly, the changes in the characteristics of the fluorescing complexes caused other agonists as well, we had studied 5 agonists [1, 17]. In their ability to cause the effect of fluorescence change, the agonists could be arranged in the following sequence:

adrenaline > acetylcholin e> GABA >
> glycine > serine.

In case of application of the method of neurons' marking using retrograde axon transport, the processes of neurons were usually well visible: their trajectories were marked with luminous granules of primulin-proteins. Under the action of agonists on the neurons, the "marked" parts of the processes were significantly lengthened, exceeding two or three diameters of the neurons.

Mathematic and program modeling of different phases of electrical impulses development in MN in framework of studied systems were done. There also were developed some mathematical and program models of systems with the use of studied effects, the principles of information coding by such systems were suggested [1, 25, 72].

Monitoring at the third time interval — long-term monitoring during few months and years. This long-term monitoring was realized by studying of changes in biological organisms. For such studyings we selected as organisms-bioindicators insects *Noctuidae (Lepidoptera)* — single insects, species and their populations. Insects were collected using light traps, field collections were done as well.

As a result of the works for the development of monitoring system in the third time interval — long-term monitoring of environment — the original IS with DB of images were developed and suggested for use in ecological scientific and academic practice, for environment protection. Detailed analysis and studying of peculiarities of biological objects and necessity to use of mathematic and other methods that were not used before became the basis for the DB development [166–170]. The series of these works were continued by the elaboration of some IS with DB, including DB of images, and electronic working places linked with DB for professionals of few specialties (ecologists, zoologists, and some others).

The complex of the works done conserning this time interval included practical development of electronic systems for monitoring of environment, for example, using monitoring of bioindicators (*Noctuidae, Lepidoptera*) in different regions of Ukraine and neighboring countries. As it was written above the biotechnical system developed for such purposes is called "EcoIS", it fragment is suggested on (Fig. 6). Developed technical ISs with databases for *Noctuidae* (*Lepidoptera*) basing on the results of their study in the mountains of Elbrus region (Caucasus, Russia) during environmental eco-monitoring in extreme conditions was described as well [1, 9, 10, 174, 175, 176, 178]. It should be noted that the adaptation of bioorganisms in extreme conditions takes place according two strategies, and strategy of adaptation of insects differs from the strategy of adaptation of mammals.

The list of the works done in the framework of the third time interval includes the following materials [1, 9, 10, 174, 175, 176, 178]:

– problems of the network IS development with databases were discussed; there were

observed IS with databases of images, ISs with distributed databases;

– problems of designing the database for ecology, according to eco-monitoring results basing on the results of the study of bioorganisms in extreme conditions were observed and discussed;

– general overview of the methods of mathematics and computer modeling in the field of environment protection, other spheres of medicine and biology were done;

– algorithms of ecomonitoring of fauna of different ecosystems with the use of the possibilities of academic IS and networks with distributed bio-organism databases were presented;

– possibility of development of IS for eco-monitoring with databases of images have been demonstrated;

– the effectiveness of the newest methods of ecological monitoring of bioorganism populations on the basis of network IS with distributed databases was demonstrated including mathematical analysis of the data obtained in conditions of monitoring points distributed on the territory of the country;

– results of the work on the development of "EcoIS" system for eco-monitoring were obtained (Fig. 7). "EcoIS" is a "system of academic destination" [1, 7, 8]. Being electronic networking system with distributed *Noctuidae* (*Lepidoptera*) databases, "EcoIS" is

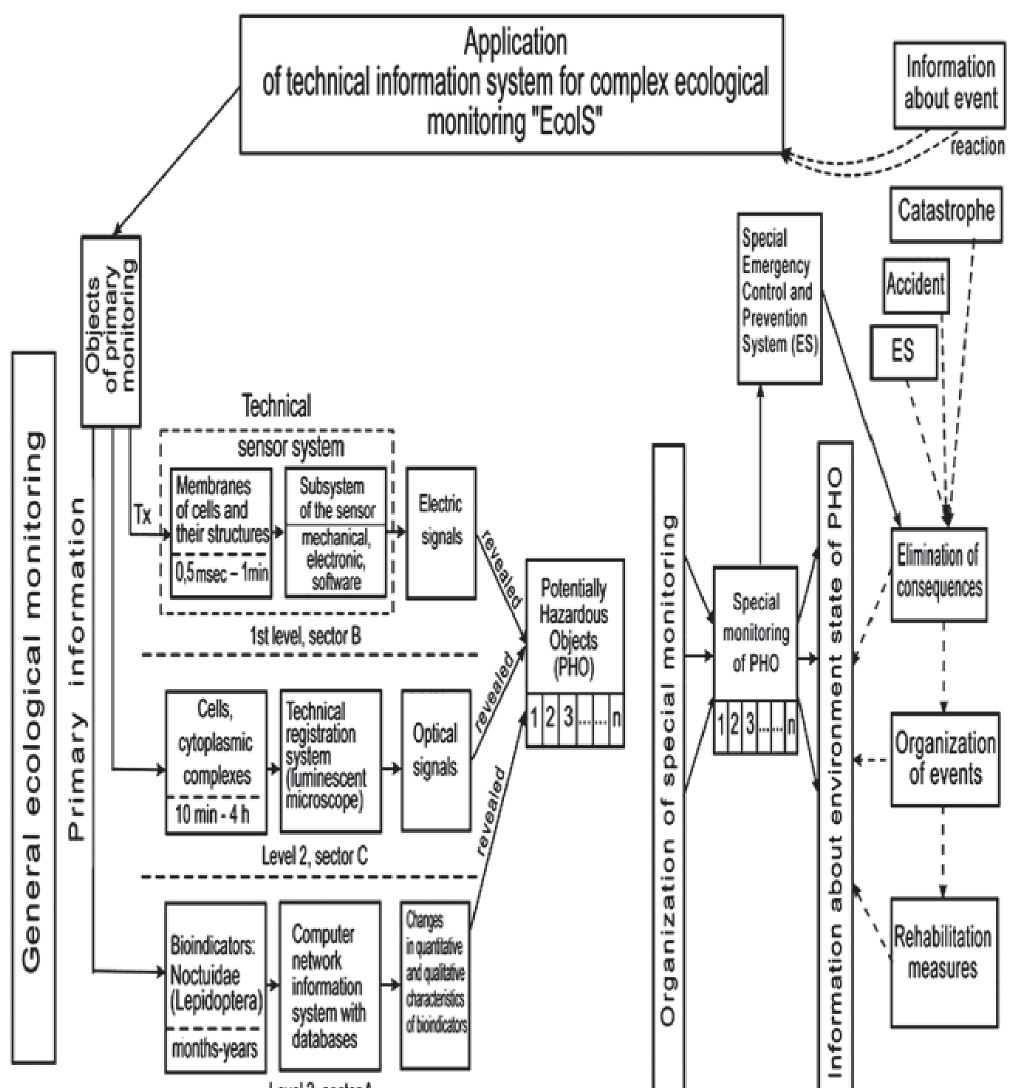

Fig. 7. **Application of the technical system "EcoIS" for monitoring of bioobjects of different levels of the hierarchy:**
the methods used in the various sectors of "EcoIS" allowed to receive qualitatively new information in comparison with the previous ones and to realise qualitatively new monitoring possibilities

possible to use for ecomonitoring in different regions of Ukraine;

– application of the developed methods and results of monitoring of bioindicators in the Striltsivskyi Steppe Preserve (Luhansk region, Ukraine) and for comparative analysis of some bioindicators in the mountain regions of Carpathians (Ukraine) and Caucasus (Russia) were demonstrated;

– authomatized electronic working places linked with DB for professionals of few specialties (ecologists, zoologists, and some others) were constructed. They became interfaces to "EcoIS".

At the end of the description of the work done it is necessary to emphasize two positons.

I. Scientific novelties of the work done were as follows:

– for the first time it was proposed the technical system of environmental data collection and processing in which the biotechnical sensors (detectors) were connected with electrical signals with measuring devices, computer means; the system also combined algorithms of data processing and methods of eco-monitoring;

– for the first time there were developed the methods and biotechnical devices — sensors (detectors), which allow measuring the influence of toxic substances much more accurately (by several orders of magnitude) compared to current ones;

– for the first time a new type of methods for quantitative and qualitative analysis of organic substances (incuding pollutants) was invented as a method, which allows to recognize approximately the chemical structure of organic compounds in dependence to their influence on transmembrane electric currents, so in dependence to physiological effects they occurred. Some patents were obtained for these methods [179–183]. These works formed the scientific basis for the development of the new technical systems for such organic substances detection and analysis.

II. Practical values of the obtained results are as follows.

– A software and analytical system were developed for ecological monitoring «EcoIS», which enabled the conducting works on eco-monitoring on various objects of Ukraine (in the regions of industrial pollution, in areas with extreme conditions, where such monitoring was not possible due to lack of funds or difficult access to these locations, and etc.).

– The inverse problem of organic chemical substances determining, the presence of pollutant molecules in the nature by their effects on the CTE-currents were solved. The theoretical dependence of the damaging effect of ecotoxins on their chemical structure was found. Such dependence might be the basis for the development of new technical expert systems for monitoring and analysis of some organic compounds in pollutants.

– There were elaborated the automatized electronic workplaces (ERM) and an improved analytical research complex for scientists of several specialties. Such ERM became interfaces for communication between human and "EcoIS" or other systems from this family.

– The described results in their different parts and at different years were implemented at the National Aviation University, the International Center for Astronomical and Medico-Ecological Studies (ICAMED) of the National Academy of Sciences of Ukraine in the Caucasus (Russia, the Kabardino-Balkaria Republic), at A. A. Bogomoletz Institute of Physiology and Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, Uman State Pedagogical University named after P. Tychyna. The obtained results were also used for monitoring of the bioorganisms of the Donbas — Striltsivskyi Steppe Preserve (Ukraine), at Ukrainian Polissia, in the extreme conditions of Ukrainian Carpathians and the Elbrus region (Caucasus, Russia).

Thus, in process of the work described above there were obtained the following results, partially defended by patents [172–183, 195–199].

1. The scientific basis was developed and the newest technical system for eco-monitoring was developed. It used a new type of the sensor groups as a technical means for the state of the environment monitoring. Accompanying laboratory, experimental methods and appropriate researches were done. The sensor model as part of a technical system for the diagnosis and testing of ecotoxins was elabrated. The corresponding software was developed.

2. The numerical characteristics of interaction for all studied toxic substances were investigated, mathematical description of the processes of CTE-currents blocking by them were performed. The general laws of the damaging action of toxic substances were established. Due to the phenolic and/or indole fragments of the molecule of a toxic substance, it interacts with the hydrophobic components of the membranes. Due to the polyamine — it interacts with the glutamate receptor (Glu-R), providing the main mechanism of currents' blocking. New methods of quantitative and qualitative analysis of studied toxic and harmful organic compounds in environment were proposed.

3. The method to amplify by 11.8 times the amplitudes of CTE-currents in neuron membranes (MN) was elaborated. It enabled to improve electric signals detection against noise backgrounds. Using the elaborated method, experimental recording of CTE-currents became more perfect. So, all further electrophysiological recordings became more perfect as well. These methods were patented [172–177].

4. The methods of optical registration of processes of neurons excitation at the molecular level of the action of 5 different agonists were elaborated and applied in the experment. The method of retrograd dye axon transport was used for this.

5. Algorithms, mathematical and program approaches for elaboration of the databases (DBs) for the developed “EcoIS” system, ERM, others, taking into account the features of bioobjects were proposed.

6. ERM — automated electronic work places — were developed on the basis of the corresponding databases for use by scientists-biologists of several specialties (ecologists, neurotoxicologists, zoologists, etc.). They became interface to the system “EcoIS”. ERM were elaborated on the basis of network technologies, their structure, functions. The developed ERMs are easy to use and quite satisfactorily meet the requirements of the relevant experts in experimental and theoretical data.

11. An example of a network technical information system based on distributed databases with information on bioindicators *Noctuidae* (*Lepidoptera*) (the system “EcoIS”) was developed for the purpose of its use for professional monitoring of bioorganisms. This system included DB with the results of ecomonitoring for some regions of Ukraine (Striltsivskyi Steppe Preserve (Luhansk region), Karpathian Mountains) and Russia (Prielbrussie). It is possible to use this system for monitoring with the aim of environmental protection. For this purpose it has to include also environmental monitoring equipment for the detection of polluting organic compounds, ecotoxins, to ensure environmental safety around industrial enterprises, including damaged as a result of accidents, man-caused and ecological disasters, in the areas of military actions, as well as accidents at chemical enterprises, other accident zones with industrial pollution.

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БІОТЕХНІЧНІ ІНФОРМАЦІЙНІ СИСТЕМИ ДЛЯ МОНІТОРИНГУ ХІМІЧНИХ РЕЧОВИН У НАВКОЛИШНЬОМУ СЕРЕДОВИЩІ: БІОФІЗИЧНИЙ ПІДХІД

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Проаналізовано новітні біотехнічні системи екологічного моніторингу довкілля, що базуються на використанні сучасних інформаційних і комп'ютерних технологій та наявних баз даних хімічних речовин. Зокрема, розглянуто такі сучасні біофізичні методи досліджень, як імітаційне та програмне моделювання, що враховують результати автора, одержані в експериментах з реєстрацією хемочутливих трансмембранних електричних струмів у нейронах у режимі фіксації потенціалу, застосуванням флуоресцентних нейронних маркерів та обліком організмів-біоіндикаторів. Розроблені системи та методи дають змогу виявити та ідентифікувати небезпечні для живих організмів речовини і зробити висновки щодо їхнього можливого біологічного впливу. Функціонування біотехнічних інформаційних систем моніторингу довкілля проаналізовано в широкому часовому діапазоні з використанням сучасних баз даних, експертних підсистем та інтерфейсів, здатних ідентифікувати різні типи хімічних речовин. Показано, що за такого системного екологічного моніторингу існує можливість вивчати та прогнозувати наслідки дії речовин упродовж тривалого часу — від перших моментів впливу на окремі клітини організму до місяців і років після впливу на весь організм.

Ключові слова: біотехнічна інформаційна система моніторингу, забруднення довкілля, біоіндикатори, бази даних.

БИОТЕХНИЧЕСКИЕ ИНФОРМАЦИОННЫЕ СИСТЕМЫ ДЛЯ МОНИТОРИНГА ХИМИЧЕСКИХ ВЕЩЕСТВ В ОКРУЖАЮЩЕЙ СРЕДЕ: БИОФИЗИЧЕСКИЙ ПОДХОД

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Проанализированы новейшие биотехнические системы экологического мониторинга окружающей среды, основанные на использовании современных информационных и компьютерных технологий и имеющихся баз данных химических веществ. В частности, рассмотрены такие современные биофизические методы исследований, как имитационное и программное моделирование, учитывающие результаты автора, полученные в экспериментах с регистрацией хемочувствительных трансмембранных электрических токов в нейронах в режиме фиксации потенциала, применением флуоресцентных нейронных маркеров и подсчетом организмов-биоиндикаторов. Разработанные системы и методы позволяют выявить и идентифицировать опасные для живых организмов вещества и сделать выводы относительно их возможного биологического воздействия. Функционирование биотехнических информационных систем мониторинга окружающей среды проанализировано в широком временном диапазоне с использованием современных баз данных, экспертных подсистем и интерфейсов, способных идентифицировать различные типы химических веществ. Показано, что при таком системном экологическом мониторинге существует возможность изучать и прогнозировать последствия действия веществ на протяжении длительного времени — от первых моментов воздействия на отдельные клетки организма до месяцев и лет после воздействия на весь организм.

Ключевые слова: биотехническая информационная система мониторинга, загрязнение окружающей среды, биоиндикаторы, базы данных.

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Russulaceae FAMILY MUSHROOMS LECTINS: FUNCTION, PURIFICATION, STRUCTURAL FEATURES AND POSSIBILITIES OF PRACTICAL APPLICATIONS

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The purpose of this paper was to analise the results of own author's research and literature date that concerned lectins of *Russulaceae* family mushrooms, which, despite their widespread, are still poorly investigated. Most studies merely reported about the determination of hemagglutinating activity and the isolation of pure lectin preparations from fresh fruit bodies of this family mushrooms.

This article provides information about lectins physiological role in mushrooms, the list of *Russulaceae* family spesies tested for hemagglutinating activity, as well as procedure of purification, molecular structure and carbohydrate specificity of isolated lectins. Particulary, the most effective methods of lectins purification of the *Russulaceae* family were highlighted.

High lability of the lectins molecules explaned the loss of the activity of these lectins during purification from raw material when standard procedures were applied, as well as the reason why these lectins were not obtained from the dried fruit bodies.

Finally, practical application of lectins of *Russulaceae* family mushrooms in medical-biological researches are described.

Key words: lectins, *Russulaceae*, hemagglutinating activity, carbohydrate specificity.

Lectins are a class of multivalent carbohydrate-binding proteins of non-immune origin, which recognize various carbohydrate-containing structures with a high degree of stereospecificity without catalytic activity [1, 2].

The carbohydrate specificity of sugars and glycoproteins is the cause of selective binding of lectins to certain types of cells and tissues of living organisms [3].

Lectins specificity towards cells (associated with the structure of glycans on their surface) manifests in the ability to agglutinate certain cells, such as erythrocytes belonging to a particular human or animal phenotypes [4], to precipitate glycans and cause various biological effects [5, 6].

Lectins were found in all kingdoms of living organisms: viruses, bacteria, plants, mushrooms and animals [2, 7]. The vast majority of commercially available lectins are obtained from plants, but lectins of true fungi also are of great interest [8, 9]. Their fruiting bodies are able quickly accumulate significant biomass, which can be used for obtaining pure lectins. Our attention was attracted to the lectins of *Russulaceae* family mushrooms, which, despite widespread occurrence of these mushrooms, are poorly investigated at present [10]. On the basis of own research and studies of other authors, this review attempts to explain the reason for this fact and outline the potential for practical application of *Russulaceae* family lectins.

Function

The family *Russulaceae* with two genera — *Lactarius* (the milk-caps) and *Russula* (the brittlegils), has a significant species diversity (there are 750 species of genus *Russula* and 450 species of genus *Lactarius* documented worldwide [11]), and consequently, a large number of potential sources of biologically active substances.

Almost all specimens of this family form mycorrhizal associations with trees and

shrubs, particularly with the main forest tree species (pine, beech, oak, spruce, fir, etc.) [12, 13]. Lectins are involved in the mycorrhizal symbiosis [14].

The physiological role of lectins in fungi is associated with the specific recognition of glycosylated structures at the level of cells, tissues and the whole organism. Lectins in mushrooms participate in the mobilization and transportation of sugars, in the process of forming fruiting bodies (the formation of primordia), in the creation of mycorrhiza and the infection process (penetration of parasitic fungi into the host organism) [15].

The fungal lectins are involved in the recognition of the symbionts during the early stages of mycorrhizae formation. In the *Lactarius deterrimus-spruce* model, the facts that there is a lectin at the surface of the mycelial hyphae and the presence of specific sites for the lectin on the roots hairs are evidence of the involvement of the lectin in the recognition of the host spruce. Thus, the morphologically very similar *L. deterrimus, L. deliciosus* and *L. salmonicolor* are associated with the the spruce *(Picea),* pine *(Pinus),* and the fir *(Abies),* respectively with a remarkable specificity [16].

Detection

Detection of lectins in mushroom extracts is most often carried out using hemagglutination inhibition assay with human and animal erythrocytes, method can be found in reference [2, 17].

A large number of basidiomycetes were screened using the hemagglutination test. Particularly, among the 104 species, collected in Poland, aqueous extracts from *L. rufus* and *L. vellereus* showed high agglutination activity and were among ten the most active. The extract from *R. cyanoxanta* had distinctly higher agglutination activity at 4 °C, than that obtained at room temperature [18].

Agglutinins were well represented by *Lactarius* species among 403 British higher fungi, but fewer by *Russula* species (Table 1) [19]. However, in a study of 110 species of Japanese fungi only *R. emetica*, one of six *Russula* species, did not exhibit hemagglutination. All species that were active against human erythrocytes also agglutinated rabbit red blood cells, except *R. violeipes* that agglutinated only human cells [19–20].

It's interesting that the ability to agglutinate human erythrocytes of all ABO groups had dried mushroom caps of 21 species of the genus *Lactarius* (out of 48 studied), which were stored in herbarium for 8–9 years! The intensity of agglutination reached 1: 64 for the *L. cremor, L. quietus, L. piperatus* extracts, and 1: 32 for the *L. torminosus, L. controversus, L. thejogalus* [21].

Lactarius and *Russula* species of the Ukrainian Carpathians also have hemagglutinating activity toward human erythrocytes. Though the extracts of investigeted fungi did not show group specificity, rabbit, dog and guinea pig erythrocytes showed approximately the same activity. Horse and dog erythrocytes were often more sensitive to *Russulaceae* lectins, while cow and goat erythrocytes frequently showed low sensitivity to these lectins [22, 23].

So, no blood group-specific lectin among *Lactarius* and *Russula* species was found. Only *R. queletii* exhibited specificity to pigeon and horse erythrocytes [18].

We have noticed a significant variability in titers of hemagglutination, depending on the age of fruiting bodies, weather and temperature conditions, even within one mycelium (measurements were performed on mycelia of *L. torminosus* and *L. pergamenus*). Activity may differ more than in 250 times! Although clear correlation between the age of mushroom and the titer of hemagglutination was not found, in general, younger mushrooms had higher hemagglutinating activity than older ones [22, 24]. Evidently, it is much easier to detect lectins in extracts at the moment of their highest activity. According to our observations, higher activity of lectins correlates with their higher content.

Other researchers, in particular [18, 19] came to the same conclusion that caps of young carpophores are usually richer in lectins.

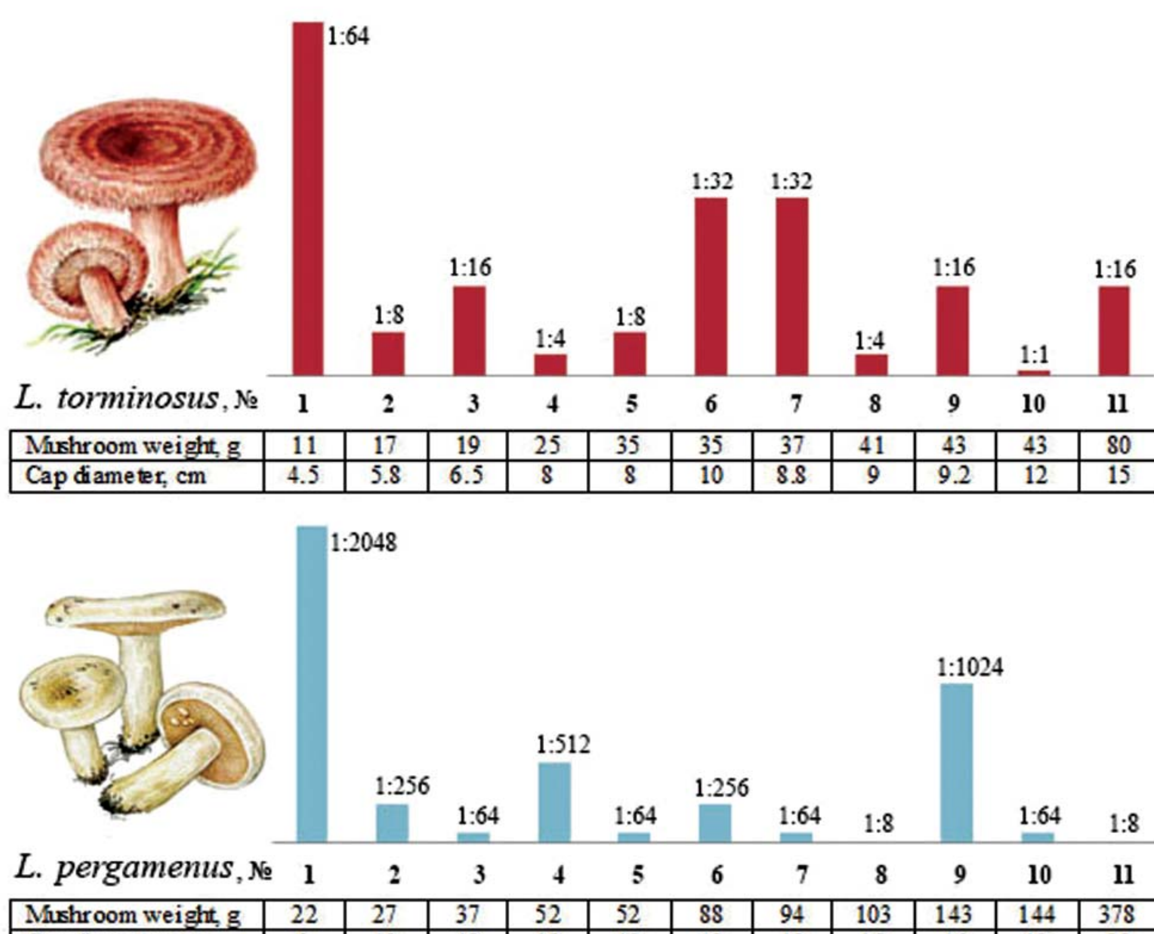
Purification

Lectins are proteins or glycoproteins that consist of one or more polypeptide chains. Procedures that are customary for purification other proteins (precipitation with salts, ion-exchange, affinity and gel chromatography, preparative electrophoresis, etc.) can be used to purified them [1, 2]. However, unlike lectins of other basidiomycota mushrooms, they have certain features. This is due to the high sensitivity of *Russolaceae* family lectins to pH changes, salt precipitation and organic solvents, drying and freezing of raw materials. At the same time, these lectins, can usually withstand heating to 65÷70 °C [22, 23]. This should be taken into consideration when purifying them.

Table 1. **List of Russulaceae family spesies tested for hemagglutinating activity**

No	*Lactarius* DC. ex S. F. Gray:	References	No	*Russula* (Fr.) S. F. Gray:	References
1	*L. aurantiacus* Fr.	[21]	1	*R. adusta* (Pers.) Fr. **	[19]
2	*L. blennius* (Fr.) Fr.	[21,19]	2	*R. aeruginea* Lindblad	[19, 23]
3	*L. controversus* (Fr.) Fr.	[21,19]	3	*R. alpina* (A. Blytt) F. H. Møller & Jul. Schäff **	[19]
4	*L. cremor* Fr.	[21]	4	*R. atropurpurea* (Krombh.) Britzelm.	[19, 23]
5	*L. decipiens* Quel.	[21]	5	*R. carminea* (J. Schaeff.) Kühner & Romagn.	[19]
6	*L. deliciosus* (L.) Gray	[18, 21, 25, 19, 22]	6	*R. claroflava* Grove	[19]
7	*L. deterrimus* Gröger	[26,19]	7	*R. cyanoxantha* (Jul. Schäff.) Fr.	[19, 23]
8	*L. flavidulus* S. Imai	[27]	8	*R. delica* Fr.	[32]
9	*L. fluens* Boud.	[19]	9	*R. emetica* (Schaeff.: Fr.) S.F. Gray*	[18]
10	*L. glaucescens* Cossl.	[19]	10	*R. farinipes* Romell.	[19]
11	*L. glyciosmus* Fr.	[21]	11	*R. flavida* Frost &Peck apud Peck	[20]
12	*L. helvus* Fr.	[21]	12	*R. foetens* (Fr.) Fr.	[23]
13	*L. laeticolor* (Imai) Imaz.	[20]	13	*R. grisea* (Pers. ex Secr.) Fr.	[19, 23]
14	*L. lignyotus* Fr.	[28,21]	14	*R. illota* Romagn.	[19]
15	*L. necator* (Bull. ex Fr.) Karst.	[22]	15	*R. laurocerasi* Melzer	[19, 20]
16	*L. pergamenus* (Fr.) Fr.	[21, 22, 24]	16	*R. lepida*	[33]
17	*L. piperatus* (Scop.) Gray	[21, 19, 29, 22]	17	*R. nigricans* (Bull. ex Merat) Fr.	[19, 23]
18	*L. porninsis* Roll.	[21]	18	*R. paludosa* Britzelm.	[19]
19	*L. pubescens* Fr.	[21]	19	*R. queletii* Fr.	[18, 19]
20	*L. quietus* (Fr.)	[21, 19, 20, 22]	20	*R. rosacea* (Pers.) S. F. Gray	[20]
21	*L. rufus* (Scop.) Fr.	[18, 19, 30, 22]	21	*R. sardonia* Fr.em Romagn. ***	[19]
22	*L. ruginosus* Romagn.	[19]	22	*R. subfoetens* Smith	[19]
23	*L. salmonicolor* R. Heim & Leclair	[21, 31]	23	*R. vesca* Fr.	[19]
24	*L. sanguifluus* Paul ex Fr.	[21]	24	*R. violeipes* Quel.	[20]
25	*L. serifluus* DC.ex Fr.	[21]			
26	*L. subvellereus* Peck	[20]			
27	*L. subzonarius* Hongo	[20]			
28	*L. tabidus* Fr.	[19]			
29	*L. thejogalus* (Bull.) Fr.	[21]			
30	*L. torminosus* (Schaeff.) Gray	[21, 19, 30, 22]			
31	*L. turpis* (Weinm.)**	[19]			
32	*L. vellereus* (Fr.) Fr.	[18, 21, 19, 22]			
33	*L. vietus* (Fr.) Fr.	[19]			
34	*L. volemus* (Fr.) Fr.	[19, 22]			
35	*L. zonarioides* K hner & Romagn	[21]			

* Hemagglutination only with trypsin treated erythrocytes
** Hemagglutination only with bromelin treated erythrocytes
*** Hemagglutination with neuraminidase treated erythrocytes



L. torminosus, №	1	2	3	4	5	6	7	8	9	10	11
Mushroom weight, g	11	17	19	25	35	35	37	41	43	43	80
Cap diameter, cm	4.5	5.8	6.5	8	8	10	8.8	9	9.2	12	15

L. pergamenus, №	1	2	3	4	5	6	7	8	9	10	11
Mushroom weight, g	22	27	37	52	52	88	94	103	143	144	378
Cap diameter, cm	8	10	12	12	13	13	13	15	16	18	28

The titres of haemagglutination in sap from *L. torminosus* and *L. pergamenus* within one mycelium

Although many *Russulaceae* family species showed agglutination activity there are only several publications on obtained lectins (Table 2).

Not always the hemagglutinating activity of *Russulaceae* lectins is remained entirely by the precipitation of lectins with ammonium sulfate and ethanol, which are most often used in the purification of protein molecules [22, 23].

It should be noted that all authors purified lectins only from raw materials harvested fresh. None of the cited studies didn't explain why these lectins were not obtained from dried fruit bodies. In own research [22, 23], it has been shown that the hemagglutination activity strongly reduces or completely disappears after drying, as well as freezing of most species of this family mushrooms.

The reason for this, in our opinion, is the loss of activity of these lectins during purification from raw material using standard procedures.

Affinity chromatography is the most effective method for the purification of the lectins. The affinity sorbents used for their isolation were: stromas of erythrocytes embedded in polyacrylamide gel [25, 26, 31], copolymer of polyvinyl alcohol with a blood group specific substance [30, 34, 29, 24], fetuin immobilized on Sepharose [28]. The purification of other lectins (*L. flavidulus, R. delica, R. foetens, R. lepida*) [27, 32, 23, 33] was carried out by a combination of ion-exchange chromatography and gel filtration. However, in our opinion, these methods are more labour-intensive and less productive than affinity chromatography.

Lectins were eluted from affinity sorbents either with 0.1 M borate buffer (pH 9.0) [28] or heated to +65 °C 1 M NaCl. Specific carbohydrate was not used to remove the lectin from a column due to the absence of interaction of these lectins with monosaccharides and low-cost di- and oligosaccharides.

The elution of lectins by acidic buffer is not recommended because it can cause loss of

Table 2. **List of isolated lectins of *Russulaceae* family species**

Species	Isolation	Structural Properties	Sugar Specificity	Refe-rences
L. lignyotus	By affinity chromatography on Sepharose 4B containing immobilized fetuin	98 kDa, Tetrameric with 22 kDa subunits	Desialyzed fetuin, desialyzed glycoprotein from edible bird's nest and desialyzed mucin from porcine submaxillary glands	[28]
*L. deliciosus**	By a combination of affinity chromatography on stromas of group O erythrocytes embedded in polyacrylamide gel and hydroxylapatite and gel filtration chromatography * Isolated from the carpophores, lectins are also expressed at the surface of the hyphae	37 kDa, Dimeric with two types of subunits (about 19,000 and 18,000)	All the lectins are most specific for DGalβ1-3DGlcNAc residues (TF antigen)	[25]
*L. deterrimus**		37 kDa, Dimeric		[26]
*L. salmonicolor**		39 kDa, Dimeric		[31]
L. rufus	By affinity chromatography on copolymer of polyvinyl alcohol with a blood group B specific substance	98 kDa, Hecameric with 17 ± 1 kDa subunits	Group-specific substances from human blood erythrocytes, asialo-BSM, asialo-ovomucoid, human and bovine thyroglobulins, orosomucoid, fetuin, transferrin, human Ig G, α-phenyl N-acetyl-D-glucosaminopyranoside, 4-nitrophenyl-β-D-galactopyranoside	[30]
L. torminosus		98 kDa, Hecameric with 17 ± 1 kDa subunits	Fetuin, asialo-BSM, BSM, group-specific substances from human blood erythrocytes, human Ig G, transferrin	[34]
L. piperatus		97±3 kDa	Group-specific substances from human blood erythrocytes, human Ig G	[29]
L. pergamenus	By a combination of ethanol precipitation, affinity chromatography on copolymer of polyvinyl alcohol and human blood B group specific substance, and ion exchange chromatography on DEAE-Toyopearl	96 kDa, Hecameric with 16 kDa subunits	The lectin weakly interacts with DGalNAc, while DGalβ1-3DGalNAc and DGalβ1-3DGlcNAc are the most probable candidates for ligands, with which the lectin interacts at disaccharides level. Among them fetuin of fetal calf serum and group-specific substances A, B, and H of human blood were the strongest	[24]

Table 2. Continued

Species	Isolation	Structural Properties	Sugar Specificity	Refe-rences
L. flavidulus	The chromatographic procedure utilized comprised anion-exchange chromatography on DEAE-cellulose, cation-exchange chromatography on CM-cellulose, anion-exchange chromatography on SP-Sepharose and gel filtration by fast protein liquid chromatography on Superdex 75	29.8 kDa, Dimeric	Lactose, *p*-nitrophenyl α-D-glucopyranoside, *p*-nitrophenyl β-D-glucopyranoside, inositol and inulin	[27]
R. delica	It was adsorbed on both SP-Sepharose and Q-Sepharose and unadsorbed on DEAE-cellulose	60 kDa, Dimeric with 30 kDa subunits	Inulin and *o*-nitrophenyl-β-D-galactopyranoside	[32]
R. foetens	By ion exchange chromatography on CM-cellulose	Not done	Asialo-ovomucoid	[23]
R. lepida	The purification scheme involved $(NH_4)_2SO_4$ precipitation, ion exchange chromatography on DEAE-cellulose and SP-Sepharose, and fast protein liquid chromatography-gel filtration on Superdex 75	32 kDa, Dimeric with 16 kDa subunits	Inulin and *o*-nitrophenyl-β-D-galactopyranoside	[33]

their activity. In particular, as our studies have shown [22, 23], *Russulaceae* lectins are quite enough stable at high temperatures, but are very sensitive to changes in pH: even a short-term reduction of pH to 4.5 often leads to a loss of 50–75% of hemagglutinating activity, and at pH 3.5 activity of many (but not all) species was completely lost. That's why the elution of almost all *Lactarius* lectins from the affinity column was carried out using 1 M NaCl solution heated to +65 °C. An attempt to eluate the *L. deterrimus* lectin by 0.1 M solution of N-acetyl-D-galactosamine, although was successful [26], did not allow the authors to obtain the expected activity of lectin. In their opinion it was due to incomplete unbounding of this carbohydrate from the lectin by dialysis.

The yield of lectins from fresh mushroom biomass varied from ≈3 mg/kg in *L. pergamenus* [24], 6.4 mg/kg in *L. deterrimus* [26] to ≈ 30 mg/kg in *L. deliciosus* [25].

Molecular structure

There is a small amount of data in the literature concerning the deep learning on molecular structure of lectins of the *Russulaceae* family mushrooms. In particular, there is no data on the crystallographic structure of lectins from mushrooms of this family in complex with a specific carbohydrate inhibitor.

According to the literature (Table 2), purified lectins consist of two (*L. deterrimus, L. salmonicolor, L. flavidulus, R. delica* and *R. lepida*) or four identical subunits (*L. lignyotus*), *L. deliciosus* lectin is also dimer, but has non-identical subunits, with a molecular weight of 18 and 19 kDa.

Except the *L. lignyotus* lectin, whose subunits are bound by disulfide bridges, other studied lectins of the *Russulaceae* family have subunits bound noncovalently.

According to our research, *L. rufus* and *L. torminosus* lectins consist of six subunits with a molecular weight of about 17 kDa,

and *L. pergamenus* — about 16 kDa. It was determined that the linkage between the individual subunits is very labile and the lectin molecule can easily disintegrate, possibly gradually, which is accompanied by loss of haemagglutinating activity. Herewith the hexamer possesses the highest specific haemagglutinating activity. Disintegration of the lectin molecule is taking place under the action of minor pH changes and even under the action of such soft factors as freezing or precipitation of the lectin-containing fraction of proteins with ammonium sulfate, not to mention about drying of mushrooms at +52 ± 2 °C [22, 23]. It should be noted that hexameric structure of a molecule is the phenomenon quite rare in lectins; not more than ten such molecules are described, the most famous is a lectin from an edible snail (*Helix pomatia*) [1, 2].

The data obtained by us explain the decrease of haemagglutinating activity by changes in the molecular structure of fungal lectins of the *Russulaceae* family, observed during drying and storage of the fruit bodies and throughout the purification of lectins. The disappearance of the hemagglutinating activity is due to the high lability of the quaternary structure of the lectin molecule; this fact probably also explains a small number of studies on lectins of a given family. However, lectins of the genus *Lactarius*, as compared with lectins of the genus *Russula*, are generally more resistant to such impacts, particularly towards drying, and therefore more promising for further research.

Carbohydrate specificity

The most important functional characteristic of lectins is their specificity to carbohydrates, which often determines the opportunities for their further use.

The literature suggests that *Russulaceae* family lectins rarely exhibit specificity to monosaccharides, and much more often to complex oligosaccharide structures, at least to disaccharides. Mostly such disaccharide is DGalβ1-3DGalNAc (TF antigen) [25, 22, 23].

The hemagglutinating activity of *L. flavidulus* lectin was inhibited by a variety of simple sugars, in particular lactose, *p*-nitrophenyl α-D- and β-D-glucopyranosides, inositol, and by the polysaccharide inulin [27]. The *L. deterrimus, L. deliciosus* and *L. salmonicolor* lectins have almost identical molecular mass and the same affinity for DGalβ1-3DGalNAc, a disaccharide which contains D-galactose and N-acetyl-D-galactosamine [25, 26, 31]. The same can be said of the *L. torminosus, L. rufus* and *L. pergamenus* lectins, the best sugars to interact with were complex oligosaccharides with minimal active disaccharide chains of DGalβ1-3DGlcNAc or DGalβ1-3DGalNAc. The best inhibitors of their activity were group-specific blood components human (ovariomucin) and human immunoglobulin G [34, 30, 24].

In case of *L. lignyotus* lectin the most effective inhibitors of its agglutinating activity were desialized fetuin, desialized glycoprotein from edible bird's nest and desialyzed mucin from porcine submaxillary glands [28].

Inhibitors of agglutinating activity of *R. delica* and *R. lepida* lectins were inulin and *o*-nitrophenyl-β-D galactopyranoside [32, 33].

Practical application

The selectivity of lectins to carbohydrate structures makes them very important tools for biochemical studying of glycoconjugates of the membrane and cell wall [35]. Thereby, due to the high specificity of binding of lectins to membrane carbohydrate structures, they have become extremely useful for the identification and differentiation of closely related species or strains of single-celled parasitic organisms. In particular, *L. controversus* and *R. nigricans* lectins characterized the variety of sugar structures of the cell walls of 114 pathogenic strains of the *Candida* genus. The lectin from *L. deliciosus* was used in researching the biology and taxonomy of the fungal organisms from the class of *Chytridiomycetes* inhabiting the alimentary canal in ruminants. *L. torminosus* lectin was used in researching glycoconjugates taking part in the identification reactions of embryonic cells in the urogenital morphogenesis in bird embryos [15].

Lectins from *R. delica* and *R. lepida* potently inhibited proliferation of HepG2 hepatoma and MCF 7 breast cancer cells [32, 33]. The lectin from *L. flavidulus* suppressed the proliferation of HepG2 hepatoma and L1210 leukemic cells [27]. Both above mentioned lectins from *L. flavidulus* and *R. delica* inhibited the activity of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase [27, 32].

The lectin of *L. deliciosus,* which is specific to DGalβ1-3DGalNAc, can be used to follow the expression the tumor-associated Thomsen-Friedenreich (TF) antigen [36].

The purified by us lectins from *L. torminosus* and *L. pergamenus* may find application as the histochemical reagents for the comparative histochemical investigation of kidneys of the newborn and adult rats [37, 38].

The key role of lectins in the formation of mycorrhiza between certain conifer tree species and *Russulaceae* family mushroom species has been proved.

The most effective method for the purification of lectins from the *Russulaceae* is affinity chromatography using sorbents with DGalβ1-3DGlcNAc structures (immobilized fetuin, blood group-specific substances). In the purification process should be taken into account high sensitivity of *Russulaceae* lectins to changes in pH and precipitation with ammonium sulfate and ethanol, their relatively thermal stability (up to + 70 °C).

The reason for the relative instability of the lectins of the *Russulaceae* family, in our opinion, is the features of the molecular structure. The molecules of these lectins consist of 2, 4 or 6 subunits, and the linkage between the individual subunits is very labile and the molecule of lectins can easily disintegrate (possibly gradually), which goes hand in hand with the loss of haemagglutinating activity.

Lectins isolated from widespread *Russulaceae* family mushrooms can find practical applications for the research of mycorrhizal symbiosis, as histochemical reagents in the detection of carbohydrate structures (for example, TF antigen), for the study of the topography of membranes and glycoconjugates of cell walls of various biological objects.

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ЛЕКТИНИ ГРИБІВ РОДИНИ *Russulaceae*: ФУНКЦІЇ, ОЧИЩЕННЯ, СТРУКТУРНІ ОСОБЛИВОСТІ ТА МОЖЛИВОСТІ ПРАКТИЧНОГО ЗАСТОСУВАННЯ

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Метою роботи є опис результатів власних досліджень та досліджень інших авторів, що стосуються лектинів грибів родини *Russulaceae*, які, незважаючи на велике поширення, на сьогодні недостатньо досліджені. Більшість робіт повідомляє лише про визначення гемаглютинувальної активності та виділення лектинових препаратів зі свіжих плодових тіл грибів даної родини.

У статті наведено інформацію про фізіологічну роль лектинів у грибах, перелік видів грибів родини *Russulaceae*, протестованих на гемаглютинувальну активність, а також процедуру очищення, структуру молекули та вуглеводну специфічність виділених лектинів. Розглянуто найбільш ефективні методи очищення лектинів родини *Russulaceae*.

Висока лабільність молекули пояснює втрату активності цих лектинів під час очищення із сировини з використанням стандартних процедур, а також неможливість їх отримання із сушених плодових тіл. Також описано практичне застосування лектинів грибів родини *Russulaceae* в медико-біологічних дослідженнях.

Ключові слова: лектини, *Russulaceae*, гемаглютинувальна активність, вуглеводна специфічність.

ЛЕКТИНЫ ГРИБОВ СЕМЕЙСТВА *Russulaceae*: ФУНКЦИИ, ОЧИСТКА, СТРУКТУРНЫЕ ОСОБЕННОСТИ И ВОЗМОЖНОСТИ ПРАКТИЧЕСКОГО ПРИМЕНЕНИЯ

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Целью работы является описание результатов собственных исследований и исследований других авторов, касающихся лектинов грибов семейства *Russulaceae*, которые, несмотря на широкое распространение, сегодня недостаточно исследованы. Большинство работ сообщает лишь об определении гемагглютинирующей активности и выделении лектиновых препаратов из сырых плодовых тел грибов данного семейства.

В статье приведена информация о физиологической роли лектинов в грибах, перечень видов грибов семьи *Russulaceae*, протестированных на гемагглютинирующую активность, а также о процедуре очистки, структура молекулы и углеводной специфичности выделенных лектинов. Рассмотрены наиболее эффективные методы очистки лектинов семейства *Russulaceae*.

Высокая лабильность молекулы объясняет потерю активности этих лектинов при очистке из сырья с использованием стандартных процедур, а также невозможность их получения из сушеных плодовых тел. Также описано практическое применения лектинов грибов семейства *Russulaceae* в медико-биологических исследованиях.

Ключевые слова: лектины, *Russulaceae*, гемагглютинирующая активность, углеводная специфичность .

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ANTIMICROBIAL ACTIVITY OF SURFACTANTS OF MICROBIAL ORIGIN

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The recent literature data about the antibacterial and antifungal activity of microbial surfactants (lipopeptides synthesized by representatives of genera *Bacillus*, *Paenibacillus, Pseudomonas, Brevibacillus*, rhamnolipids of bacteria *Pseudomonas, Burkholderia, Lysinibacillus* sp., sophorolipids of yeasts *Candida* (*Starmerella*) and *Rhodotorula*), and our own experiments data concerning antimicrobial activity of surfactants synthesized by *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 and *Nocardia vaccini* IMV B-7405 were presented. The analysis showed that lipopeptides were more effective antimicrobial agents compared to glycolipids. Thus, the minimum inhibitory concentrations (MIC) of lipopeptides, ramnolipids and sophorolipids are on average (μg/ml): 1–32, 50–500, and 10–200, respectively. The MIC of surfactants synthesized by the IMV B-7241, IMV Ac-5017 and IMV B-7405 strains are comparable to those of the known microbial lipopeptides and glycolipids. The advantages of glycolipids as antimicrobial agents compared with lipopeptides were the possibility of their synthesis on industrial waste and the high concentration of synthesized surfactants. The literature data and our own results indicate the need to study the influence of microbes' cultivation conditions on the antimicrobial activity of the final product.

Key words: microbial lipopeptides, rhamnolipids and sophorolipids, antibacterial and antifungal activity.

Biodegradation and non-toxic microbial surfactants are used in many fields due to their surface active and emulsifying properties, antimicrobial and antiadhesive activity. They are a useful alternative to standard chemical surfactants in various industrial, medical and nature conservation technologies [1–3].

Microbial surfactant research has a long history. In 1968 it was found that *Bacillus subtilis* AMS-H2O-1 could produce surfactin [4], in 1977 *B. subtilis* DS-104 was shown to produce iturin [5], and the first reports of rhamnolipids came from as early as 1940's [6], while their bactericidal properties were discovered in early 1970's [7]. However, despite this, the detailed studies of their antimicrobial properties commenced quite recently.

In 1997, Vollenbroich et al. established that the linopeptide produced by *B. subtilis* OKB105 at 0.032 mg/ml inhibits the growth of *Mycoplasma hyorhinis* and *Mycoplasma orale*, which can cause inflectional disease of the urinary tract. This was the first research into the antimicrobial action of that surfactin [8].

In 2001, Abalos et al. revealed antifungal action of seven homologues of rhamnolipids of *Pseudomonas aeruginosa* AT10, which at low concentrations (16–32 μg/ml) inhibited growth of fungi belonging to the genera *Aspergillus, Penicillium, Aureobasidium,* and of the phytopathogens *Botrytis* and *Rhizoctonia* [9].

In 2003, the rhamnolipids of *P. aeruginosa* 47T2 NCBIM 40044 were shown to have antibacterial properties [10]. Thus, minimal inhibiting concentrations (MIC) of these surfactants against some bacteria of the genera *Serratia, Enterobacter, Klebsiella, Staphylococcus* were 0.5–32 μg/ml. Reports [8–10] were the impulse for further research

of the antimicrobial action of microbial surfactants [11–13].

One reason for such interest to microbial surfactants as antimicrobial agents is the pathogen resistance to widespread antibiotics and chemical biocides [11, 13].

Compared to the well-known antimicrobial compounds, microbial surfactants have a number of advantages [1, 2, 11, 13]. They are biodegradable and non-toxic, which prevents environmental pollution and allergies. They can be implemented in a wide range of pH, temperature and other environmental factors, due to their stable physical and chemical properties. Also, their action mechanism is based on the disruption of the cytoplasmic membrane, decreasing the possibility of microorganism resistance [5, 8, 10, 11].

The high interest to the microbial surfactants is evidenced by the many publications about these products of microbial synthesis. A few literature reviews were published in the last five years on the properties and perspectives of the practical implementation of microbial surfactants [1, 3, 14–19]. Those reviews mostly focused on certain surfactant types (rhamnolipids, lipopeptides, sophorolipids etc.) with emphasis on certain properties of these compounds. For example, Zhao et al. [17] pay attention mostly to the anti-inflammatory, antitumour, antiviral, and antiplatelet properties of lipopeptides, their interaction with biofilms, while the antibacterial effect is not considered at all and the antifungal is discussed briefly. The review [15] provides not only the specifics of the chemical composition but also the information about antimicrobial activity of lipopeptides, but the information is of almost a ten years ago. Similarly, Cortés-Sánchez Ade et al. [14], while analyzing antimicrobial properties of glycolipids, largely refer to the data of 2005–2010.

This review aims to summarize literature of the last several years on the antimicrobial potential of various surfactant substances of microbial origin.

Lipopeptides of Bacillus sp. as antimicrobial agents

The bacteria of the genus *Bacillus* are among the most studied sources of lipopeptides. The lipopeptydes are grouped into three families of cyclic compounds: surfactin, iturin and fengicin, differing in the number and sequence of the amino acids they include, as well as in the length of the acyl chain [15, 16]. Differences in the chemical composition and construction determine the range of their biological action. Thus, iturin and fengicin have antifungal properties while surfactin with a shorter acyl chain is characterized by a wider range of antibacterial action [15, 16].

Antibacterial action. In 2015, Torres et al. [20] established antimicrobial activity of the surfactant complex of *Bacillus subtilis* subsp. *subtilis* CBMDC3f, which contains four surfactin homologues and one for each iturin and fengicin. When the complex was added to cell suspension of *Listeria monocytogenes* 01/155 at 0.5 mg/ml, the number of viable cells dropped two orders of magnitude after 25 minutes. A similar effect towards *Bacillus cereus* MBC1 and *Staphylococcus aureus* ATCC 29213 was seen at higher concentrations of lipopeptide complex (1–2 mg/ml). The authors state that surfactants of similar composition produced by other strains of *Bacillus licheniformis* or *B. subtilis* were active only against *B. cereus* and *S. aureus*, without antagonistic activity against the genus *Listeria* [20].

Sharma et al. [21] studied antimicrobial activity of lipopeptides produced by *Bacillus pumilus* DSVP18 on potato peel substrate. Minimum inhibiting concentration against *B. cereus* MTCC 430, *Escherichia coli* MTCC 1687, *Salmonella enteritidis* MTCC 3219, and that against *S. aureus* MTCC 5021 was 30 μg/ml.

Surfactin of *Bacillus amyloliquefaciens* ST34 showed antimicrobial activity against a range of both Gram-negative (*Escherichia coli* ATCC 13706, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 10031, *Serratia* sp. SM14, *Enterobacter* sp. E11) and Gram-positive (*B. cereus* ST18, *Enterococcus* sp. C513, *Micrococcus* sp. AQ4S2, *S. aureus* C2) bacteria [22]. At the concentration of surfactin 0.26 mg/ml, zones of bacterial growth inhibition were 13–17 mm.

Chen et al. [23] isolated from the sediments of Bohai Sea a strain of *Bacillus licheniformis* MB01 which produces a complex of surfactin and fatty acids showing antibacterial activity against *E. coli, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio harveyi, Pseudomonas aeruginosa, S. aureus, Proteus species*. For example, its MIC against *V. parahaemolyticus* was 50 μg/ml [23].

Strain *B. subtilis* SK.DU4 synthesizes the complex of bacteriocin-like peptide and iturin-like lipopeptide with 15 carbon atoms in the acyl chain [24]. The bacteriocin-like peptide had antimicrobial action against *Micrococcus luteus* MTCC106 and *Listeria monocytogenes*

MTCC839 (growth inhibition zone 12 and 14 mm, respectively). If only the inturin-like lipopeptide was present, the zone of growth inhibition was 11 mm in both test cultures. If the mixture of bacitracin and lipopeptide was used, the zone of *M. luteus* MTCC106 and *L. monocytogenes* MTCC839 growth inhibition increased to 15 and 17 mm, respectively.

The study of Zhou et al. [25] is one of the first concerning dependence of surfactin antimicrobial activity on the carbon source in the culture medium of *B. subtilis* HH2, as well as the stability of antimicrobial action in a wide range of temperature (60–121 °C), pH (1–12), and in the presence of trypcin (100–300 μg/ml, pH 8) and pepsin (100–300 μg/ml, pH 2). It was found that surfactin synthesized on a mixture of glucose (0.33 %) and cellulose (0.67 %) had higher antimicrobial activity (at 0.4 mg/ml surfactin, the growth inhibition zones of *E. coli* CCTCC AB 212358 and *S. aureus* CCTCC AB 91053 were 16 and 14 mm, respectively). Lipopeptide obtained on medium with 1 % glucose, had low antimicrobial effect. Antimicrobial activity of surfactin remained constant at 60–100 °C, pH 2–11, and in the presence of trypsin and pepsin.

Due to synthesis of surfactin, bacteria of the genus *Bacillus* are considered promising in controlling the growth of such phytopathogens as *P. syringae* (causes root infection of arabidopsis), *Xanthomonas axonopodis* pv. *glycines* (bacterial pustule of soybean), and phytopathogen mycoplasms *Spiroplasma citri* and *Acholeplasma laidlawii,* which cause etiolation in citruses, clover phyllody and phytoplasma disease in solanaceous crops, respectively [15, 16].

B. subtilis 9407 synthesizes the complex of lipopeptides, the main one being C13-C16 surfactin A [26]. This complex showed of the antimicrobial effect against *Acidovorax citrulli* MH21 the causative agent of pumpkin bacterial blotch (growth inhibition zone 18 mm). To prove the role of surfactin in inhibition this pathogen, the authors obtained a mutant strain unable of synthesize lipopeptide. The mutant had no antimicrobial activity. Besides *A. citrulli* MH21, lipopeptides of strain 9407 showed antimicrobial effect on other phytopathogenic bacteria: *Pseudomonas syringae* pv. *tomato* DC3000, *Xanthomonas campestris* pv. *campestri*s Xcc 8004, *Pectobacterium carotovora* subsp. *carotovora* Ecc 09, *Pectobacterium atrosepticum* SCRI1043 (growth inhibition zones 10–18 mm) [26].

In 2018 [27] was reported about a sea isolate *Bacillus pumilus* SF214 wich produced pumilacidin (the mixture of cyclic heptapeptides linked to fatty acids of different lengths). The lipopeptide inhibited *S. aureus* ATCC 6538 (in the presence of supernatant, growth inhibition zone was 10 mm.

Antifungal activity. In the publications on the antifungal activity pay the most attention to the effect of these surfactants on phytopathogenic fungi. Since we provided the information on antifungal effect of lipopeptides produced by rhizosphere and endophytic bacteria of the genus *Bacillus*, which are promising for control the number of phytopathogenic fungi, what we reported in the review [28], we shall now pay attention to studies which have appeared after then. The lipopeptide antifungal activity is determined by analyzing such parameters as MIC [29–34], degree of the fungal growth inhibition [35, 36], and the diameter of fungal growth inhibition zone [37].

The data on MIC of lipopeptides produced by bacteria of the genus *Bacillus* against fungi and yeast are summarized in Table 1. According to the data, the highest antifungal activity is shown for *B. subtilis* RLID 12.1 lipopeptides. MIC against yeasts of the genera *Cryptococcus* and *Candida* was only 1–20 μg/ml, that orders of magnitude lower than MIC of other lipopeptides against fungi. Notably, the antimicrobial activity of lipopeptides of *Bacillus* sp. AR2 depends on the carbon source in the culture medium [20]. The strain AR2 was found to produce the mixture of homologues of iturin, fengicin and surfactin. If the strain was grown in medium with sucrose, glycerol, sorbitol and maltose the prevailing fraction in the complex was C15 surfactin. However the most active antifungal agents were lipopeptides produced on sucrose. Sarwar et al. [35] studied the degree of growth inhibition of phytopathogenic fungi *Fusarium moniliforme* KJ719445, *Fusarium oxysporum (*the strain was not specified), *Fusarium solani* SAN1077, *Trichoderma atroviride* P150907 for the action of lipopeptides synthesized by bacteria of the genus *Bacillus.*

It was found that lipopeptides of *B. amyloliquefaciens* FZB42, *B. subtilis* NH-100 and *B. subtilis* NH-217 inhibited fungal growth by 83–87, 79–80, and 76–79% respectively.

Lipopeptides synthesized by *Bacillus* XT1 CECT 8661 added at 2–10 mg/ml inhibited the growth of *Botrytis cinerea* by 19–72%, and maximum degree of inhibition

Table 1. **Minimum inhibitory concentrations of *Bacillus* sp. lipopeptides against fungi**

Test culture		Lipopeptide producer	MIC, µg/ml	Refe-rences
Genus	Species, strain			
Alternaria	*Alternaria solani*	*Bacillus subtilis* CU 12	150	[30]
	Alternaria alternata MTCC 2724	*Bacillus* sp. AR2	500–750*	[34]
	Alternaria citri MTCC 4875	*Bacillus* sp. AR2	500–750*	[34]
Fusarium	*Fusarium oxysporum* f. sp. *iridacearum*	*Bacillus subtilis* BBG125	10	[33]
	Fusarium sambucinum	*Bacillus subtilis* CU 12	100	[30]
	Fusarium solani ATCC 36031	*Bacillus* sp. AR2	250–750*	[34]
	Fusarium oxysporum MTCC 7229	*Bacillus* sp. AR2	250–750*	[34]
	Fusarium solani	*Bacillus subtilis* SPB1	3000	[31]
Rhizoctonia	*Rhizoctonia bataticola*	*Bacillus subtilis* SPB1	40	[32]
	Rhizoctonia solani	*Bacillus subtilis* SPB1	4000	[32]
Rhizopus	*Rhizopus stolonifer*	*Bacillus subtilis* CU 12	100	[30]
Verticillium	*Verticillium dahliae*	*Bacillus subtilis* CU 12	100	[30]
Cladosporium	*Cladosporium cladosporioides* ATCC 16022	*Bacillus* sp. AR2	750–2000*	[34]
Scopulariopsis	*Scopulariopsis acremonium* ATCC 58636	*Bacillus* sp. AR2	125–500*	[34]
Microsporum	*Microsporum gypseum* MTCC 4522	*Bacillus* sp. AR2	125–500*	[34]
Trichophyton	*Trichophyton rubrum* MTCC 2961	*Bacillus* sp. AR2	750–2000*	[34]
Botrytis	*Botrytis cinerea*	*Bacillus* XT1 CECT 8661	8000	[36]
Cryptococcus	*Cryptococcus* spp.	*Bacillus subtilis* RLID 12.1	1–16	[29]
Candida	*Candida* spp.	*Bacillus subtilis* RLID 12.1	2–20	[29]

Note.* — different MIC values dependent on the carbon source in the culture medium.

was seen at the highest studied surfactant concentration [36].

For the action surfactin of *B. amyloliquefaciens* ST34 at concentration 0.26 mg/ml, growth inhibition zones in different strains of *Candida albicans* and *Cryptococcus neoformans* were in the range of 13–15 mm [22].

In our review [28] we reported an increased synthesis of antifungal lipopeptides (in particular, fengicin and iturin) in response to the presence of phytopathogenic fungi in the medium of producer cultivation. Zihalirwa Kulimushi et al. [37] studied the effect of a lipopeptide complex (surfactin, fengicin and iturin) produced by *B. amyloliquefaciens* S499 on the phytopathogenic fungus *Rhizomucor variabilis,* and the possibility of inducing the antifungal compounds synthesis in the presence of a pathogen in the culture medium of strain S499. Experiments showed that co-culturing *B. amyloliquefaciens* S499 and *Rhizomucor variabilis* led to an almost three-fold increase in fengicin content and increased the antifungal effect [37].

The another interesting research [38] showed that *Bacillus amyloliquefaciens* UCMB5113 syntesized the mixture of linear fengicins, whereas they commonly occur only in the cyclic form [15, 16]. Linear fengicins were divided into 14 fractions, all fractions showed antagonistic activity against *Alternaria brassicicola*, *Alternaria brassicae*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Verticillium longisporum*; but the fraction 9 had the highest antifungal effect. According to the analysis, it belonged to the family of C15-fengicin. The authors suppose that all other fractions have shorter acyl chains and so are less active.

Antimicrobial effect of lipopeptides produced by other microorganisms

Representatives of the genera *Paenibacillus* [16, 39–41], *Pseudomonas* [42–46],

Brevibacillus [47], *Corynebacterium* [48], *Aneurinibacillus* [49], *Streptomyces* [50], even *Propionibacterium* [51], *Citrobacter* and *Enterobacter* [52] also synthesises lipopeptides.

High antimicrobial activity was revealed for lipopeptide surfactants of strain *Paenibacillus* sp. MSt1, isolated from the peat beds of tropical forests. Thus, its MIC was (μg/ml) 1.5 against *E. coli* ATCC 25922; 25 — methicillin resistant strain *S. aureus* ATCC 700699, and 12.5 — *C. albicans* IMR [39].

Huang et al. [40] established high antimicrobial activity of paenibacterin of *Paenibacillus thiaminolyticus* OSY-SE. MIC of the lipopeptide against strains *E. coli, P. aeruginosa, Acinetobacter baumannii, K. pneumoniae, S. aureus* and *E. faecalis* were fairly low: 8–16 μg/ml, comparable to the MIC of such antibiotics as polymixin B and vancomycin.

In 2017, was reported about strain *Paenibacillus* sp. OSY-N that produce the mixture of lipopeptides BMY-28160, permetin A, a novel cyclic lipopeptide and its linear analogues (paenipeptins A, B and C) [41]. Differences in the compound content underlie their different biological effect. Thus far, the highest antimicrobial effect was seen in paenipeptin C (contains C8-acyl chain and isoamino acid): MIC against Gram-positive (*B. cereus* ATCC 11778, *Listeria innocua* ATCC 33090, *S. aureus* ATCC 25923, *S. aureus* ATCC 6538) and Gram-negative (*E. coli* K-12, *E. coli* ATCC 25922, *Salmonella enterica* ser. Typhimurium LT2, *S. enterica* ser. Typhimurium LT2) bacteriae were 2–4 and 0.5–2 μg/ml, respectively. The authors explain such activity of paenipeptin C, unlike other lipopeptides, by a longer acyl chain, and presence of unusual amino acids and their conformation.

Although bacteria of the genus *Pseudomonas* are more known as sources of glycolipids [1, 2, 7, 9, 10, 12, 14], there are data on their ability to produce lipopeptides, too. As early as 1970's the structure of lipopeptide viscosin was established (the compound was produced by *Pseudomonas fluorescens)*, with antimicrobial effect [42] of such magnitude that intensive research of its biological properties lasted until 2000's [43]. Currently, viscosin has been established to have an antimicrobial effect against 94 Gram-negative and 72 Gram-positive bacteria and 95 fungal species [44].

Ma et al. [45] established that *Pseudomonas* sp. CMR5C produced orfamide B and G, with the same amino acid sequence but different acyl chain lenth: C14 for orfamide B and C16 for orfamide G. Irrespectively of the acyl chain length, orfamide had no antifungal effect against *Magnaporthe oryzae* VT5M1, however at 50 μmole/ml the appressorium of *M. oryzae* VT5M1 did not develop.

Pseudomonas aeruginosa MA-1 grown on olive oil (4 %) produced lipopeptides in the high concentration of 12.5 g/l [46] of low antimicrobial effect; the growth inhibition zone of *S. aureus* ATCC 43300 did not exceed 7–9.5 mm at surfactant concentration of 0.5–5 g/l.

The lipopeptide brevibacillin (produced by *Brevibacillus laterosporus* OSY-I1) has high antimicrobial effect on Gram-positive bacteria (MIC 2–4 μg/ml) [47]. Notably, its MIC for Gram-negative bacteriae was higher than 32 μg/ml.

Dalili et al. [48] studied the antimicrobial effect of coryxin, produced by *Corynebacterium xerosis* NS5 [48]. It was found that coryxin had low antimicrobial activity against Gram-negative bacteria (MIC for strains *E. coli* and *P. aeruginosa* were 3120 and 10 000 μg/ml, respectively). However, MIC of this lipopeptides against Gram-positive bacteria *S. aureus* and *Streptococcus mutans* were significantly lower (190 μg/ml).

The aneurinifactin, produced by sea bacteria *Aneurinibacillus aneurinilyticus* SBP-11 A, had significantly higher antimicrobial activity compared to coryxin [49]. Its MIC against strains *E. coli* MTCC 443 and *S. aureus* MTCC 96 was 8 μg/ml, and *P. aeruginosa* MTCC — 16–424 μg/ml.

The study [50] described the lipopeptide produced by *Streptomyces amritsarensis* sp. MTCC 11845T, which at 10 μg/ml showed antibacterial activity to Gram-positive bacteria. The growth inhibition zones for *B. subtilis* MTCC 619, *Staphylococcus epidermidis* MTCC 435 and *Mycobacterium smegmatis* MTCC 6 were 21, 17, 15 mm, respectively. Meanwhile there was no antimicrobial activity to Gram-negative bacteria and fungi, perhaps because of a short (C12) acyl chain of the lipopeptide.

While bacteria of the genus *Propionibacterium* are known sources of organic acids and vitamins, recent research [51] established that *Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674 produces the lipopeptide surfactant inhibiting *Rhodococcus erythropolis* and *B. cereus:* MIC for both was 25 mg/ml.

Strains *Citrobacter* sp. S-3, S-6 and S-7, *Enterobacter* sp. S-4, S-5, S-9 S-10, S-11 and

S-12 were isolated from polluted soil. They [52] produced the complex of lipopeptides with antimicrobial effect to Gram-positive and Gram-negative bacteria. The strains S-3 and S-11 were shown to produce fractions Fr-c and Fr-e with β-hydroxy fatty acids of chain length C14 and C17, respectively. Thus they can be classified as belonging to the fengicin and iturin families. However the antimicrobial effect was seen only in the purified lipopeptide fraction Fr-c with the shorter acyl chain. Its MIC were 12, 15 and 16 µg/ml against Gram-positive test cultures *Micrococcus luteus* MTCC106, *S. aureus* MTCC1430 and *S. epidermidis* MTCC435, and 20 and 32 µg/ml against Gram-negative test cultures *Serratia marcescens* and *P. aeruginosa* ATCC27853, respectively. Notably no of all lipopeptides had an antifungal effect on *C. albicans* MTCC1637.

A summary of lipopeptides antibacterial activity is shows in Table 2, composed to compare MIC of different lipopeptides for the same test cultures. The lipopeptides produced by bacteria of the genus *Paenibacillus* showed the highest antimicrobial activity, a moderate activity — surfactants of the genus *Bacillus*, and lipopeptides of such atypical producer as *Corynebacterium* and *Propionibacterium* were not active enough.

According to recent literature, the antimicrobial activity of lipopeptides depends on their content and on the test culture (species and strain). Usually, higher antifungal activity is seen in lipopeptides with longer (C16–C18) acyl chains, and compounds with fewer carbons atoms (C7–C14) in the fatty acid chain have antibacterial effect. However, currently there is not enough information in the literature, on the basis of which it would be possible to do correct conclusions about the influence of the chemical composition of lipopeptides on their antimicrobial activity. Table 2 contains more higher MIC of lipopeptides than previously described [15, 16], perhaps because the reported data [15, 16] are given for individual substances but not for the complexes analyzed in our review.

Antimicrobial activity of rhamnolipids

A glycolipids has a carbohydrate part which might be rhamnose, trehalose, sophorose etc., and a lipid chain. Accordingly, they are classified into rhamno- trehaloso-, sophorolipids, etc. [1, 2, 14, 18, 53]. Currently, rhamnolipids are the most studied of them. Only in the last few years there were published several reviews [54–60] dedicated to the increasing rhamnolipid biosynthesis, new avenues and problems of their application in various industrial and medical practices.

In a rhamnolipids, one or two rhamnoses are bound to one, two or seldom three molecules of β-hydroxyalyphatic acids. Depending on the number of carbohydrate and fatty acid molecules, the rhamnolipids can be grouped into mono-rhamno-mono-lipids, mono-rhamno-di-lipids, di-rhamno-mono-lipids and di-rhamno-di-lipids [58, 60]. Over sixty rhamnolipid homologues are produced by microorganisms of the genus *Pseudomonas* (*P. chlororaphis, P. alcaligenes, P. putida, P. stutzeri,* etc.), and strains of *P. aeruginosa* are the main rhamnolipid sources. Lately, there were reports of rhamnolipid-synthesizing abilities in bacteria of the genera *Acinetobacter (A. calcoaceticus), Enterobacter, Pantoea, Burkholderia, Myxococcus* [58–60].

The effect of rhamnolipid on bacteria

According to Tedesco et al., rhamnolipids are probably produced by many microorganisms [61]. The rhamnolipid-producing strains of microbiota belonging to *Psychrobacter, Arthrobacter* and *Pseudomonas* were isolated from the Ross Sea (Antarctica). Monorhamnolipids at concentration 1 mg/ml inhibited the growth of pathogenic strains of *Burkholderia (*Table 3). Given the high antimicrobial activity of rhamnolipids of *Pseudomonas* BTN 1, the next step was separation of the rhamnolipid complexes into fractions. This yielded three kinds of monorhamnolipids with different lipid chain length. For each fraction, the researchers were determined the minimum inhibitory and minimum bactericidal concentrations (MBC).

The fractions 1 and 2 of monorhamnolipids with shorter acyl chains were most active. Thus, MIC of these fractions against *B. cenocepacia* LMG 16656, *B. metallica* LMG 24068, *B. seminalis* LMG 24067, *B. latens* LMG 24064 and *S. aureus* 6538P were about 1.56–12.5 µg/ml, and MBC did not exceed 200 µg/ml.

Chebbi et al. [62] isolated from engine oil-polluted soil the strain *P. aeruginosa* W10, which produced 9.7 g/l rhamnolipids on a medium with 2% glycerol. However, the antimicrobial effect of the surfactants turned out to be relatively low. Thus, MIC of rhamnolipid complex of strain W10 against the pathogenic strains *E. coli* ATCC 25922, *S. aureus (*MRSA) ATCC 43300 and *C. albicans* ATCC 10231 were 37.50, 9.37 and 2.34 mg/ml, respectively.

The effect of mono- and dirhamnolipids produced by *Burkholderia thailandensis*

Table 2. **Antibacterial activity of lipopeptides against some microorganisms**

Test culture	Lipopeptide source	MIC, μg/ml	References
Escherichia coli O157:H7 ATCC 43889	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
Escherichia coli ATCC 25922	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
	Paenibacillus sp. MSt1	1.5	[37]
	Paenibacillus thiaminolyticus OSY-SE	8	[38]
Escherichia coli O157:H7 EDL 933	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
	Paenibacillus thiaminolyticus OSY-SE	8	[38]
	Bacillus laterosporus OSY-I1	32	[40]
Escherichia coli 2276	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Escherichia coli MTCC 443	*Aneurinibacillus aneurinilyticus* SBP-11	8	[42]
Escherichia coli K-12	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5	[39]
	Bacillus laterosporus OSY-I1	>32	[40]
Escherichia coli MTCC 1687	*Bacillus pumilus* DSVP18	30	[21]
*Escherichia coli**	*Corynebacterium xerosis* NS5	3120	[39]
Staphylococcus aureus (methicillin-resistant)	*Bacillus laterosporus* OSY-I1	1	[40]
Staphylococcus aureus ATCC 6538	*Bacillus laterosporus* OSY-I1	1–2	[40]
Staphylococcus aureus ATCC 25923	*Paenibacillus* sp. OSY-N (paenipeptin C)	2–4	[39]
Staphylococcus aureus ATCC 6538	*Paenibacillus* sp. OSY-N (paenipeptin C)	4–8	[39]
Staphylococcus aureus (methicillin-resistant)	*Paenibacillus* sp. OSY-N (paenipeptin C)	8	[39]
Staphylococcus aureus MTCC 96	*Aneurinibacillus aneurinilyticus* SBP-11	8	[42]
Staphylococcus aureus (methicillin-resistant)	*Enterobacter* sp. S-11	15	[44]
*Staphylococcus epidermidis**	*Enterobacter* sp. S-11	16	[44]
Staphylococcus aureus ATCC 700699	*Paenibacillus* sp. MSt1	25	[37]
Staphylococcus aureus MTCC 5021	*Paenibacillus* sp. OSY-N (paenipeptin C)	16–32	[39]
	Paenibacillus thiaminolyticus OSY-SE	32	[38]
	Bacillus pumilus DSVP18	30–35	[21]
Staphylococcus aureus ATCC 43300	*Paenibacillus thiaminolyticus* OSY-SE	32	[38]
*Staphylococcus aureus**	*Corynebacterium xerosis* NS5	190	[41]
Bacillus cereus ATCC 11778	*Bacillus laterosporus* OSY-I1	2–4	[40]
	Paenibacillus sp. OSY-N (paenipeptin C)	4	[39]
Bacillus cereus ATCC 14579	*Bacillus laterosporus* OSY-I1	1,0	[40]
	Paenibacillus sp. OSY-N (paenipeptin C)	8	[39]
Bacillus cereus MTCC 430	*Bacillus pumilus* DSVP18	30–35	[21]
*Bacillus cereus**	*Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674	25 000	[43]
Bacillus subtilis MTCC 619	*Aneurinibacillus aneurinilyticus* SBP-11	16	[42]
Listeria monocytogenes OSY-8578[h]	*Bacillus laterosporus* OSY-I1	1–2	[40]

Table 2. Continued

Test culture	Lipopeptide source	MIC, μg/ml	References
Listeria innocua ATCC 33090	*Bacillus laterosporus* OSY-I1	1–2	[40]
	Paenibacillus sp. OSY-N (paenipeptin C)	2–4	[39]
Listeria monocytogenes Scott A	*Bacillus laterosporus* OSY-I1	1	[40]
	Paenibacillus thiaminolyticus OSY-SE	2	[38]
	Paenibacillus sp. OSY-N (paenipeptin C)	4–8	[39]
Pseudomonas aeruginosa ATCC 27853	*Paenibacillus* sp. OSY-N (paenipeptin C)	1–2	[39]
	Paenibacillus thiaminolyticus OSY-SE	8	[38]
	Bacillus laterosporus OSY-I1	>32	[40]
Pseudomonas aeruginosa ATCC 999	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Pseudomonas aeruginosa ATCC 2325	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Pseudomonas aeruginosa MTCC 424	*Aneurinibacillus aneurinilyticus* SBP-11	16	[42]
*Pseudomonas aeruginosa**	*Enterobacter* sp. S-11	30	[44]
*Pseudomonas aeruginosa**	*Corynebacterium xerosis* NS5	10 000	[41]
Klebsiella. pneumoniae 2461	*Paenibacillus thiaminolyticus* OSY-SE	4	[38]
Klebsiella pneumoniae MTCC 7162	*Aneurinibacillus aneurinilyticus* SBP-11	4	[42]
Klebsiella pneumoniae 2463	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Klebsiella pneumoniae ATCC 700603	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Klebsiella pneumoniae 2317	*Paenibacillus thiaminolyticus* OSY-SE	64	[38]
Enterococcus faecalis ATCC 51299	*Bacillus laterosporus* OSY-I1	4–8	[40]
Enterococcus faecalis 2731	*Paenibacillus thiaminolyticus* OSY-SE	8	[38]
Enterococcus faecalis ATCC 29212	*Paenibacillus thiaminolyticus* OSY-SE	16	[38]
	Paenibacillus sp. OSY-N (paenipeptin C)	32	[39]
Enterococcus faecalis ATCC 700802	*Paenibacillus thiaminolyticus* OSY-SE	64	[38]
Salmonella enterica ser. Typhimurium LT2	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
Salmonella enterica ser.Typhimurium DT104	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
Salmonella enteritidis MTCC 3219	*Bacillus pumilus* DSVP18	30–35	[21]
Salmonella typhimurium DT 109	*Bacillus laterosporus* OSY-I1	>32	[40]
Acinetobacter baumannii ATCC BAA-747	*Paenibacillus thiaminolyticus* OSY-SE	2	[38]
Acinetobacter baumannii 2315	*Paenibacillus thiaminolyticus* OSY-SE	2	[38]
Alicyclobacillus acidoterrestris ATCC 49025	*Bacillus laterosporus* OSY-I1	0.5–1	[40]
Alicyclobacillus acidoterrestris	*Bacillus laterosporus* OSY-I1	1	[40]
*Streptococcus agalactiae**	*Paenibacillus* sp. OSY-N (paenipeptin C)	0.5–1	[39]
*Streptococcus mutans**	*Corynebacterium xerosis NS5*	25 000	[41]
Lactobacillus plantarum ATCC 8014[f]	*Bacillus laterosporus* OSY-I1	1	[40]

Table 2. Continued

Test culture	Lipopeptide source	MIC, µg/ml	Refe-rences
Lactococcus lactis ATCC 11454[g]	*Bacillus laterosporus* OSY-I1	2	[40]
Clostridium difficile A515[c]	*Bacillus laterosporus* OSY-I1	4–8	[40]
*Rhodococcus erythropolis**	*Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674	25 000	[43]
*Serratia marcescens**	*Enterobacter* sp. S-11	20	[44]
Vibrio cholerae MTCC 3906	*Aneurinibacillus aneurinilyticus* SBP-11	16	[42]
*Vibrio parahaemolyticus**	*Bacillus licheniformis* MB01	50	[23]
*Micrococcus luteus**	*Enterobacter* sp. S-11	12	[44]
*Enterobacter aerogenes**	*Paenibacillus* sp. OSY-N (paenipeptin C)	2–4	[39]
Paenibacillus larvae ATCC 9545	*Bacillus pumilus* DSVP18	30–35	[21]
*Yersinia enterocolitica**	*Paenibacillus* sp. OSY-N (paenipeptin C)	0,5–1	[39]

Note: * — strain number not provided.

Table 3. **Effect of rhamnolipids produced by the Arctic Sea bacteria on strains of *Burkholderia***

Test culture	Inhibition of test cultures (%) in the presence of rhamnolipids, produced by				
	Pseudomonas BTN 1	*Psychrobacter* BTN2	*Psychrobacter* BTN15	*Psychrobacter* BTN5	*Arthrobacter* BTN 4
Burkholderia diffusa LMG 24065	100	75	77	77	63
Burkholderia metallica LMG 24068	92	70	71	77	64
Burkholderia cenocepacia LMG 16656	100	78	87	84	57
Burkholderia latens LMG 24064	100	53	75	58	41
Burkholderia seminalis LMG 24067	100	43	67	40	56

E264 (ATCC 700388) on glycerol, on their antimicrobial activity was studied in [63]. Chemical analysis of the rhamnolipids showed that strain E264 synthesizes the mixture of dirhamnolipids and monorhamnolipids in the ratio 3:1. Further research showed that dirhamnolipids have higher antimicrobial effect than monorhamnolipids. Meanwhile the highest antimicrobial activity was found in supernatant with unpurified rhamnolipid mixture which might be explained by synergy of the fractions or the presence of other compounds besides rhamnolipids with antimicrobial effect.

Aleksic et al. [64] studied antimicrobial activity of both the complex of rhamnolipids produced by *Lysinibacillus* sp. BV152.1 and its separate fractions. It was found that all fractions of strain BV152.1 rhamnolipids had the same weak antimicrobial effect against *P. aeruginosa* PAO1, *P. aeruginosa* DM50, *S. aureus* ATCC 25923, *S. aureus* MRSA and *S. marcescens* ATCC 27117. Their MIC against all test cultures were 500 µg/ml.

The report [65] describes the isolation of a strain identified as *P. aeruginosa LCD12* which synthesizes the complex of mono- and dirhamnolipids, from samples of raw petroleum. The authors studied antimicrobial activity of the surfactant complex and of its constituents. It was found that MIC of all studied rhamnolipids against *Streptococcus epidermidis, B. subtilis, S. aureus* and *E. coli* were close: 4; 4; 16 and 4 µg/ml, respectively.

The data on rhamnolipid antimicrobial activity are summarized in Table 4.

Table 4. **Minimum inhibitory concentrations of rhamnolipids**

Test culture	Producer	MIC, μg/ml	References
Staphylococcus aureus 6538P	*Pseudomonas* BTN 1	1.56–3.12	[61]
Staphylococcus aureus	*Pseudomonas aeruginosa* LCD12	16	[65]
Staphylococcus aureus ATCC 25923	*Lysinibacillus* sp. BV152.1	500	[64]
*Staphylococcus aureus** (methicillin-resistant)	*Lysinibacillus* sp. BV152.1	500	[64]
Staphylococcus aureus ATCC 25923	*Pseudomonas aeruginosa* C2	650	[66]
Staphylococcus aureus ATCC 43300 (methicillin-resistant)	*Pseudomonas aeruginosa* W10	9 370	[62]
Staphylococcus capitis SH6	*Pseudomonas aeruginosa* W10	18 750	[62]
Pseudomonas aeruginosa PAO*1*	*Lysinibacillus* sp. BV152.1	500	[64]
Pseudomonas aeruginosa DM50	*Lysinibacillus* sp. BV152.1	500	[64]
Bacillus subtilis	*Pseudomonas aeruginosa* LCD12	4	[65]
Bacillus licheniformis CAN55	*Pseudomonas aeruginosa* W10	1500	[62]
Escherichia coli	*Pseudomonas aeruginosa* LCD12	4	[65]
Escherichia coli K8813	*Pseudomonas aeruginosa* C2	550	[66]
Esherchia coli ATCC 25922	*Pseudomonas aeruginosa* W10	37 500	[62]
Streptococcus epidermidis	*Pseudomonas aeruginosa* LCD12	4	[65]
Streptococcus oralis	*Burkholderia thailandensis* E264	150	[63]
Streptococcus sanguinis	*Burkholderia thailandensis* E264	150	[63]
Neisseria mucosa	*Burkholderia thailandensis* E264	150	[63]
Actinomyces naeslundii	*Burkholderia thailandensis* E264	300	[63]
Serratia marcescens ATCC 27117	*Lysinibacillus* sp. BV152.1	500	[64]
Candida albicans ATCC 10231	*Pseudomonas aeruginosa* W10	2 340	[62]

Data in Table 5 show that the antibacterial activity of rhamnolipids as well as lipopeptides (Table 2) depends on the test culture (both species and strain) and on the complex of surfactants. Lipopeptides are more efficient antibacterial agents compared to rhamnolipids (Tables 2 and 5).

In a number of recent studies, the antibacterial activity of rhamnolipids was determined by the agar diffusion technique but not the MIC [22, 67–69]. Thus, supernatant (15 μl, with rhamnolipid concentration 0.57 g/l) obtained by culturing *P. aeruginosa* P1R16 on olive oil, the growth inhibition zones were the following: 11 mm for *E. coli* ATCC 25922, 25 mm for *P. aeruginosa* ATCC 27853, 12 mm for *S. aureus* ATCC 25923 and *B. cereus* CCT0198, and 22 mm for *Ralstonia solanacearum* 1226 [67].

In the presence 1.12 mg/ml rhamnolipids of *P. aeruginosa* SARCC 697 the diameters of growth inhibition zones for bacterial test cultures were (mm): 13.5 for *E. coli* ATCC 417373; 29.3 for *E. coli* ATCC 13706; 13.5 for *Klebsiella pneumoniae* ATCC 10031; 8.3 for *K. pneumoniae* P3; 20.3 for *Salmonella typhimurium* ATCC 14028; 14 for *Salmonella enterica* SE19; 14 for *Serratia marcescens* ATCC 13880; 13.7 for *S. aureus* ATCC 25923; and 11.5 for *S. aureus* C2 [22]. Growth inhibition zone for methicillin-resistant strain *S. aureus* ATCC 43300 under the effect of rhamnolipids produced by *P. aeruginosa* 47T2 on the mixture of waste sunflower and olive oil was 10 mm [68].

Oluwaseun et al. [69] compared the antimicrobial activity of rhamnolipids of *P. aeruginosa* C1501 and Tween 80. The

Table 5. **Action of surface-active substances synthesized by *A. calcoaceticus* IMV B-7241, *N. vaccinii* IMV B-7405 and *R. erythropolis* Ac-5017 on some microorganisms**

Strain	Carbon source in the culture medium	Minimum inhibitory concentration (μg/ml) against					
		Bacillus subtilis BT-2	*Enterobacter cloaceae* C-8	*Staphylococcus aureus* BMS-1	*Proteus vulgaris* PA-12	*Escherichia coli* IEM-1	*Candida albicans* D-6
A. calcoaceticus IMV B-7241	Ethanol	14	56	14	14	28	N.d.
	Purified glycerol	4	2	4	N.d.	2	2
	Waste of biodiesel production	16	4	8	N.d.	4	16
	Refined sunflower oil	50	25	14	1.8	0.9	25
	Waste sunflower oil	20	20	2.5	2.5	1.3	40
N. vaccinii IMV B-7405	Purified glycerol	45	180	90	90	45	45
	Waste of biodiesel production	15	120	15	60	30	30
	Refined sunflower oil	20	160	80	80	10	40
	Waste sunflower oil	18	140	70	70	9	35
R. erythropolis IMV Ac-5017	Ethanol	60	240	N.d.	N.d.	15	>480
	Purified glycerol	15.6	N.d.	62.5	62.5	250	N.d.
	Waste of biodiesel production	62.5	N.d.	125	31	125	N.d.

Note. N.d. — not determined

research showed that surfactants of strain C1501 were more effective antimicrobial agents compared to the chemical analogue. Thus, growth inhibition zones for *S. aureus, B. cereus* and *E. coli* with addition of 3 % rhamnolipid solution were 20–22 mm, and that of Tween at similar concentrations was only 5 mm.

Rhamnolipids action on fungi. Our paper [28] provides information on the antifungal activity of rhamnolipids aimed to manage the spread of phytopathogenic fungi, so our current review shall focus on further work.

Yan et al. [70] studied the effect of rhamnolipids of *P. aeruginosa* ZJU-211 on the phytopathogenic fungus *Alternaria alternata.* They found that at 125 μg/ml surfactant, growth of the fungus was inhibited only by 26.6%, and at 250 μg/ml rhamnolipids, by 40%. Raising the rhamnolipids concentration to 400–1000 μg/ml was followed by inhibition of the pathogenic spore germination by 64–81.7%. Treating tomatoes, infected with *A. alternata,* with the mixture of rhamnolipids (500 μg/ml) and laurel oil (500 μg/ml) decreased the degree of infection to 43 %.

At 200 μg/ml, the surfactant complex and fractions of mono- and dirhamnoliipds of *P. aeruginosa* KVD-HM52 inhibited the growth of *F. oxysporum* NCIM1072 by 95 and 84%, respectively [71]. MIC of purified rhamnolipids against the micromycete was only 50 μg/ml.

Another study [72] considered the antifungal activity of rhamnolipids produced by *P. aeruginosa* No. 112 against *Aspergillus niger* MUM 92.13 and *Aspergillus carbonarius* MUM 05.18. It was established that the dirhamnolipids were responsible for the antifungal activity, while monorhamnolipids demonstrated weak inhibiting action. Besides that, the authors showed that adding NaCl to purified mono- and dirhamnolipids increased their antifungal effect. Thus, the mixture of dirhamnolipids of 0.375 g/l and 875 mM

NaCl fully inhibited growth of test cultures of *A. niger* MUM 92.13, while pure dirhamnolipid solution did it only by 40 %. Adding salt at the same concentration to monorhamnolipid solution was followed by inhibition of test culture only by 40 %, and monorhamnolipids without salt did not inhibit the fungal growth at all. The effect of added salt was explained by NaCl repairing structure of rhamnolipids which was disrupted in extraction from the culture medium.

Thus, research of antimicrobial activity of rhamnolipids is still fruitful. Though rhamnolipids are less efficient than lipopeptides in their antimicrobial action, they have a number of some advantages: firstly, the higher productivity of producers, and secondly, the possibility of synthesis on industrial waste, which decreased their cost.

Sophorolipid effect on microorganisms

Main producers of sophorolipids are yeasts of the genera *Candida (Starmerella), Rhodotorula,* and *Wickerhamomyces* [73]. A sophorolipid has a hydrophobic part (fatty acid) and a hydrophilic one (sophorose disaccharide with a β-1,2 bond), and sophorose can be acetylated on the 6' and/or 6'' position. The carboxyl group of the fatty acid can be free forming acid (non-lactone) structure or etherified on the 4'' position forming the lactone variant [73].

Most recent publications focused on the antimicrobial effect of sophorolipids produced by *Candida (Starmerella) bombicola* ATCC 22214 [74–79]. Thus, the authors of [74] studied antimicrobial properties of the glycolipids produced on glucose and lauryl alcohol (10%, v/v). They showed that the yeast culture on the lauryl alcohol produced lactone sophorolipids, which unlike surfactants obtained on glucose fully inhibited the growth of Gram-negative (*E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027) and Gram-positive (*S. aureus* ATCC 6358, *B. subtilis* ATCC 6633) bacteria and of the yeast *C. albicans* ATCC 20910, at concentration 5–10 µg/ml. The data showed that the hydrophobic substrates are more suitable for production of sophorolipids with high antimicrobial activity.

Zhang et al. [75] analysed the antimicrobial activity of sophorolipids produced by *C. bombicola* ATCC 22214 on glucose with added palmitic, stearic and oleic acids as precursors. Irrespectively of the culture conditions, sophorolipids almost did not vary in antimicrobial activity against *Salmonella* spp. and *Listeria* spp.

In the paper [76] it was established that sophorolipids produced by *C. bombicola* ATCC 22214 on coconut oil had higher antimicrobial activity against *E. coli* and *S. aureus,* than if produced on corn oil. Quite probably the different antimicrobial activity of sophorolipids is caused by different length of acyl chain, yet the authors did not stress it.

Elshikh et al. [77] studied the effect of sophorolipids of *C. bombicola* ATCC 2221, on the oral pathogens. MIC of the sophorolipids against *Streptococcus mutans* DSM-20523, *Streptococcus oralis* DSM-20627; *Actinomyces naeslundii* DSM-43013, *Neisseria mucosa* DSM-4631 and *Streptococcus sanguinis* NCTC 7863 were 195, 97.5, 195, 97.5 and 195 µg/ml, respectively.

Solaiman et al. [78] studied the effect of culture condition of *S. bombicola* ATCC 22214 on its sophorolipid antimicrobial action on microbes destroying salt hides. They cultured the microbial source on medium with glucose (10 g/l) with co-substrate (2 g/l) of palmitic, stearic and oleic acids (the sophorolipids were referred to as SL-p, SL-s, SL-o). The experiments showed that MIC of SL-p and SL-o against Gram-positive (*B. licheniformis, B. pumilus, Bacillus mycoides, Enterococcus faecium, Aerococcus viridans, Staphylococcus xylosus, Staphylococcus cohnii)* and Gram-negative (*Pseudomonas luteola, Enterobacter cloacae, Enterobacter sakazakii* and *Vibrio fluvialis)* bacteria were the same (19.5 µg/ml), and MIC of SL-s were lower (4.88–9.76 µg/ml).

Later [79] the same authors studied antimicrobial action of sophorolipids of *S. bombicola* ATCC 22214 on bacteria of the genera *Lactobacillus* and *Streptococcus,* which cause dental caries. The growth of *Lactobacillus acidophilus* ATCC 4356 and *Lactobacillus fermentum* ATCC9338 was fully inhibited at 1.3 and 1.0 mg/ml sophorolipids, respectively. Meanwhile the MIC of the studied compounds against *Streptococcus mutans* ATCC 25175, *Streptococcus salivarius* ATCC 13419 and *Streptococcus sobrinus* ATCC 33478 were only 20–38 µg/ml.

In 2017, sophorolipids produced by *Rhodotorula babjevae* YS3 on a medium with glucose (10 g/l) were shown to have antifungal effect [80]. MIC against *Colletotrichum gloeosporioides* was 62 µg/ml. Comparatively, MIC against *Fusarium verticilliodes, Fusarium oxysporum* f. sp. pisi was 125 µg/ml, while that against *Corynespora cassiicola* and

Table 6. **Advantages and disadvantages of different microbial surfactants as antimicrobial agents**

Surfactant	Advantages	Disadvantages
Rhamnolipids	Possible synthesis on industrial waste; high surfactant content	Producers belong to conditionally pathogenic microorganisms; antimicrobial activity not high enough
Lipopeptides	Low minimum inhibiting concentrations against a wide range of pathogenic microorganisms	Low content of produced surfactants; narrow range of substrates for surfactant synthesis (mostly carbohydrates); antimicrobial activity depends on culture conditions
Sophorolipids	Synthesis on cheap substrates (waste oil, oil production waste); High antimicrobial activity at low surfactant concentrations	Low product yield relative to substrate; sources belong to conditionally pathogenic microorganisms; antimicrobial activity depends on culture conditions
Complex of amino- and glycolipids of strains IMV B-7241, IMV B-7405 and IMV Ac-5017	Synthesis on waste (waste oil, waste of biodiesel production); High antimicrobial activity at low surfactant content	Antimicrobial activity depends on the culture conditions

Trichophyton rubrum was much higher (2000 and 1000 μg/ml, respectively).

Therefore, the antimicrobial activity of sophorolipids is higher than that of rhamnolipids. Sophorolipids have a wide range of antimicrobial action on Gram-negative and Gram-positive bacteria and fungi. Publications of the recent years seldom show that sophorolipid antimicrobial activity depends on the culture conditions, such as the carbon source and the presence of precursors for biosynthesis.

Antimicrobial activity of Acinetobacter calcoaceticus IMV B-7241, Rhodococcus erythropolis IMV Ac-5017 and Nocardia vaccinii IMV B-7405 surfactants

We have already established [81] that chemically the surfactants of *R. erythropolis* IMV Ac-5017 are a complex of glyco- (trehalose mono- and dimycolate), neutral (cetyl alcohol, palmitic acid, methyl ester of n-pentadecane acid, mycolic acids) and phospholipids (phosphatidylglycerol, phosphotidylethanolamine). Glyco- and aminolipids were found in the surfactant of *A. calcoaceticus* IMV B-7241, and *N. vaccinii* IMV B-7405 produces a complex of neutral, glyco- and aminolipids [81].

Table 5 presents the MIC of surface-active substances produced by strains IMV Ac-5017, IMV B-7241 and IMV B-7405 on various carbon substrates against bacteria and yeasts. The data show that the antimicrobial activity of *A. calcoaceticus* IMV B-7241, *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017 surfactants depends on the culture conditions, which agrees with data obtained by other researchers in the recent reports [25, 34, 74, 76, 78]. Notably, the surfactants we studied had no higher MIC then described elsewhere.

* * *

We analysed the recent literature on the antimicrobial properties of suface-active substances produced by different groups of microorganisms as an alternative for antibiotics, chemical biocides and desinfectants. The as-yet few papers and our own results do support the necessity of studying the influence of culture conditions on antimicrobial activity of the synthesized surfactants.

The well-known microbial surfactants are compared in Table 6. It shows that the microbial surfactants have their advantages and disadvantages. A strong advantage is the possibility for culturing on industrial waste, which not only lowers the production cost but helps utilize waste of other industries.

The dependency of the substances' antimicrobial activity on the culture conditions can be regulated by chemical modification [82, 83], by genetically [58, 84, 85] and methabolically [86, 87] engineering strains, and by implementing physiological approaches described in [88–90].

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АНТИМІКРОБНА АКТИВНІСТЬ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН МІКРОБНОГО ПОХОДЖЕННЯ

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Метою роботи було проаналізувати літературу останніх років щодо антибактеріальної та антифунгальної активності мікробних поверхнево-активних речовин (ПАР) (ліпопептидів, синтезованих представниками родів *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Brevibacillus*, рамноліпідів бактерій родів *Pseudomonas*, *Burkholderia*, *Lysinibacillus*, софороліпідів дріжджів родів *Candida* (*Starmerella* та *Rhodotorula*), а також дані власних експериментальних досліджень антимікробної активності ПАР, синтезованих *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 і *Nocardia vaccinii* IMB B-7405. Проведений аналіз показав, що ліпопептиди є ефективнішими антимікробними агентами порівняно з гліколіпідами. Мінімальні інгібувальні концентрації (МІК) ліпопептидів, рамноліпідів і софороліпідів становлять у середньому (мкг/мл): 1–32, 50–500 і 10–200 відповідно. МІК поверхнево-активних речовин, синтезованих штамами IMB B-7241, IMB Ac-5017 і IMB B-7405, — у межах, визначених для відомих ліпопептидів та гліколіпідів. Перевагами гліколіпідів як антимікробних агентів порівняно з ліпопептидами є можливість їх синтезу на промислових відходах і висока концентрація синтезованих ПАР. Нечисленні дані літератури і власні результати авторів свідчать про необхідність проведення досліджень щодо впливу умов культивування на антимікробну активність цільового продукту.

Ключові слова: мікробні ліпопептиди, рамноліпіди та софороліпіди, антибактеріальна та антифунгальна активність.

АНТИМИКРОБНАЯ АКТИВНОСТЬ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ МИКРОБНОГО ПРОИСХОЖДЕНИЯ

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Целью работы был анализ данных литературы последних лет относительно антибактериальной и антифунгальной активности микробных поверхностно-активных веществ (ПАВ) (липопептидов, синтезированных представителями родов *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Brevibacillus*, рамнолипидов бактерий родов *Pseudomonas*, *Burkholderia*, *Lysinibacillus*, софоролипидов дрожжей родов *Candida* (*Starmerella* и *Rhodotorula*), а также собственных экспериментальных исследований антимикробной активности ПАВ, синтезированных *Acinetobacter calcoaceticus* IMB B-7241, *Rhodococcus erythropolis* IMB Ac-5017 и *Nocardia vaccinii* IMB B-7405. Проведенный анализ показал, что липопептиды являются более эффективными антимикробными агентами по сравнению с гликолипидами. Минимальные ингибирующие концентрации (МИК) липопептидов, рамнолипидов и софоролипидов составляют в среднем (мкг/мл): 1–32, 50–500 и 10–200 соответственно. МИК поверхностно-активных веществ, синтезированных штаммами IMB B-7241, IMB Ac-5017 и IMB B-7405, находятся в пределах, установленных для известных липопептидов и гликолипидов. Преимуществами гликолипидов как антимикробных агентов по сравнению с липопептидами являются возможность их синтеза на промышленных отходах и высокая концентрация синтезированных ПАВ. Немногочисленные данные литературы и собственные результаты авторов свидетельствуют о необходимости проведения исследований влияния условий культивирования продуцентов на антимикробную активность целевого продукта.

Ключевые слова: микробные липопептиды, рамнолипиды и софоролипиды, антибактериальная и антифунгальная активность.

EXPERIMENTAL ARTICLES

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DIAGNOSTIC CHARACTERISTICS OF THE ELISA TEST FOR THE HEPATITIS B VIRUS SURFACE ANTIGEN DETECTION

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The aim of the work was to define the diagnostic ability of the enzyme immunoassay test system DIA-HBsAg (PJSC "SPC "Diaprof-Med""), in which the principle of analysis is based on biotin-streptavidin amplification of a specific signal.

The assay performance was studied on WHO Second International Standard for HBsAg, subtype adw2, genotype A (NIBSC code: 00/588) in concentration 0.006 IU/ml; on Capricorn HBsAg standard subtypes ad and ay in concentration 0.006 ng/ml and 0.004 ng/ml respectively. All 14 members of the HBsAg Low Titer Performance Panel PHA 106 (BBI, USA) were detected in DIA-HBsAg with high OD/CO ratio 11.9–40.7.

The DIA-HBsAg sensitivity were similar to the sensitivity of Roche COBAS and Murex HBsAg 3.0 when tested on the HBsAg Mixed Titer Performance Panel PHA 206 (BBI, USA) which consisted of sera with various HBsAg concentrations.

The DIA-HBsAg has correctly detected low reactive members of the HBsAg Verification Panel VHA 601 (BBI, USA) with OD/CO ratio 21.0–40.7 whereas the negative member OD/CO was 0.4.

In the evaluation of 174 cross-reactive serum specimens one false positive result was obtained out of 8 sera reactive for IgM to HSV-1/2. The DIA-HBsAg specificity on 1 177 blood donors` specimens was 99.9%.

Key words: ELISA, diagnostics, hepatitis B, analytical and diagnostic sensitivity, specificity.

Hepatitis B is an infectious liver disease with various clinical manifestations [1]. The infection is characterized by severity, high lethality, chronic forms with the development of cirrhosis and liver carcinoma [2]. The infectious agent is a DNA virus (HBV- hepatitis B virus), which is widely distributed because of its incredible resistance to various physical and chemical factors [3]. The HBV virus has infected approximately 350 million people worldwide, including 1.5 million in Ukraine [4]. Creating new highly sensitive tests for the HBV diagnosis will significantly reduce the spread of the infection.

The surface HBV antigen, HBsAg is a main serological marker in hepatitis diagnostics. Only a small amount of HBsAg takes part in virion formation while the rest persists in the infected organism [5]. In acute hepatitis B, the antigen is found in blood one-two weeks after the infection occurred and disappears one-two months later. If it circulates in the body for more than six months, the illness has become chronic [6, 7]. Since it appears before the clinical symptoms

of the disease and the increase in the activity of aminotransferases, the diagnosis of HBV includes an obligatory blood test for HBsAg [8, 9]. However, most ELISA kits for HBsAg determination can only detect it in concentrations of 0.1–0.05 IU/ml [10, 11]. At lower concentrations of the antigen, for example during early stages of the disease, the analysis can give false negative results before HBsAg is eliminated in the blood or in the cases of concurrent infections.

The aim of our research was to study qualitative parameters of the ELISA kit DIA-HBsAg with sensitivity level of 0.01 IU/ml for the diagnostics of HBsAg HBV. The kit is manufactured by SPC Diaproph-Med (Ukraine).

Materials and Methods

ELISA kits

ELISA kit DIA-HBsAg was constructed as a two-stage sandwich. The immunosorbent and biotinylated conjugate included mouse monoclonal antibodies to various immunodominant HBsAg sites. The specific signal is amplified at the next stage of the reaction, when biotinylated antibodies to HBsAg bind to the streptavidin conjugate labeled with high polymer horseradish peroxidase. To detect the reaction we used mono-component TMB/substrate (3, 3′, 5, 5′-tetramethylbenzidine in citrate buffer with hydrogen peroxide). The reaction was terminated using 0.5 M HCl solution. Analysis of the results is carried out by measuring the optical density of the liquid in the wells in a two-wave mode at 450/620 nm, it's proportional to the HBsAg concentration in the serum/plasma. Immunoassay was performed using thermoshaker according to the Instruction for the DIA-HBsAg kit. Results of ELISA were considered at ratio of optical density (OD) to the cut off value (CO). Serum was considered positive at OD/CO ≥1,0 or negative at OD/CO < 1,0.

To confirm of HBsAg in test sera of the patients with hepatitis B we used ELISA kits — Immunite HBsAg (Diagnostic Products Corporation, USA), ДС-ІФА-HBsAg-0,01 (RPS Diagnostic systems, Russia), Вектогреп B-HBs-антиген (RPS Vector-Best, Russia). The assays were carried out according to the instructions for use.

HBsAg standards

Second International standard HBsAg subtype adw2, genotype A (NIBSC, code 00/588) was diluted with blood serum from healthy donors which was previously tested for the absence of HBsAg, to concentrations of 0.02 IU/ml, 0.01 IU/ml, 0.006 IU/ml and 0.004 IU/ml.

Commercial standard ДС-СО-HBsAg (Russia) was diluted by the manufacturer relative to the Second International standard HBsAg (NIBSC) to 20 IU/ml. The antigen was used in concentrations of 0.02 IU/ml, 0.01 IU/ml, 0.006 IU/ml and 0.004 IU/ml.

Standard HBsAg Capricorn, subtypes ad and ay (USA) were diluted by blood serum without HBsAg to concentrations of 0.01 ng/ml, 0.006 ng/ml and 0.004 ng/ml.

Test blood sera

The diagnostic sensitivity of DIA-HBsAg kit was determined using:

– standard serum panel HBsAg Low Titer Performance Panel (Modified) PHA 106 M (BBI), consisting of 15 samples, 14 of which with low concentrations of HBsAg and 1 (sample No. 7) was negative;

– standard serum panel HBsAg Mixed Titer Performance Panel PHA 206 (BBI) including of 25 samples, 23 of which with different HBsAg concentrations and 2 (No.1 and 25) negative;

– standard HBsAg Verification Panel VHA 601 (BBI), in which 5 sera were weakly positive for HBsAg and 1 (sample No.6) was negative;

– 22 blood sera patients with hepatitis B, 8 of which were additionally diluted by blood sera of healthy donors.

The specificity parameter of the test kit was studied using:

– 174 blood sera with cross-reactive components which can lead to false positives in analysis for hepatitis B, including:

30 samples with IgM/IgG to HCV;
32 samples with IgG to HSV1/2;
8 samples with IgM to HSV1/2;
16 samples with IgM/IgG to HSV1/2, IgM/IgG to CMV;
16 samples with IgG to CMV;
24 samples with IgG to CMV, IgG to HSV1/2;
8 samples with IgM to CMV;
8 samples with IgM/IgG to CMV, IgG to HSV1/2;
8 samples with IgM/IgG to CMV;
24 samples from pregnant women,

and 1177 blood sera of nonselective donors.

The test kit specificity was calculated according to the following formula:

$$Specificity = \frac{TN}{TN+FP} \times 100\%, \quad (1)$$

where TN is the amount of true negative results; FP is the amount of false positive results.

In the tables and pictures the results of typical experiment are presented.

Results and Discussion

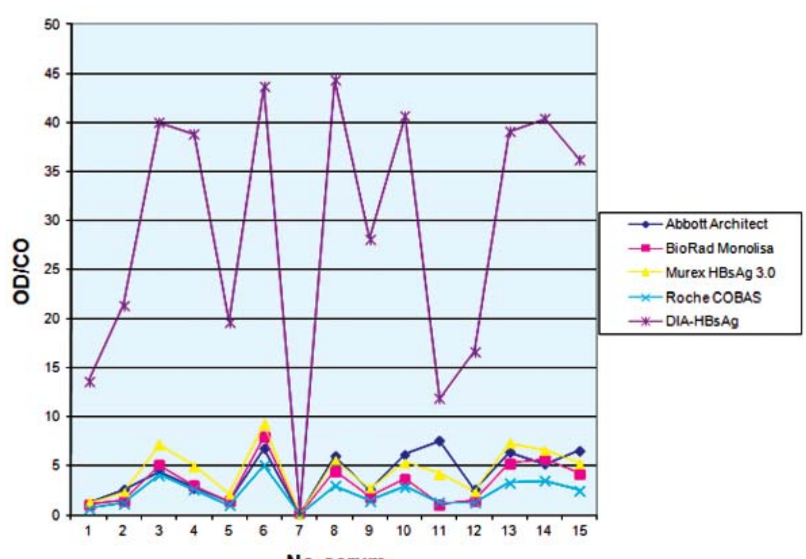
The investigation in the DIA-HBsAg kit different commercial standards of the surface antigen of hepatitis B virus established that it detects Second International standard HBsAg (NIBSC) and Russian standard ДС-СО-HBsAg, diluted by it, in concentration 0.006 IU/ml (Table 1). Test kit was able to detect the standard HBsAg Capricorn subtype ad at 0.006 ng/ml and the subtype ay at 0.004 ng/ml.

To determine diagnostic sensitivity of the DIA-HBsAg kit, we tested samples from serum panel PHA 106 M (BBI) with low concentrations of HBsAg (Figure).

Analysis results in commercial test kits were taken from the passport on the panel. The DIA-HBsAg kit identified all 14 sera with

Table 1. **Performance of DIA-HBsAg kit in tests on various HBsAg standards**

HBsAg standards	HBsAg concentration	ELISA results OD/CO
Second International standard HBsAg (NIBSC, code 00/588)	0.02 IU/ml	3.4
	0.01 IU/ml	1.8
	0.006 IU/ml	1.1
	0.004 IU/ml	0.7
ДС-СО-HBsAg	0.02 IU/ml	3.7
	0.01 IU/ml	1.9
	0.006 IU/ml	1.2
	0.004 IU/ml	0.8
Standard HBsAg Capricorn (USA):		
Subtype ad	0.01 ng/ml	1.7
	0.006 ng/ml	1.0
	0.004 ng/ml	0.7
Subtype ay	0.01 ng/ml	3.0
	0.006 ng/ml	1.9
	0.004 ng/ml	1.2



Comparative analysis results HBsAg Low Titer Performance Panel (Modified) PHA 106 M (BBI) in different ELISA kits ($P < 0.05$)

HBsAg as positive with high OD/CO ratio (11.9–40.7), and the one without HBsAg as negative. The data showed the ability of the DIA-HBsAg kit to identify low concentration of HBsAg to be significantly higher than in test kits comparing leading foreign manufacturers (Abbott Architect, BioRad Monolisa Plus, Murex HBsAg 3.0, Roche COBAS).

Table 2 presents the results for PHA 206 (BBI) serum panel with 25 samples containing various ratios of HBV DNA and HBsAg (23 samples) or negative for HBV (No.1 and 25) according to the panel passport. The data on commercial kits were taken from the panel's passport. The DIA-HBsAg kit revealed the HBV surface antigen in all positive samples not inferior in its diagnostic ability to commercial analogues. A false-negative result was obtained in the analysis of serum No. 22 in the Roche COBAS kit, while Murex HBsAg 3.0 and DIA-HBsAg identified this sample positive with OD/CO ratio of 1.9 and 8.9 respectively.

The qualitative parameters of industrially-manufactured ELISA kits

Table 2. **Results of identification of the antigen in samples of HBsAg Mixed Titer Performance Panel PHA 206 (BBI) using various test systems**

Sample №	**Test system**			
	HBV DNA PCR Roche Amplicor Monitor	Roche COBAS	Murex HBsAg 3.0	DIA-HBsAg
	results			
	copies/ml	OD/CO		
1	<300	0.1	0.4	0.3
2	>2×10^5	**59.0**	**>max**	**40.9**
3	>2×10^5	**47.4**	**35.9**	**42.5**
4	>2×10^5	**74.5**	>max	42.0
5	>2×10^5	**74.5**	**>max**	**42.3**
6	>2×10^5	**74.5**	**>max**	**41.1**
7	2×10^5	**44.6**	**31.9**	**41.9**
8	1×10^5	**25.3**	26.1	46.4
9	2×10^5	**32.8**	**30.9**	**46.2**
10	4×10^4	**10.8**	**11.5**	**39.5**
11	7×10^4	**9.8**	**11.1**	**38.1**
12	2×10^5	**16.0**	15.3	38.7
13	1×10^5	**10.6**	**13.2**	**40.6**
14	5×10^3	**7.8**	**7.4**	**42.4**
15	5×10^4	**3.6**	**5.6**	**35.6**
16	4×10^3	**4.9**	4.8	36.7
17	<300	**1.2**	**2.0**	**7.4**
18	2×10^4	**2.2**	**3.6**	**43.2**
19	6×10^4	**2.7**	**4.1**	**34.2**
20	2×10^4	**4.6**	5.2	40.0
21	2×10^5	**5.7**	**4.7**	**42.0**
22	<300	0.5	**1.9**	**8.9**
23	5×10^3	**2.1**	**2.3**	**25.6**
24	9×10^2	**1.2**	1.6	16.6
25	<300	0.2	0.5	0.3

Table 3. **Serum panel test results for HBsAg Verification Panel VHA 601 (BBI) using DIA-HBsAg test kit**

№	HBsAg analysis (from the panel passport)	IEA results for DIA-HBsAg kit, OD/CO
1	Weakly positive	45.0
2	Weakly positive	43.5
3	Weakly positive	37.1
4	Weakly positive	35.4
5	Weakly positive	21.0
6	Negative	0.4

designed for diagnostics of various infections are determined using the special commercial verification serum panels. They allow to determine the ability of the ELISA kits to distinguish weakly positive samples from negative ones.

In our study, the diagnostic ability of the DIA-HBsAg kit was tested on the commercial serum verification panel VHA 601 (BBI). The panel included 6 samples of which 5 had low concentration of HBsAg and one was negative for HBV. DIA-HBsAg kit identified the surface antigen of HBV in all weakly positive blood sera with OD/CO 21.0–45.0 (Table 3). Sample No.6, without HBsAg, was identified as negative with OD/CO 0.4.

The high diagnostic capability of DIA-HBsAg for standard serological samples, including the ones with low HBsAg content, was further corroborated by testing clinical material obtained from hepatitis B patients (Table 4). We used 22 blood sera, pre-checked for HBsAg using commercial kits. To lower HBsAg content, 8 samples were diluted with sera of healthy donors after which the HBsAg in them was confirmed again.

In investigating of undiluted sera from hepatitis B patients OD/CO was 35.3–51.2. The value OD/OC barely changed (48.1–39.7) for sample No.1 and secondary samples obtained by its dilution 10 and 1000 times. In the analysis of weakly positive samples obtained after dilution of serum No. 13 in 150–1600 times, the ratio of OD/CO was in the range of 1.2–18.1.

Thus, research of diagnostic sensitivity of the DIA-HBsAg kit on standard serum panels and blood serum samples from patients with hepatitis B patients with different virus surface antigen titers showed the kit's ability to detect HBsAg in low concentrations common for the early stages of the disease.

The diagnostic specificity of the DIA-HBsAg kit was tested on 1177 sera of nonselective donors. The ability of the test kit to correctly analyze sera without HBsAg but with various cross-reactive reagents that can cause false positive results was established by testing the sera with specific antibodies to HCV, HSV1/2, CMV, and sera of blood of pregnant women (Table 5).

One sample was determined positive when investigated in DIA-HBsAg 1177 sera from nonselective donors from various units of the Ukrainian Blood Service. At the same time, ДС-ІФА-HBsAg-0,01 and Вектогреп B-HBs-антиген identified it as negative. The kit's specificity, calculated according to formula 1, was 99.9%.

In the investigating of 174 sera with potential cross-reactivity in DIA-HBsAg kit was obtained single false positive result for 1 serum out of 8 studied with antibodies of class M to the herpes simplex virus. The specificity parameter in thise experiment was 99.4%.

Therefore, the DIA-HBsAg kit for detecting the main serological marker of hepatitis B — HBsAg, which appears early in the acute stage and persists in the chronic form, has a sufficiently high analytical and diagnostic ability. The ELISA kit employs amplificatory method of boosting a specific signal combined with high-polymer enzyme label. This construction allows to detect low HBsAg concentration - International standard HBsAg (NIBSC) in concentration 0.006 IU/ml, standard HBsAg Capricorn subtypes ad and ay in concentration 0.006 ng/ml and 0.004 ng/ml, respectively. Comparative research showed that DIA-HBsAg kit was able to detect small HBsAg concentrations more reliably than its leading foreign analogues produced by Abbott Architect, BioRad Monolisa Plus,

Table 4. **Results of testing DIA-HBsAg kit against blood sera of hepatitis B patients with varying HBsAg content**

№	Serum (and dilution)	Test kit	IEA results in the DIA-HBsAg kit (OD/CO)
1	1	Immulite HBsAg	48.1
2	1 (1/10)		47.3
3	1 (1/100)		47.5
4	1 (1/1000)		39.7
5	2		45.2
6	3		48.5
7	4		46.0
8	5		45.5
9	6		47.6
10	7		43.4
11	8		35.3
12	9		37.8
13	10	ДС-ІФА-HBsAg-0,01	48.7
14	11		39.1
15	12		48.2
16	13	Векторгеп В-HBs-антиген, ДС-ИФА-HBsAg	38.3
17	13 (1/150)		18.1
18	13 (1/200)		12.7
19	13 (1/400)		3.9
20	13 (1/800)		1.9
21	13 (1/1600)		1.2
22	14		51.2

Table 5. **Specificity values for DIA-HBsAg kit obtained analyzing sera with interferent components from nonselective donors**

Samples	Number of samples	Number of positive results	Number of negative results	Specificity
Interfering components in blood sera:				99.4%
IgM/IgG to HCV	30	0	30	
IgG to HSV1/2	32	0	32	
IgM to HSV1/2	8	1	7	
IgM/ IgG to HSV1/2 IgM/IgG to CMV	16	0	16	
IgG to CMV	16	0	16	
IgG to CMV IgG to HSV1/2	24	0	24	
IgM to CMV	8	0	8	
IgM/ IgG to CMV IgG to HSV1/2	8	0	8	
IgM/ IgG to CMV	8	0	8	
Pregnant women's blood sera	24	0	24	
Total:	174	1	173	
Nonselective donors' blood sera	1177	1	1176	99.9%

Murex HBsAg 3.0, Roche COBAS. The high diagnostic ability of the kit was confirmed also by the results obtained for hepatitis B patients. The kit correctly distinguishes weakly positive sera of from negative ones. Testing 1177 sera of nonselective donors established the DIA-HBsAg kit specificity to be 99.9%. The qualitative parameters of the kit allow it to be widely used in specific diagnostics of hepatitis B and to detect both early and chronic stages of the disease, making donated blood safer.

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ДІАГНОСТИЧНА ХАРАКТЕРИСТИКА ELISA ТЕСТ-СИСТЕМИ ДЛЯ ВИЗНАЧЕННЯ ПОВЕРХНЕВОГО АНТИГЕНУ ВІРУСУ ГЕПАТИТУ В

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Метою роботи було визначити діагностичну здатність імуноензимної тест-системи DIA-HBsAg (НВК «Діапроф-Мед»), в якій принцип аналізу засновано на біотинстрептавідиновій ампліфікації специфічного сигналу. Діагностикум виявляє 2-й Міжнародний стандарт HBsAg, субтип adw2, генотип А (NIBSC, code: 00/588) в концентрації 0,006 МО/мл, стандарт HBsAg Capricorn субтипу ad та ay (USA) — 0,006 нг/мл і 0,004 нг/мл відповідно.

Під час дослідження панелі сироваток PHA 106 M (BBI), 14 зразків якої містять низькі концентрації HBsAg, тест-система виявила його у всіх позитивних сироватках з високим співвідношенням ОГ/cut off —11,9–40,7.

За результатами аналізу панелі сироваток PHA 206 (BBI) з різними концентраціями HBsAg діагностична здатність тест-системи не поступалася її закордонним аналогам — Roche COBAS і Murex HBsAg 3.0.

Діагностикум коректно розділяв слабопозитивні зразки верифікаційної панелі VHA 601 (BBI) — ОГ/cut off 21,0–40,7 від негативного (ОГ/cut off — 0,4).

У процесі дослідження 174 сироваток крові з кросреактивними компонентами на гепатит В отримано один хибнопозитивний результат під час аналізу 1 зразка з 8 досліджених, які містили IgM до HSV1/2. Специфічність тест-системи за результатами аналізу 1177 донорів становила 99,9%.

Ключові слова: імуноензивна тест-система, діагностика, гепатит В, аналітична і діагностична чутливість, специфічність.

ДИАГНОСТИЧЕСКАЯ ХАРАКТЕРИСТИКА ELISA ТЕСТ-СИСТЕМЫ ДЛЯ ОПРЕДЕЛЕНИЯ ПОВЕРХНОСТНОГО АНТИГЕНА ВИРУСА ГЕПАТИТА В

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Целью работы было определение диагностической способности иммуноэнзимной тест-системы DIA-HBsAg (НПК «Диапроф-Мед»), в которой принцип анализа основан на биотинстрептавидиновой амплификации специфического сигнала. Диагностикум выявляет 2-й Международный стандарт HBsAg, субтип adw2, генотип А (NIBSC, code: 00/588) в концентрации 0,006 МЕ/мл, стандарт HBsAg Capricorn (USA) субтипы ad и ay — 0,006 нг/мл и 0,004 нг/мл соответственно.

При исследовании панели сывороток PHA 106 M (BBI), 14 образцов которой содержат низкие концентрации HBsAg, тест-система выявила его во всех положительных сыворотках с высоким соотношением ОП/cut off — 11,9–40,7.

По результатам анализа панели сывороток PHA 206 (BBI) с различными концентрациями HBsAg диагностическая способность тест-системы не уступала ее зарубежным аналогам — Roche COBAS и Murex HBsAg 3.0.

Диагностикум корректно разделял слабоположительные образцы верификационной панели VHA 601 (BBI) — ОП/cut off 21,0–40,7 от отрицательного (ОП/cut off — 0,4).

При исследовании 174 сывороток крови с кросс-реактивными компонентами на гепатит В получен один ложноположительный результат при анализе 1 образца из 8 исследованных, которые содержали IgM к HSV1/2. Специфичность тест-системы по результатам анализа 1177 доноров составила 99,9%.

Ключевые слова: иммуноэнзимная тест-система, диагностика, гепатит В, аналитическая и диагностическая чувствительность, специфичность.

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INFLUENCE OF C_{60}-FULLERENE AQUEOUS COLLOID SOLUTION ON LIVER AND PANCREAS MORPHOLOGICAL STATE AND BLOOD AMINOTRANSFERASES OF RATS WITH EXPERIENCED ACUTE CHOLANGITIS

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Aim of the work was to investigate the suspended C_{60}-fulleren effect on liver and pancreas state under intraperitoneal and intragastrial administration on rat experimental cholangitis model. Acute cholangitis was simulated by a single ingestion of α-naphthyl isothiocyanate — ANIT. C_{60}-fullerene aqueous colloid solution (C_{60}FAS, 0.15 mg/ml) was administered to animals at a volume containing C_{60}-fullerene at a dose of 0.5 mg/kg body weight in 24 and 48 h after ANIT administration. After 72 hof the experiment, the animals were euthanized. Blood serum ALT and AST activities were measured, the liver and pancreas states were analyzed by light-microscopy level. It was found that intragastrial and intraperitoneal administration of C_{60}FAS contributes to the correction of negative effects in the liver and pancreas caused by the induction of acute cholangitis. This was proved by the normalization of ALT activity, reduction of pancreatic parenchymal edema and liver fibrosis, and increased blood flow in these organs. Application of C_{60}FAS could improve the state of the liver and pancreas under acute cholangitis in rats.

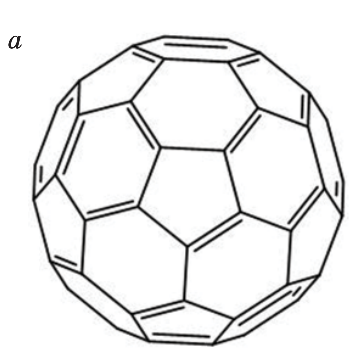
Key words: C_{60}-fullerenes, acute cholangitis.

Primary sclerosing cholangitis (PSC) is a chronic cholestatic disease characterized by intrahepatic and extrahepatic bile ducts inflammation,obliteration and fibrosis [1], resulting in biliary cirrhosis, portal hypertension and hepatic failure. The etiology of PSC is unknown. Genetic predisposition (in particular hla b8, dr3, drw52) [2] and environmental factors, including bacterial and viral infections, are considered to be risk factors. Most often this pathology is associated with autoimmune diseases (primary biliary cirrhosis, rheumatoid arthritis, autoimmune hepatitis, systemic sclerosis, systemic lupus erythematosus, cystic fibrosis) and inflammatory bowel disease (70–80% of cases).

PSC-prognosis is extremely unfavorable due to following complications: portal hypertension accompanied by bleeding and functional renal insufficiency (hepatorenal syndrome), spontaneous bacterial peritonitis, chronic cholestasis accompanied by weakness, itching, steatorrhea, deficiency of fat-soluble vitamins (A, D, E and K) and osteoporosis. Specific complications of PSC include bacterial cholangitis (15–35%), cholelithiasis (25–56%), chronic pancreatitis (20–23%) and cholangiocarcinoma (6–18%). The survival rate of patients in the last case does not exceed 12 months [1].

PSC-therapy is symptomatic and includes medications for treatment itching and jaundice (choleretics, sorbents), antibiotics (for the treatment of infections), diet and vitamin supplements, immunosuppressants and anti-inflammatory drugs. The only effective method

a



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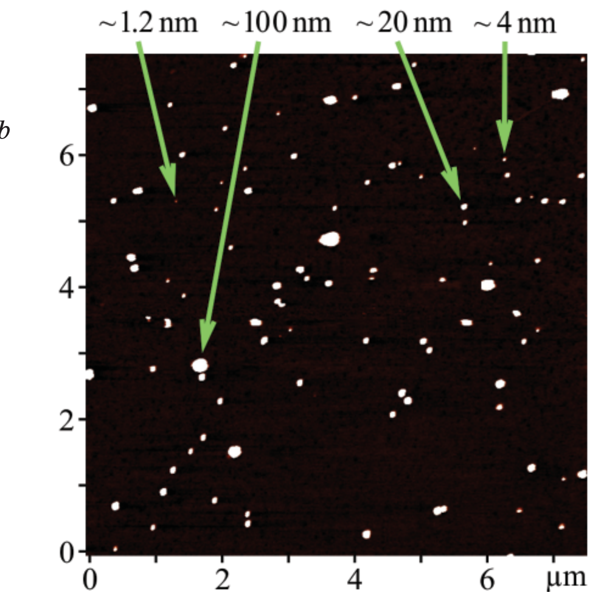

Fig. 1. **C_{60} fullerene (*a*), AFM image (tapping mode) of C_{60} fullerene particles on the mica surface: arrows indicates the height of individual nanoparticles (*b*)**

for PSC treating is liver transplantation, but PSC relapse after transplantation occurs in 15–20% of patients, and the 5-year survival rate is only 66–72% [3].

PSC, as any inflammatory disease, is accompanied with oxidative stress, increased levels of lipid peroxidation products and antioxidants deficiency. Therefore, the use of the latter is promising to correct at least the disease symptoms, remaining, however, beyond the attention of researchers. Numerous studies have shown the anti-inflammatory effects of natural antioxidants (vitamins, minor amino acids, polyunsaturated fatty acids, plant extracts) *in vitro*, but effectiveness of those in *in vivo* systems is highly questionable. On the other hand, artificial compounds, in particular fullerene, have clearly defined properties and are involved in a number of cellular processes [4–9], which determines their selective action and a more pronounced therapeutic potential. C_{60}-fullerene can effectively scavenge free radicals and thus act as antioxidants [10, 11], revealing anti-inflammatory properties [12, 13].They are non-toxic in *in vitro* and *in vivo* systems acting in physiological concentrations [6, 8, 14] and are capable to be accumulated in the liver [15]. The last makes them very attractive for direct impact on this organ. Thus, C_{60}-fullerene may be considered as potential therapeutics for effective prevention and treatment of liver diseases associated with oxidative stress.

Given the foregoing, the aim of the study was to investigate the morphofunctional state of the liver and pancreas in rats received suspended C_{60}-fullerene under α-naphthyl isothiocyanate-induced acute cholangitis condition.

Materials and Methods

The studywas conducted on 32 white outbred male rats with an average body mass 198 ± 10 g, which were kept under standard vivarium conditions. All experiments were conducted in compliance with bioethics principles, legislative norms and provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), General Ethical Principles for Experiments on Animals, adopted by the First National Bioethics Congress (Kyiv, 2001), and approved by an institutional review committee (protocol № 2 dated April 25, 2017, ESC "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv).

Acute cholangitis was simulated by a single ingestion of α-naphthyl isothiocyanate (ANIT, Sigma, USA) at a dose of 100 mg/kg dissolved in sunflower oil (total volume of 0.1 ml). ANIT is a hepatotoxin which depending on dose and duration of administrationcauses liver damage, such as intrahepatic cholestasis, acute cholestatic hepatitis, sclerosing cholangitis, biliary fibrosis and cirrhosis [16]. The mechanism of action of

this toxicant is associated with a specific lesion of the epithelial cells of intralobular bile ducts. Cholangiocytes' injury causes bile ducts obstruction with detritus or cells excessive growth with subsequent sclerotic degeneration resulting in bile flow stop and periportal inflammation [16]. The systemic result is blood serum and urine bilirubin increase (mainly due to directbilirubin) and serum aminotransferases and alkaline phosphatase activity raise, which corresponds to the biochemical manifestations of acute and chronic sclerosing cholangitisin humans.

A highly stable pristine C_{60}-fullerene aqueous solution (C_{60}FAS) with purity of more than 99.95% has been prepared and characterized according to [17, 18]. Briefly, this method is based on transferring C_{60}-fullerene from organic solution into the aqueous phase by ultrasonic treatment. The maximal concentration of C_{60}-fullerene in water was 0.15 mg/ml. Themorphological state of C_{60}-fullerene in aqueous solution was monitored using atomic force microscopy and measurement of small-angle neutron scattering (Fig. 1) [19]. Concentrated C_{60}-FAS contained both single C_{60} moleculesand their nanoparticles (aggregates) with sizes of 1.2–100 nm which is in agreement with our previous results [17, 20].

Animals received C60FAS intraperitoneally or intragastrially in a dose equal to 0.5 mg / kg body weight C_{60}-fullerene in 24 and 48 h after ANIT administration. All manipulations with the animals in comparison groups were conducted similarly to the animals of the experimental ones, including the appropriate solutions administration. There were 4 experimental groups (n = 8): 1 — control; 2 —acute cholangitis; 3 — cholangitis and C_{60}FAS intraperitoneal administration; 4 — cholangitis and C_{60}FAS intragastrial administration. Since the effect of C_{60}FAS on healthy animals has been studied and described in our previous studies [9, 21], we did not include the description of groups of healthy animals receiving C_{60}FAS in the current manuscript.

In 72 h after the start of the experiment the animals were killed by inhalation of CO_2 and subsequent cervical dislocation.The blood for biochemical analysis was collected immediately after the sacrifice from the femoral vein, left for 20 min to form a clot and then centrifuged 8 min at 1000 g. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in blood serum using standard reagent kits (Filisit diagnosis, Ukraine) and expressed in μmol sodium pyruvate per ml of blood serum per h.

The fragments of liver and pancreas were harvested immediately after the sacrifice and fixed in Bouin mixture for 14 days for histological assay. Then, they were embedded into paraffin, sliced into 5 m sections, stained with hematoxylin and eosin [22] and examined under the light microscope (Olympus BX-41, OlympusEuropeGmbH, Japan). Liver centrilobular and periportal zones, exocrine and endocrine part of the pancreas were assessed separately.

Statistical processing of data was carried out using one-way analysis of variance (ANOVA) with the Tukey post hoc test [23]. The difference between compared groups was considered significant at $P \leq 0.05$.

Results and Discussion

The yellowness of the peritoneum and mucous membranes resulting from the accumulation of bilirubin in plasma blood and tissues, edema and liver granularity suggesting the micronodular fibrosis were observed atthe autopsies ofall animals from the cholangitis group (group 2). On liver micropreparations (Fig. 2, *B*) one can see fibrotically alteredportal tracts with surrounding cellular inflammatory infiltrate, sites with violation of the parenchymal limiting plate and portal-portal linking septs, suggesting the acute cholangitis. Atrophic and degenerative changes in the bile duct epithelium and somebile ducts replaced by fibrous cords (scars) were observed. Lymphoid follicles and fibrotic loci were detected in parenchyma. Also, thrombosis of some blood vessels including central veins, sinusoids dilation and a marked increase in the number of leukocytes in vessels were detected. Blood serum ALT and AST were higher than those in control group (by 81% and 75% respectively) (Fig. 3) indicating cytolysis of hepatocytes and cholestasis, which may be the result of bile ductsobstruction.

The pancreas of rats in this grouprevealed significant structural changes (Fig. 4, *B*): largeareas ofsecretory acinidestruction; dystrophic changes of acinar tissue; cellular and intercellular edema, which sometimes caused the destruction of both individual pancreatic cells and whole acini; cytoplasm zonation loss in pancreatic cellsfrom areas with destroyed structure. Observed changes could indicate a violation of

pancreaticexocrine parenchyma functional state and the development of acute pancreatitis. The gland microcirculatory also underwent changes: there was a stasis and sometimes expressed thrombosis of small blood vessels, sinusoid hemocapillaries dilation and sometimes microtrombosis. The pancreatic endocrine apparatus wasn't changedsignificantly,pancreatic endocrinocytes did not differ from control ones.

In rats received C60FAS (groups 3 and 4) the jaundice of mucous membranes and peritoneum was less pronounced compared to non-treated animals (group 2), but liver edema and micronodular fibrosis persisted (Figs. 2, *C*, *D*). The area of the liver fibrotically altered parenchyma was smaller compared to non-treated animals, portal-portal linking septsweren't detected. However, necrotic loci and loci of hypereosinophilic cells were observedin fibrotically altered sites. Also blood vessels overflow was markedly higher compared to control, sometimes even blood stasis occurred. Occasionally zones with dark inclusions were detected, which might be caused by C_{60} fullerene accumulation. It should be noted, that rats received C_{60}FAS intragastrially (group 4) demonstrated significantly less expressedsigns of periportal inflammation in comparison with those received C_{60}FASintraperitoneally (group 3). Manifestations of necrosis in fibrotically altered sites in this group also were less common and less frequent compared to group 3. ALT activity was restored to control values in rats received C_{60}FAS by both ways, although AST activity remained unchanged (Fig. 3), which mightindicate partial persistence of cholestasis. Thus, C_{60}FAS inhibited the symptoms of acute cholangitis but did not prevent hepatocyte cytolysis completely.

Blood stasis in small vessels, wall thickening and edema of middle blood vessels were revealed in the pancreas of animals received C_{60}FAS (Figs. 4, *C*, *D*). The vast majority of exocrine pancreatic cells had a normal structure, although there were occasional sites with signs of acinus dystrophy and cells with cytoplasmic vacuolation. The cytoplasm of most cells was clearly delineated into basophilic and acidophilic zones, the nuclei had a rounded form, indicating the normal functional activity of the cells. The endocrine part of the pancreas did not undergo significant changes. However, significantly greater interacinar edemawas detected in animals received C_{60}FAS intraperitoneally compared to those received C_{60}FAS intragastrially. Summarizing, C_{60}FAS contributed to mitigate of pancreatic injury due to ANIT-induced acute cholangitis.

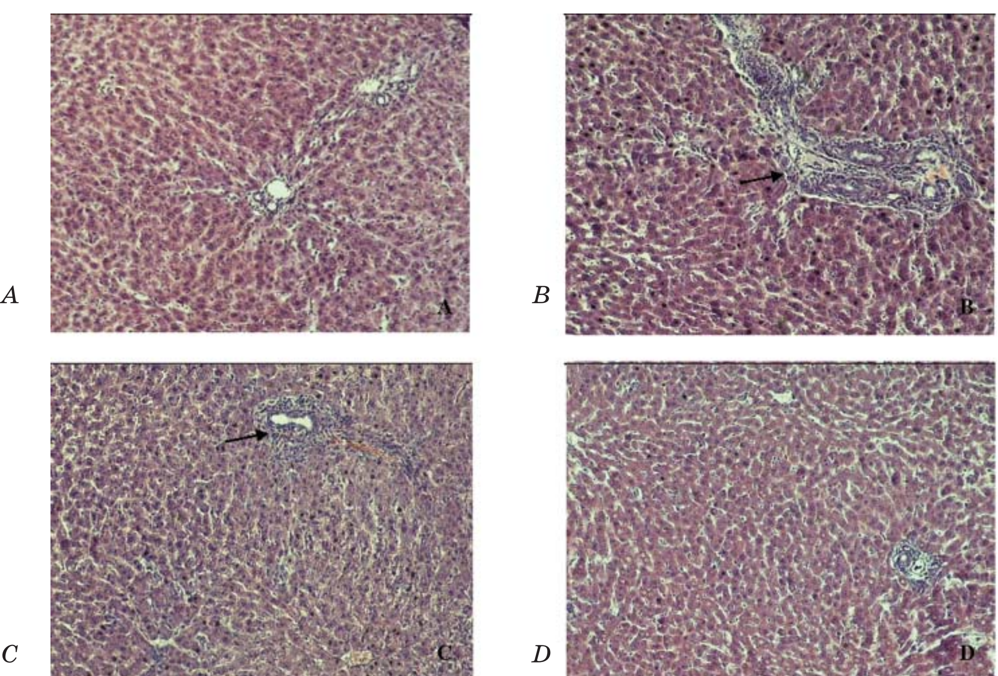

Fig. 2. **Microphotographs of liver of rats experienced acute cholangitis and treated with C60FAS: control (*A*), acutecholangitis (*B*), cholangitis+C_{60}FAS intraperitoneal (*C*) and intragastrial (*D*) administration. Hematoxylin-eosin staining, ×100 magnification; the arrows indicate fibrotically altered portal hepatic tracts with surrounding diffuse cell inflammatory infiltrate**

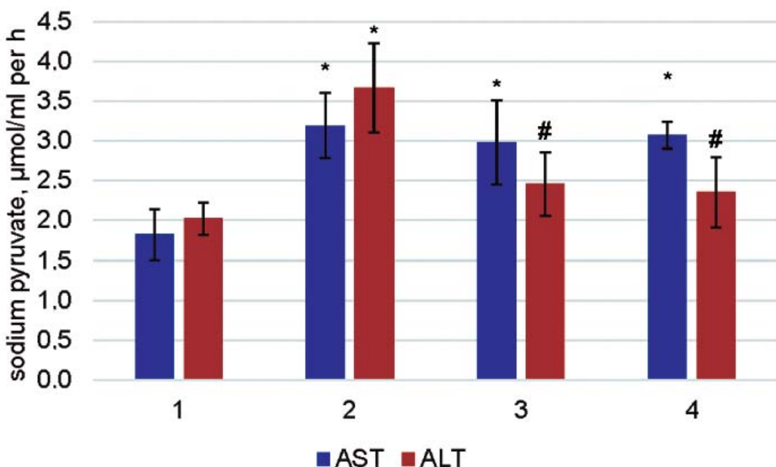

Fig. 3. **Blood serum ALT and AST activities of rats experienced acute cholangitis and treated with C_{60}FAS:** control (*1*); acute cholangitis (*2*); cholangitis+C_{60}FAS intraperitoneal (*3*) and intragastrial (*4*) administration;
*$P \le 0.05$ compared to control, #$P \le 0.05$ compared to acute cholangitis group

One of the mechanisms involved in the pathogenesis of PSC is an abnormal immune response, which leads to the activation of auto-reactive T and B lymphocytes and the subsequent production of numerous inflammatory mediators [24]. As a result, the liver undergoes significant destructive impacts of, in particular, excessive amounts of bile acids (having detergent properties), toxins, inflammatory mediators, autoimmune complexes. This eventis the cause of oxidative stress development [25]. Thus, patients with PSC demonstrate significantly increased lipid and protein peroxidation, cholesterol auto-oxidation and downregulation of antioxidant defense system [26–28]. In addition, ANIT-induced liver damage in rats (a valid model of human sclerosing cholangitis) is accompanied by oxidative stressdevelopment and is widely used to study hepatoprotective efficacy of different compounds including ones possessing antioxidant properties [29–31].

Biliary pathology is the most common cause of acute pancreatitisand exacerbation of chronic one. The main mechanism of biliary pancreatitis is bile influx into the pancreatic duct (due to the pressure difference in common bile duct and pancreatic duct under the biliary hypertension) with subsequent interactionof bile with pancreatic enzymes and bacteria. Hence, the release of bound bile acids and the activation of pancreatic enzymes immediately in the pancreatic duct systemoccur. As a result, its protective barrier is damaged and the parenchyma of the gland is affected. Based on clinical and pathological studies the association of PSC with pancreatic disease was proven [32], and the joint autoimmune component of both diseases was noted [33].

C_{60}-fullerene is a molecular form of carbon having a spheroidal structure. Due to the presence of π-conjugated double bonds between hexa- and pentagonal structures on the surface, C_{60}-fullerene is capable to scavenge reactive oxygen species (ROS) effectively [4, 11, 14]. Its unique properties include strong electron acceptor activityand high polarization and hydrophobic ability, which enables them to effectively bind free radicals not only in the extracellular space, but also penetrate into the cell [5, 10, 34]. Hence, C_{60}-fullerene are capable not only to scavenge free radicals directly [10], but also to be involved in the regulation of intracellular signaling pathways associated with ROS overproduction [4, 11]. An important feature of C_{60}-fullerene is its relatively low toxicity [11], non-immunogenicity [4] and the ability to be accumulated in liver [6, 15], which makes this compound attractive for selective impacton liver and treatment of this organ's diseases associated with oxidative stress. In addition, the ability of C_{60}-fullerene to suppress colonic inflammation under systemic and topical application and to correct its systemic effects [9], to prevent the development of toxic hepatitis [12] and to realize antitumor properties [10] were demonstrated in many studies.

One of the leading roles in the development of autoimmune inflammation belongs to oxidative stress. The last is accompanied by a violation of the pro-and antioxidant balance and overproduction of ROS and reactive

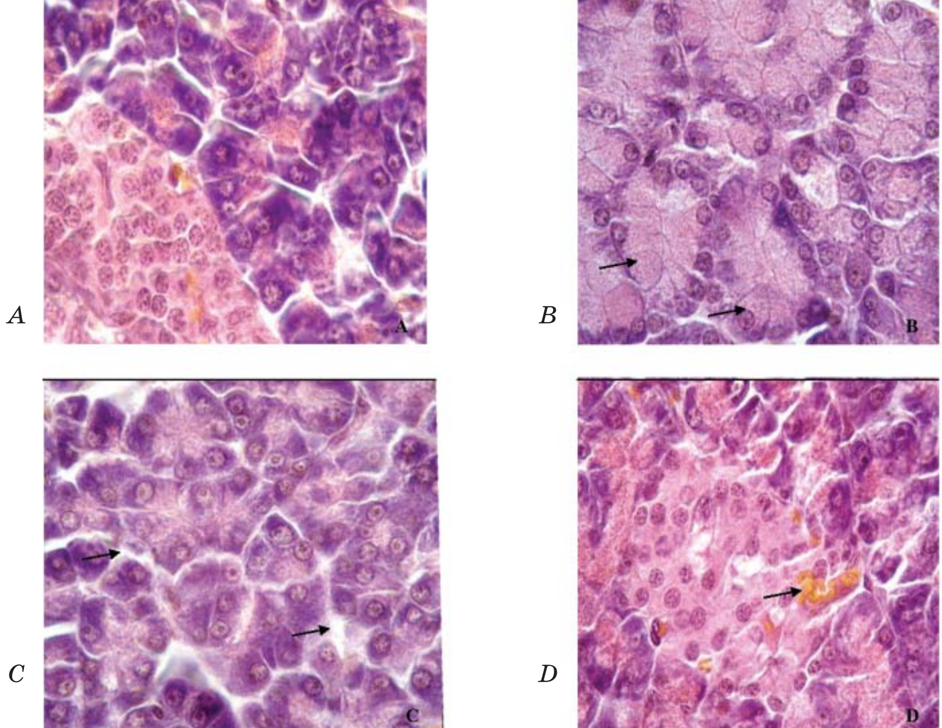

Fig. 4. **Microphotographs of pancreasof rats experienced acute cholangitis and treated with C_{60}FAS:** control (*A*); acutecholangitis (*B*);cholangitis+C_{60}FAS intraperitoneal (*C*) and intragastrial (*D*) administration. Hematoxylin-eosin staining, ×400 magnification; the arrows indicate acinar cellsedema andcytoplasm zonation loss (*B*), interacinar edema (*C*), blood vessels overflow (*D*)

nitrogen species. The consequence is the initiation of cell phagocytosis and apoptosis leading to tissue damage and intracellular autoantigensrelease, which in turn results in the production of autoantibodies. In addition, the oxidative modification of macromolecules, in particular DNA, in apoptotic cellsleads to the formation of new epitopes, which causes the production of a wide range of polyspecific autoantibodies and the escalation of the autoimmune response process [34, 35].

As mentioned above, most researchers suggest the autoimmune cause of PSC [36, 37], therefore it is logical and reasonable to assume the efficacy of antioxidant use for correction at least the diseasesymptoms. Therefore, we suppose that C_{60}FAS suppressed the symptoms of acute cholangitis and pancreatitis precisely because of its antioxidant properties. Indeed, the only drug approved for the treatment of PSC–ursodeoxycholic acid–possesses antioxidant properties and normalizes the content of reduced glutathione in liver tissue and blood serum by activating of γ-glutamylcysteinesynthase [38], which in turn can cause a positive systemic effect on the organism and particularly on the pancreas. In addition, in our previous studies we demonstrated the ability of C_{60}-fullerene to suppress lipid and protein peroxidation and to upregulate the antioxidant enzymes in liver under an inflammatory process in the organism [9].

It should be noted that intragastrial administration of C_{60}-fullerene in a dose of 10 mg/kg body weight causes an increase ofCYP 2B1 enzyme activity in liver, reduces serum blood uric acid, increases serum blood urea and enhances the small intestinal wallpermeability for macromolecules [39]. This can testify the occurrence of some local and systemic toxic effects of the chemical in case of its ingestion. This fact might be an explanation of more expressed positive effect of C_{60}FAS intraperitoneal administration on liver and pancreascompared to intragastrialone. In addition, incomplete absorption of C_{60}-fullerene in the gastrointestinal tract might occur, and, correspondingly, a lower dose of the compound enters into the systemic circulation. This assumption may be confirmed by the data of low absorption of C_{60}-fullerene from the gastrointestinal tract [36] and by information of the dose-dependent mannerof C_{60}-fullerene hepatoprotective activity when realized due to their antioxidant properties [14].

Consequently, C_{60}FAS when administered intragastrally or intraperitoneally could correct the negative effects of ANIT-induced acute cholangitis on liver and pancreas. The prove is the normalization of blood serum ALT and diminishing of pancreatic parenchyma's edema and liver fibrosis.

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ВПЛИВ ВОДНОГО КОЛОЇДНОГО РОЗЧИНУ C_{60}-ФУЛЕРЕНУ НА МОРФОЛОГІЧНИЙ СТАН ПЕЧІНКИ, ПІДШЛУНКОВОЇ ЗАЛОЗИ ТА АМІНОТРАНСФЕРАЗНУ АКТИВНІСТЬ СИРОВАТКИ КРОВІ ЩУРІВ ЗА ГОСТРОГО ХОЛАНГІТУ

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Метою роботи було дослідити вплив C_{60}-фулерену на морфологічний стан печінки та підшлункової залози за інтраперитонеального та інтрагастрального введення на моделі експериментального холангіту щурів. Гострий холангіт відтворювали одноразовим інтрагастральним введенням α-нафтил-ізотіоціанату — ANIT. Водний колоїдний розчин C_{60}-фулерену (C_{60}FAS, 0,15 мг/мл) вводили тваринам в об'ємі, еквівалентному кількості C_{60}-фулерену на 0,5 мг/кг маси тіла, через 24 та 48 год після введення ANIT. Через 72 год після початку досліду тварин піддавали евтаназії. У крові тварин вимірювали рівень АЛТ та АСТ. Печінку і підшлункову залозу аналізували на світлооптичному рівні. Встановлено, що інтрагастральне та інтраперитонеальне введення C_{60}FAS сприяє корекції негативних ефектів у печінці та підшлунковій залозі, спричинених індукцією гостого холангіту. Свідченням цього є нормалізація рівня активності АЛТ, зменшення набряку паренхіми підшлункової залози і фіброзу печінки, а також посилення кровонаповнення цих органів. Застосування C_{60}FAS позитивно впливає на морфологічний стан печінки і підшлункової залози у щурів з індукованим гострим холангітом.

Ключові слова: C_{60}-фулерен, гострий холангіт.

ВЛИЯНИЕ ВОДНОГО КОЛЛОИДНОГО РАСТВОРА C_{60}-ФУЛЛЕРЕНА НА МОРФОЛОГИЧЕСКОЕ СОСТОЯНИЕ ПЕЧЕНИ, ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ И АМИНОТРАНСФЕРАЗНУЮ АКТИВНОСТЬ СЫВОРОТКИ КРОВИ КРЫС ПРИ ОСТРОМ ХОЛАНГИТЕ

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Целью работы было исследовать влияние C_{60}-фуллерена на морфологическое состояние печени и поджелудочной железы при интраперитонеальном и интрагастральном введении на модели экспериментального холангита крыс. Острый холангит воспроизводили однократным интрагастральным введением α-нафтилизотиоцианата — ANIT. Водный коллоидный раствор C_{60}-фуллерена (C_{60}FAS, 0,15 мг/мл) вводили животным в объеме, эквивалентном количеству C_{60}-фуллерена на 0,5 мг/кг массы тела, через 24 и 48 ч после введения ANIT. Через 72 ч после начала опыта животных подвергали эвтаназии путем цервикальной дислокации после анестезии. В крови животных измеряли уровень АЛТ и АСТ. Печень и поджелудочную железу анализировали на светооптическом уровне. Установлено, что интрагастральное и интраперитонеальное введение C_{60}FAS способствует коррекции негативных эффектов в печени и поджелудочной железе, вызванных индукцией острого холангита. Свидетельством этого является нормализация уровня активности АЛТ, уменьшение отека паренхимы поджелудочной железы и фиброза печени, а также усиление кровенаполнения этих органов. Применение C_{60}FAS положительно влияет на морфологическое состояние печени и поджелудочной железы у крыс с индуцированным острым холангитом.

Ключевые слова: C_{60}-фуллерен, острый холангит.

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RAPE BIOMASS (*Brassica napus*) AS RAW MATERIALS FOR BIOBUTANOL PRODUCTION

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The aim of the work was to investigate the accumulation of butanol by *Clostridium* strain producers using meshed green rape biomass as substrate. The accumulation of butanol by producer strains was studied using rape as substrate in the doses of 5–30 g/l. The cells were precipitated in an ultracentrifuge, the supernatant was distilled, and fermentation products were determined. The presence of solvents in the culture fluid was determined by gas chromatography. The biggest accumulation of butanol was produced by the strain *Clostridium* sp. IMB B-7570 on 2.3 g/l mashed rape biomass. The optimal inoculum concentration for maximum accumulation of butanol using rape biomass was 10% of the volume of fermentation liquid. The greatest accumulation of butanol (2.9 g/l) was obtained in optimal culture conditions and at 10 g/l dry rape biomass in the fermentation medium. Thus, the present study showed that mashed rape biomass was assimilated by *Clostridium* sp. strains. The accumulation of butanol depended on *Clostridium* strain, the amount of inoculum, the concentration and degree of grinding of the substrate.

Key words: biobutanol, *Clostridium,* plant biomass, rape.

Lately, producing liquid organic compounds from the renewable raw materials such as plant biomass became a hotter topic once again. Butanol (butyl alcohol) and ethanol are among such compounds [1, 2]. A significant amount of biomass is produced in agriculture [3], which can be used as raw materials for the bioconversion processes [4]. Various microorganisms are capable of growing on a substrate containing lignin and cellulose, producing substances like butanol, ethanol, acetone, etc. [5]. In a classic acetone-butanol-ethanol (ABE) fermentation process, the solvents are generated in a ratio of 3:6:1. Butanol is accumulated in the culture fluid if the concentration of sugar-containing substrate is at least 2 % volumetric. That is caused by the inhibiting influence of butanol on the culture growth and development. The solvent ratio changes if the culture substrates contain lignin and cellulose. The butanol concentration also changes, it depends on the amount of available carbohydrate medium (cellulose and hemicellulose) [6]. The aim of present work was to study the butanol accumulation by the producer strains of the genus *Clostridium* on a substrate of rape raw biomass.

Materials and Methods

The study objects were the strains *Clostridium acetobutylicum IMB B-7407 (*IFBG C6H), *C. tyrobutylicum* IFBG C4B and *Clostridium* sp. IMB B-7570 of the "Collection of strains of microorganisms and lines of plants for food and agricultural biotechnology" of the State institution "Institute of food biotechnology and genomics" of the National Academy of Sciences of Ukraine (henceforth, Collection); green

biomass of rape *Brassica napus (*National Scientific centre "Institute of mechanization and electrification of agriculture", Ukrainian Academy of Agricultural sciences). Samples were cultured in flasks with liquid medium or in Petri dishes. The chosen inoculum medium was glycerol medium as follows (g/l): glycerol (analytical reagent grade) — 20; yeast extract — 1.0; $(NH_4)_2SO_4$ — 0.6; $(NH_4)_2HPO_4$ — 1.6; pH 6.5. The medium was sterilized for 30 minutes at 1 atm and used for accumulation and introducing standardized doses of active bacteria to the fermentation medium. The inoculum was cultured during 24 hours, the accumulation of bacteria was evaluated by feculence of culture. After fermentation, the remaining glycerol and alcohol concentration were determined in inoculum [7].

Microorganisms were cultured on solid substrate in an anaerostat "AE 01" (Russian Federation) under nitrogen atmosphere. The anaerostat was placed in a thermostat at 35±10 °C. The rape biomass was dried at 30±10 °C for 48 hr. The dried biomass was mashed in a laboratory mill "Cyclone MSH 1" (Ukraine). The fractions were measured (20, 60, 100, 150, and 200 mesh) with "Millipore RETSCH sieve shakers" meshes (U.S.A.). Moisture of the raw material was evaluated with a RADWAG MA 50/C/1 (Poland) moisture analyzer. The major components of rape biomass were identified using the following normative protocols: lignin [8], cellulose [9], moisture [10], protein [11], hemicellulose [12]. To produce rape biomass mash, 20.0 g of dry biomass were added to 1 l of water and sterilized at 2 atm for 2 hr. To determine the optimal concentration of substrate, rape biomass mash was prepared in concentrations ranging from 5 to 30 g/l with a pitch of 5 g/l. The inoculum was cultured in 500 ml flasks in 250 ml culture medium. The flasks were stopped with concentrated sulfuric acid plugs, weighted and kept in thermostats at 35±10 °C. After fermentation (72 hr of culturing) cells were precipitated using the ultracentrifuge "Labofuge 400R" (Germany) at 13000 rpm for 10 min. After culturing the fermentation products were extracted from the culture fluid. The presence of ethanol, acetone and butanol in the culture fluid was determined using gas chromatograph with flame ionization detector. The 3 m column was packed with carbowax 1500 on N-A-W-DMCS Chromatone (0.20–0.25 mm), the column temperature was 60±2 0C, the temperature of oven 160±5 0C. The flow ratio of Nitrogen to Hydrogen to air was 1:1:10.

All experiments were performed in triplicate. Statistical data analysis was conducted using Microsoft Excel software. Difference between two average values was considered significant at $P < 0.05$.

Results and Discussion

The major components of rape biomass were studied to find the potential Carbon sources in it (Fig. 1). The major components of rape biomass include: 31% protein, 27% cellulose, and 3% hemicellulose. Other components include 13% lignin, which was not assimilated by microorganisms. The obtained results reveal which share of rape biomass can be assimilated by bacteria of the genus *Clostridium*.

Butanol accumulation was studied by the producer strains IMB B-7570, IFBG C4B and IFBG C6H cultured on a substrate of rape biomass (Fig. 2). After the fermentation by producer strains, three main ABE products were identified in the culture fluid (acetone, butanol and ethanol). It is shown that butanol accumulation was the strongest (2.3 g/l) at rape biomass mash as substrate and *Clostridium* sp. IMB B-7570 as the producer strain. In that case, acetone was present in low amounts (0.5 g/l), similarly to ethanol (0.1 g/l). The IMB B-7570 strain was used in further research due to its superior ability to produce butanol on rape biomass substrate.

The effect of grinding the substrate to various mesh sizes on butanol accumulation was analyzed using rape biomass as substrate and IMB B-7570 strain as butanol producer (Fig. 3). It can be seen in Fig. 3 that butanol accumulated in concentrations up to 0.1 g/l if the rape biomass was ground minimally. Butanol concentration in the culture fluid increased with the level of grinding of the rape biomass. The highest butanol accumulation

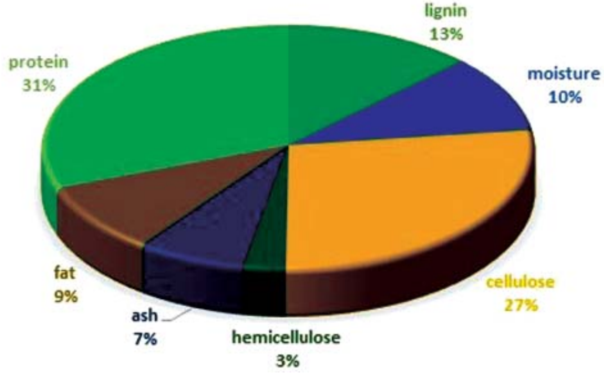

Fig. 1. **Macrocomponent composition of rape biomass**

was observed at substrate ground to 200 mesh (0.076 mm).

The obtained data reveal that the accessibility of raw material increased with substrate milling. That effect can be caused by grinding decreasing crystal zones of cellulose and increasing the amorphic zones which are easily destroyed by enzymes. In bacteria of the genus *Clostridium* the enzymes capable of cleaving cellulose are part of the extracellular multi-enzyme complex, cellulosome [6, 13].

The amount of cellulosomes is proportional to the number of bacteria in the fermentation medium. The number of bacteria in the beginning of fermentation depends on the quality and quantity of the inoculum. That is why, for the standardization of the amount of bacteria introduced into the inoculum, liquid nutrient medium with water-soluble carbon sources is used.

Thus, the effect of the concentration of IMB B-7570 strain inoculum was studied on the accumulation of butanol using rape biomass as a substrate (Fig. 4). As a result, it was shown that the concentration of inoculum introduced into the fermentation medium significantly influences the accumulation of butanol. It was shown that the amount of produced butanol increases proportionally with an increase in the concentration of inoculum material from 5 to 10% of the volume of fermentation mixture.

Increasing the concentration of inoculum to 15–20% caused the accumulation of butanol to drop. Further increasing the inoculum concentration in the fermentation mixture, in general, inhibited the synthesis of butanol in the medium. Inhibition of the butanol synthesis under increasing the inoculum concentration can be due to increased accumulation of primary metabolites of ABE fermentation (butyric, lactic and acetic acids). The optimum concentration of the inoculum added to the fermentation medium was 10%. Then the largest amount of butanol was accumulated, 2.5 g/l. Subsequent studies were conducted at precisely that inoculum concentration.

However, the concentration of inoculum was not the only key factor in the accumulation of butanol. Another important factor that influenced the growth and development of microorganisms and the ABE process was the concentration of available carbon source. The biomass of dried rape without seeds was used as a carbon source.

We studied the effects of various concentrations of rape biomass in a fermentation medium on the accumulation of butanol (Fig. 5). The obtained data indicate that the accumulation of butanol in the fermentation medium increased proportionally to in the concentration of rape biomass (substrate) from 5 to 10 g/l. When the amount of dried milled rape biomass increased from 15.0 to 30.0 g/l, the accumulation of butanol decreased.

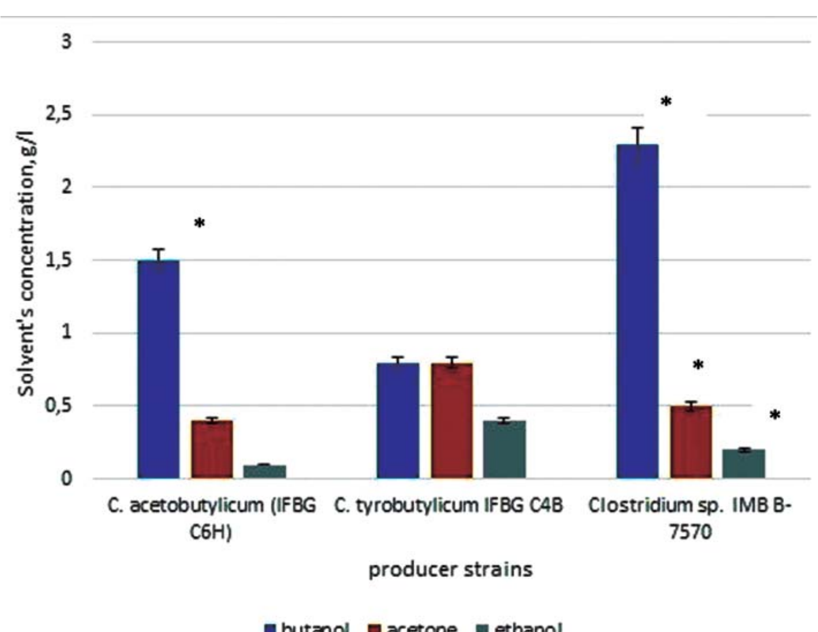

Fig. 2. **Accumulation of solvents by strains producing butanol while using rape as substrate**
**Hereinafter:* $P < 0.05$ compared to control, native medium used as control

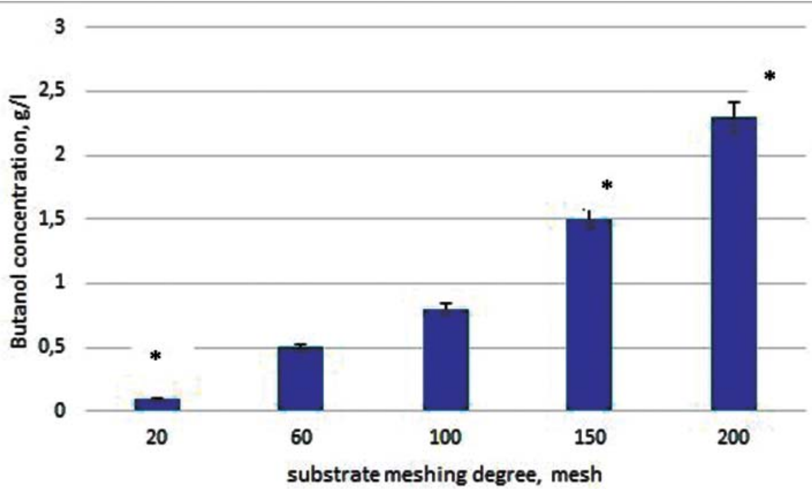

Fig. 3. **The effect of different grinding size on butanol accumulation**

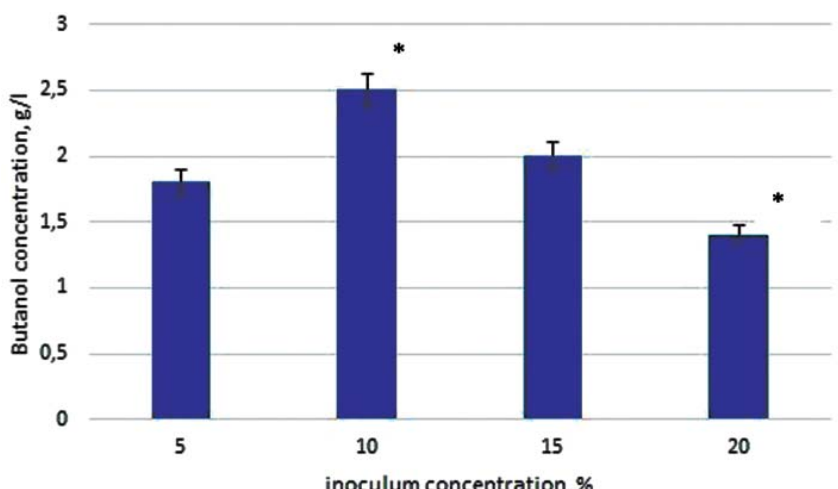

Fig. 4. **The effect of various concentraations of inoculum on butanol accumulation**

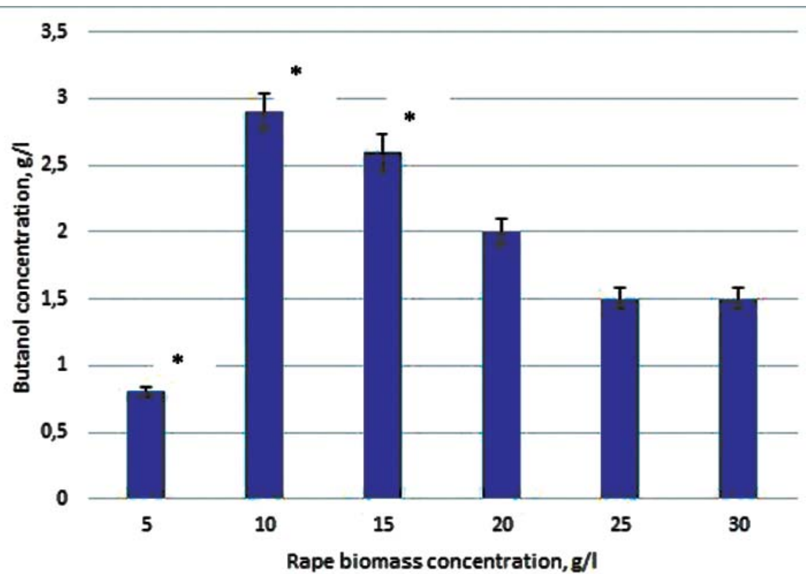

Fig. 5. **The effect of biomass concentration on butanol accumulation**

Our results indicate that increasing the concentration of carbon substrate reduces the bioavailability of the substrate. The largest accumulation of butanol (2.9 g/l) in the fermentation medium was obtained at a concentration of dry milled rape biomass of 10.0 g/l.

Thus according to the obtained results, pre-treated rape biomass was converted by the strains of *Clostridium* sp. The subsequent accumulation of butanol depended on the strain, amount of inoculum, and degree of grinding and concentration of the substrate. The largest amount of butanol (2.9 g/l) was produced by *Clostridium* sp. IMB B-7570 on the substrate of milled 200 mesh (0.076 mm) rape biomass at a concentration of 10.0 g/l.

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БІОМАСА РІПАКУ (*Brassica napus*) ЯК СИРОВИНА ДЛЯ ОТРИМАННЯ БІОБУТАНОЛУ

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Метою роботи було дослідити накопичення бутанолу штамами-продуцентами роду *Clostridium* з використанням подрібненої зеленої біомаси ріпаку як субстрату. Для вивчення накопичення бутанолу штамами-продуцентами наважку біомаси відбирали ваговим методом у діапазоні 5–30 г/л. Клітини осаджували за допомогою ультрацентрифугування, супернатант переганяли та визначали продукти бродіння. Наявність розчинників у культуральній рідині визначали за допомогою газової хроматографії. Найбільше накопичення бутанолу спостерігали за використання штаму *Clostridium* sp. IMB B-7570 та подрібненої біомаси ріпаку (2,3 г/л) як субстрату. Показано, що оптимальна концентрація посівного матеріалу для максимального накопичення бутанолу з використанням біомаси ріпаку становила 10% від об'єму ферментаційної рідини. Виявлено, що найбільше накопичення бутанолу (2,9 г/л) було за оптимізації умов культивування та концентрації 10 г/л сухої біомаси ріпаку у ферментаційному середовищі. Таким чином, проведені дослідження показали, що подрібнена біомаса ріпаку асимілювалась штамами *Clostridium* sp., накопичення бутанолу залежало від штаму, кількості посівного матеріалу, концентрації та ступеня подрібнення субстрату.

Ключові слова: біобутанол, *Clostridium*, рослинна біомаса, ріпак.

БИОМАССА РАПСА (*Brassica napus*) В КАЧЕСТВЕ СЫРЬЯ ДЛЯ ПОЛУЧЕНИЯ БИОБУТАНОЛА

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Целью работы было исследовать накопление бутанола штаммами-продуцентами рода *Clostridium* с использованием измельченной зеленой биомассы рапса в качестве субстрата. Для исследования накопления бутанола штаммами-продуцентами с использованием рапса навеску биомассы отбирали весовым методом в диапазоне 5–30 г/л. Клетки осаждали с помощью ультрацентрифугирования, супернатант перегоняли и определяли продукты брожения. Присутствие растворителей в культуральной жидкости определяли с помощью газовой хроматографии. Наибольшее накопление бутанола наблюдалось с использованием штамма *Clostridium* sp. IMB B-7570 и измельченой биомассы рапса (2,3 г/л) в качестве субстрата. Показано, что оптимальная концентрация посевного материала для максимального накопления бутанола с использованием биомассы рапса составляла 10% от объема ферментационой жидкости. Выявлено, что наибольшее накопление бутанола (2,9 г/л) наблюдалось при оптимизации условий культивирования и концентрации сухой биомассы рапса у ферментационной среде 10 г/л. Таким образом, проведенные исследования показали, что измельченная биомасса рапса ассимилировалась штамамми *Clostridium* sp., при этом накопление бутанола зависело от штамма, количества посевного материала, концентрации и степени измельчения субстрата.

Ключевые слова: биобутанол, *Clostridium*, растительная биомасса, рапс.

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EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS BY HUMAN ENDOMETRIAL MULTIPOTENT MESENCHYMAL STROMAL/STEM CELLS *in vitro* UNDER HYPOXIA CONDITIONS

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The aim of the study was to investigate the level of estrogen (ESR1, ESR2) and progesterone (PGR) receptors expression in the primary culture of endometrial multipotent mesenchymal stromal/stem cells endometrial multipotent mesenchymal stem cells and during *in vitro* cultivation under different athmospheric oxygen content. The dynamics of changes in the level of expression of the sex hormones receptors in the primary culture and during cultivation under different oxygen content in the athmosphere was shown.

Key words: human endometrium, multipotent mesenchymal stromal cells, expression of the receptors to the sex hormones, estrogen, progesterone.

Currently the problem of the reproductive function of the person violation is very relevant.

The endometrium plays a key role in the implantation process of the embryo. The uniqueness of the endometrium is not only in the ability to cyclic self-renewal of the cellular composition, but also in the ability to respond to changes in the hormonal status [1–3].

Functional and structural maturity of the endometrium is formed during the menstrual cycle in conditions of dynamic fluctuation of the level of steroid hormones of the ovaries — estrogens and progesterone [4] .The normal development of the endometrium and changes during the luteal phase are key to the successful implantation of the embryo [5, 6].

It is proved that determining the role in implantation is played by not only the concentration of steroid hormones acting on the tissue-targets of the reproductive system, but also the morphological structure and receptivity of the endometrium, that is, the number of functionally mature receptors for steroid hormones [7–11].

It is known that the concentration of oxygen in the tissues of the body and the niche of stem cells is much lower than atmospheric 20%. Hypoxia is one of the key factors that negatively affects the viability of transplanted cells and reduces their therapeutic potential. In addition, the concentration of oxygen in the atmosphere of the incubators during *in vitro* cultivation can affect the morphofunctional characteristics of cells in the culture.

The aim of the study was to investigate the level of estrogen (ESR1, ESR2) and progesterone (PGR) receptors expression in the primary culture of the endometrial multipotent mesenchymal stem cells (eMMSCs) and during *in vitro* cultivation under different atmospheric oxygen content.

eMMSCs obtaining and cultivating

Endometrial samples ($n = 5$) were obtained by biopsy in the proliferative phase of the menstrual cycle from women with endometrial hypoplasia. The age of patients was 34 ± 3.3 years. In all cases, the voluntary informed consent was signed. The fragments of the endometrium were dissociated by enzymatic treatment for 50 minutes in a solution of 0.1% collagenase IA and 0.1% pronase with the addition of 2% FBS (fetal bovine serum). The resulting suspension of cells was cultured in DMEM/F12 medium with addition of 10% FBS, 2 mM glutamine and 1 µg/ml of FGF-2 (all — Sigma, USA) in multigas incubators at 37 °C, absolute humidity, 5% CO_2 and 5% and 20% concentration of O_2. eMMSCs were selected as a cell fraction that adhered to the plastic after 24–48 hours after transferring the suspension to a culture vessel.

qRT-PCR

Total RNA was isolated from cells using TRIzol reagent (Sig ma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Two µg isolated RNA were reverse transcribed to cDNA with RevertAid Reverse Transcriptase, RiboLock RNAase Inhibitor, and Oligo (dT) 18 anchored primer (all Thermo Scientific, USA). qRT-PCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) on a sequential detection system 7500 (Applied Biosystems, CA, USA) and analyzed by 7500 System SDS Software (version 1.3.1).The sequence of primers used in this study and PCR cycling conditions are listed in S2 Table. Expression of the TATA-box binding protein (TBP) was used as an endogenous control for standardization. Ct values were determined for the internal control (TBP) and the test genes at same threshold level in the PCR curves of the exponential phase. Relative quantification (comparative Ct (ddCt) method) was used to compare the expression level of the tested genes with the internal control and was represented in relative units. Dissociation curve analysis was performed after each run to check the specificity of the reaction. Three reactions (each in triplicate) were run for each gene, and the standard error of mean was calculated.

We have shown that cell populations derived from a minimal human endometrial biopsy were in line with the minimal criteria for the determination of MMSCs proposed by the International Society for Cellular Therapy [12]. The cells in culture were adhered to plastic under standard cultivation conditions and expressed at high levels of CD90+CD105+CD73+, in the absence of expression of hematopoietic markers CD34-CD45-HLA-DR-. Also, eMMSCs possessed the ability to direct trilinear differentiation in the adipo-, osteo- and chondrogenic directions, with the acquisition of the corresponding morphofunctional features [13].

The level of estrogen and progesterone receptors expression was determined in primary cultures, as well as in the course of cultivation over several passages (P0, P1, P3, P5) with 5% and 21% of O_2 content in the atmosphere.

The results of the ESR-1 expression level study are shown in Fig. 1. The level of estrogen-1 receptors expression (ESR1) during the cultivation was dynamically varied. In the primary culture of eMMSCs during P0, there was a significantly higher expression level of ESR1 under cultivation with 5% O_2 in the atmosphere, but this tendency was not maintained. An increase in the level of ESR1expression at oxygen content of 21% was observed already during P1. Subsequently, the level of expression was aligned and decreased in both groups of comparison.

The results of the ESR2 expression level study are presented in Fig. 2.

In the primary culture of eMMSCs during P0, the expression level of ESR2 was also higher in cultivation with 5% of O_2

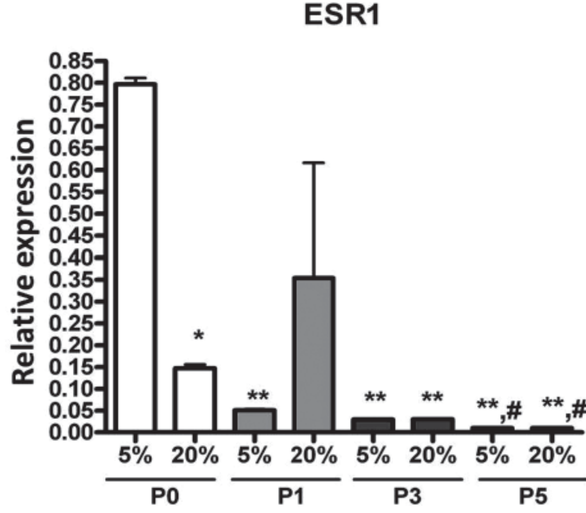

Fig. 1. **Expression of ESR1 estrogen receptor of eMMSCs in 20% and 5% O_2 cultivation with respect to the TBP household gene:**
* $P < 0.05$ in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0;
# — $P < 0.05$ compared with 5% and 20% of O_2, respectively, during P1, P3

in the atmosphere. The tendency towards equalization during cultivation was also maintained. It should be noted, that in contrast to the stable low expression of ESR1 in both groups during P5, an increase in the expression level of ESR2 in this passage was observed. It should also be noted that the cultivation of eMMSCs at 20% of O_2 during P5 results in a significant increase in the expression level of ESR2 compared to the primary culture and earlier passages.

Fig. 3 shows the results of the study of the expression level of eMMSCs progesterone (PGR) during cultivation.

The highest values of the level of the receptor to progesterone expression were noted in the primary culture of eMMSCs during cultivation with 5% of O_2 in the atmosphere. There was a tendency to decrease the expression of PGR in both groups during cultivation. There was no significant difference between the groups during P1, P3 and P5. However, the level of PGR expression in the primary culture during P0 was significantly higher when cultivated with 5% than 20% of oxygen in the atmosphere.

It should be noted that the level of estrogen and progesterone receptors expression differed in cultures depending on the donor.

Given the results of numerous clinical studies, the need to support the ART programs in the luteal phase with progesterone is beyond doubt. The glandular component of the endometrium is extremely sensitive to the action of progesterone and reacts in the first half of the luteal phase. The stromal component is less sensitive, reacts in the second half of the luteal phase and requires higher levels of progesterone.

It is known that estradiol and progesterone are the only hormones that are necessary to achieve the receptivity of the endometrium. Hormonal profile in DRT programs has features: too high levels of estradiol in the stimulation phase and before the prescription of a trigger dose of chorionic gonadotrophin, a sharp fall in estradiol levels after oocyte aspiration, lowered levels of progesterone in the luteal phase of the program. Due to clinical trials in different countries, it has been established that estradiol may not be added in the luteal phase in donation programs, but there is not enough data to suggest that this does not negatively affect the receptivity of the endometrium, the frequency of pregnancy and miscarriage.

Today, in clinical practice, the physiological levels of estradiol in the luteal phase are simulated and it is traditionally added to treatment regimens. Obviously, it is necessary to develop more precise criteria for the prescription of estradiol. One such criterion may be the ratio of the concentration of estradiol and progesterone, it is a better indicator than just the concentration of these hormones in blood plasma. However,

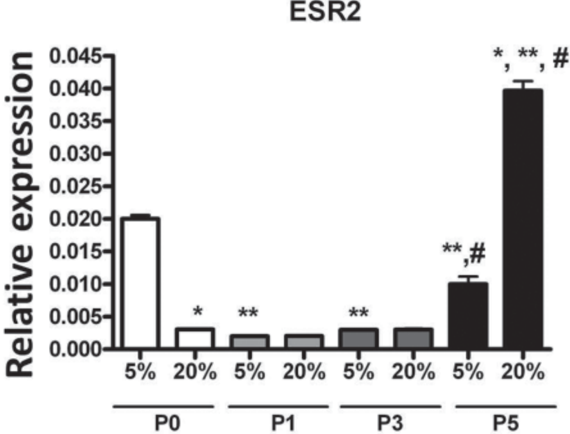

Fig. 2. **Expression of the ESR2 estrogen receptor of eMMSCs in 20% and 5% of O_2 cultivation with respect to the TBP household gene:**
* — P <0.05 in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0;
# — $P < 0.05$ compared with 5% and 20% of O_2, respectively, during P1, P3

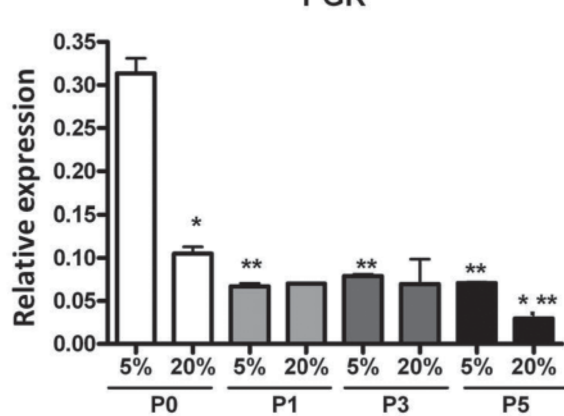

Fig. 3. **Expression of the PCR progesterone receptor of eMMSCs in 20% and 5% of O_2 cultivation with respect to the TBP household gene:**
* — $P < 0.05$ in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0

the concentration of hormones in the blood plasma does not provide exhaustive answers, because the presence of the hormone does not indicate its likely work, because for this purpose, the maturity of the target tissue and the willingness to respond to the stimulus are needed. That is why the study of the dynamics of vibration of receptors for hormones in eMMSCs *in vitro* is a promising field of study, as it can become the basis for optimizing the existing protocols for managing patients with unsuccessful ART attempts, which additionally applied cellular technologies to restore endometrial receptivity.

The work is experimental, therefore, it only represents the results of our research and does not outline the clinical picture in general, since it is the prerogative of gynecologists.

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ЕКСПРЕСІЯ РЕЦЕПТОРІВ ЕСТРОГЕНІВ ТА ПРОГЕСТЕРОНУ МУЛЬТИПОТЕНТНИМИ МЕЗЕНХІМАЛЬНИМИ СТРОМАЛЬНИМИ/СТОВБУРОВИМИ КЛІТИНАМИ ЕНДОМЕТРІЮ ЛЮДИНИ *in vitro* ЗА УМОВ ГІПОКСІЇ

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Метою роботи було дослідити рівень експресії рецепторів до естрогенів (ESR1, ESR2) та прогестерону (PGR) у первинній культурі ендометріальних мультипотентних мезенхімальних стромальних/стовбурових клітин і впродовж культивування *in vitro* за різного вмісту кисню в атмосфері. Показано динаміку зміни рівня експресії рецепторів до статевих гормонів у первинній культурі та протягом культивування за різного вмісту кисню в атмосфері.

Ключові слова: ендометрій людини, мультипотентні мезенхімальні стромальні клітини, експресія рецепторів до статевих гормонів, естроген, прогестерон.

ЭКСПРЕССИЯ РЕЦЕПТОРОВ ЭСТРОГЕНОВ И ПРОГЕСТЕРОНА МУЛЬТИПОТЕНТНЫМИ МЕЗЕНХИМАЛЬНЫМИ СТРОМАЛЬНЫМИ/СТВОЛОВЫМИ КЛЕТКАМИ ЭНДОМЕТРИЯ ЧЕЛОВЕКА *in vitro* В УСЛОВИЯХ ГИПОКСИИ

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Целью работы было исследовать уровень экспрессии рецепторов к эстрогенам (ESR1, ESR2) и прогестерона (PGR) в первичной культуре эндометриальных мультипотентных мезенхимальных стромальных/стволовых клеток и в течение культивирования *in vitro* в условиях разного содержания кислорода в атмосфере. Показана динамика изменения уровня экспрессии рецепторов к половым гормонам в первичной культуре и во время культивирования в условиях разного содержания кислорода в атмосфере.

Ключевые слова: эндометрий человека, мультипотентные мезенхимальные стромальные клетки, экспрессия рецепторов к половым гормонам, эстроген, прогестерон.

AUTHOR RULES

General information

Results of scientific research and survey on different directions of current biotechnology, short communications, articles on biotechnology history, and new publications on biotechnology and adjoining branches of science are published in English and have identical summaries in Ukrainian and Russian languages.

Bioethical norms

"*Biotechnologia Acta*" is focuses on the rules recommended by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and International Committee of Medical Journal Editors (ICMJE).

All procedures describing experiments involving laboratory animals, any biomaterials of human origin including donors and/or patients should be carried out guided by the bioethics norms. Experimental record should be reviewed and approved by the National Commissions on Bioethics and meet the International Standards.

Basic requirements to the articles are novelty, relevance and validity of the given facts, reproducibility of the experimental data on the reported methods and drawing up of the manuscript according to the journal requirements.

Article submission

The following documents are provided via *E-mail* biotech@biochem.kiev.ua:

- full text of the article (including a summary in three languages, tables, figures and their captions placed as mentioned in the text and a list of references as well). This file should be named after the first author (family_name.doc);
- color illustrations;
- full names, first names and patronymics of all authors and full postal addresses of their institution should be given, including post codes, in Ukrainian, Russian, and English languages. The corresponding author/s should be indicated, including their e-mail and telephone;
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General requirements to manuscripts

The editors reserve the right to return the manuscripts that are not in accordance with the following instruction.

The date of the paper receipt is the date of arrival of its last, correct version to the editorial office.

The materials should be submitted in the Microsoft Word-supported format, page size of A4, book-oriented, font Times New Roman, at 14 point size, of one and a half spacing.

The manuscript (including tables, list of references, figures, and figure captions) should not exceed 30 pages (60 thousand symbols) for review articles, 20 pages (40 thousand symbols) for experimental one, 8 pages for short reports, 5–6 pages for current events and reviews. Figure volume is 1/4 of the article. Pages should be numbered consecutively, including tables, references, figure captions, summaries, etc. All tables, figures and their captions should have a concise title and serial number. The notes on the table are placed straight under the table. Footnotes can be used to explain abbreviations.

Figures should be in one of the electronic formats *.jpg or *.tif in color.

The articles are reviewed by two independent experts who are appointed by the Editorial Board.

Section article

Experimental articles should have the following scheme:

- ***Title page***;
- ***Abstracts***;
- ***Text of the article***;
- Acknowledgements (where applicable);
- Data concerning financial support (where applicable);
- ***References.***

Title page:

- UDC (Universal Decimal Classification);
- article title;
- full names of all the authors;
- name and postal address of the institution which each author works in;
- *e-mail* of the corresponding author.

Article title should be brief (not more than 8–10 significant words), informative, contain keywords and cover adequately the content of the article.

Names of all authors should be given in full. Each author should list an associated department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country.

Name of the institution: If there are some authors and they work in different institutions, it needs to show in Arabic (indices) the names of the authors of the appropriate institutions.

Abstracts:

Abstracts up to 1,500 characters with spaces should contain:

- article title;
- names of all authors;
- text of the abstract for experimental articles should be carefully structured and contain the following sections: aim, methods, results, conclusion;
- keywords (not more than ten).

In addition, an abstract should:

- explain how the study was done which includes any model and organisms were used, without getting into methodological specifics;
- summarize the most important results and their significance.

Abstracts should not include:

- citations;
- abbreviations (except standard).

Text of the article

Experimental articles should have the following sections (headlines):

- introduction (not entitled);
- materials and methods;
- results and discussion;
- conclusion (not entitled).

The relevance and purpose of the study is formulated in the introduction.

Methods or methodology of the work should be described in a case if they are distinct in novelty.

On the common techniques it is enough to give references to publications. All the designations and denominations of physical units should be given in SI International System. Amino acids are indicated by abbreviated symbols of three letters or in single letter format FASTA.

Names of the enzymes and their codes should be given according to the recommendations of the International Biochemical Society (Enzyme Nomenclature — Electron version: http://www.chem.qmul.ac.uk/iubmb/enzyme.

Names of companies and countries producers of the reagents and materials used in the experiments should be given. The number and species of the used animals as well as the methods of anaesthetization and euthanasia should be defined.

All quantitative data require statistical analysis. Its results should be given in the tables and figures. Data validity is shown as follows: * — $P < 0.05$.

Latin name of the genus and species of the organisms should be given in *italics*. Mentioning firstly, it is given full specific name (preferably with indication of taxon author), and subsequently the name of the genus could be given shortly with one letter, unless it concerns species belonging to different genera with the same first letter. Latin names of taxons are given according to current sources. It needs to use only standard abbreviations of names for measures, physical, chemical and mathematical variables and terms, all other abbreviations have to be interpreted.

If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

References

Only published or accepted manuscripts should be included in the reference list. Manuscripts that have been submitted but not yet accepted should not be cited. References must be listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, citations should be indicated by the reference number in square brackets.

The authors are responsible for the correctness of the original sources.

Citation of published and readily accessible materials to readers is only available: articles, books, conference proceedings, dissertations, patents.

It is desirable that the manuscript has no more than 20 references in experimental work, 100 references in review, and refer to recent years.

Editorial staff has no responsibility for correctness and completeness of the given bibliographic data.

Recommendations of the "Biotechnologia Acta" journal for the references compilation:

Published papers:

Examples:

Pirog T. P., Shevchuk T. A., Shulyakova M. A., Tarasenko D. O. Influence of citric acid on synthesis of biosurfactants of *Rhodococcus erythropolis* IMV Ac-5017. *Mikrobiol. Zh.* 2011, 73(5), 21–27. (In Ukrainian).

Liang G., Klose R. J., Gardner K. E., Zhang Y. Yeast Jhd2p is a histone H3 Lys4 trimethyl demethylase. *Nature Struct. Mol. Biol.* 2007, 14(2), 243–245.

Rodrigues L. R. Inhibition of bacterial adhesion on medical devices. *Adv. Exp. Med. Biol.* 2011, V. 715, P. 351–367.

Skochko A. B., Konon A. D., Pirog T. P. Research of antiadhesion properties of surface-active substances of *Acinetobacter calcoaceticus* IMV B-7241. *Kharchova promyslovist*. 2012, N13, P. 77–80. (In Ukrainian).

Examples of paper descriptions with DOI:

Klyuchko O. M. On the mathematical methods in biology and medicine. *Biotechnol. acta.* 2017, 10 (3), 31–40. https://doi.org/10.15407/biotech10.03.031

Conferences:

Riabinina A. A., Berezina E. V., Usoltseva N. V. Surface Tension and Lyotropic Mesomorphism in Systems Consisting of Nonionogenic Surfactant and Water. *Lyotropic Liquid Crystals and Nanomaterials: Proceedings of the Seventh International Conference. Ivanovo: Ivanovskiy Gosudarstvennyy Universitet*, 2009. (In Russian).

Theses:

Kordyum V., Suhorada E., Ruban T. Informational conditioning as the mechanism of permanent organism reconstruction. Abstracts of the *1st World Congress of Regenerative Medicine, Leipzig, Germany*, 22–24 October 2003.

Electronic journal articles:

Hindorff L. A. (all authors) A Catalog of Published Genome-Wide Association Studies. Available at http://www.genome.gov/gwastudies (accessed, September, 2012).

Description of the electronic encyclopedia articles:

Containerization. In Encyclopedia Britannica. Retrieved May 6, 2008, from http://search.eb.com

Links to Web resources:

Pravila Tsitirovaniya Istochnikov (Rules for the Citing of Sources) Available at: http://www.scribd.com/doc/1034528 (accessed 7 February 2011).

Links to online resource of scientific and technical reports:

Deming D., Dynarski S. The lengthening of childhood. (NBER Working Paper 14124). *Cambridge, MA: National Bureau of Economic Research*. Retrieved July 21, 2008, from http://www.nber.org/papers/w14124

Book:

McCartney E. J. Optics of the atmosphere. *N. Y.: Willey*. 1977, 400 p.

Book chapter:

Trachtenberg I. M., Levitsky E. L. Genetic toxicology. *Genetic medicine.* Zaporojan S.B. (Ed.). *Odessa State University*. 2008, P. 183–221. (In Russian).

Monograph:

Lugovskoy E. V. The Molecular Mechanisms of Fibrin Formation and Fibrinolysis. *Kyiv: Naukova dumka.* 2003. 219 p. (In Russian).

Dissertation abstracts:

N. Kawasaki. Parametric study of thermal and chemical nonequilibrium nozzle flow. M.S. thesis, Dept. Electron. Eng., *Osaka Univ*., Osaka, Japan, 2003.

J.O. Williams. Narrow-band analyzer. *Ph.D. dissertation, Dept. Elect. Eng., Harvard Univ., Cambridge, MA.* 2010.

Dissertation:

Young R. F. Crossing boundaries in urban ecology: Pathways to sustainable cities *(Doctoral dissertation). Available from ProQuest Dissertations & Theses database.* 2007. (UMI No. 327681).

Encyclopaedia, dictionary:

Sadie S., Tyrrell J. (Eds.). The new Grove dictionary of music and musicians (2nd ed., Vols. 1–29). *New York, NY: Grove.*

Patent:

Wilkinson J. P. Nonlinear resonant circuit devices. *U.S. Patent 3 624 125*, July 16, 1990.

NOTE: Use "issued date" if several dates are given.

Short messages

Smaller articles by volume that have an implicit novelty and relevance for biotechnology are published in "*Biotechnologia Acta*" journal. These articles are passed expedite reviewing and published in a short time. The total volume of a short message is limited to 10 typewritten pages, number of drawings and/or tables should not exceed 3, and a list of used literature sources should not contain more than 15.

Sections of the short messages are similar to that of the original article but are not shown with headings and subheadings, the results could be given together with the discussion.

Author for correspondence should justify the expediency of such an extraordinary publication in a letter to the editor. In case of acceptance, such work could be published within 3–4 months.

Notes

Authors can correct only found mistakes in the made up pages without making additional changes and supplements.

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ПРАВИЛА ДЛЯ АВТОРІВ

Загальна інформація

Журнал *«Biotechnologia Acta»* публікує результати наукових досліджень з різних напрямів сучасної біотехнології, оглядові, експериментальні статті, нові методики, короткі повідомлення і статті, присвячені історії біотехнології, а також соціальним, правовим, моральним і етичним проблемам цієї галузі науки.

Статті в журнал приймаються українською, російською та англійською мовами, публікуються англійською та супроводжуються ідентичними за змістом резюме російською та українською мовами.

Біоетичні норми

Журнал орієнтується на правила, рекомендовані Європейською конвенцією про захист хребетних тварин, що використовуються для дослідних та інших наукових цілей (Страсбург, 1986), а також Міжнародним комітетом редакторів медичних журналів (ICMJE).

Усі процедури в експериментах із залученням лабораторних тварин, будь-яких матеріалів із організму людини або за участю донорів і/або пацієнтів потрібно проводити, керуючись нормами біоетики. Описуючи експерименти з лабораторними тваринами, слід зазначити, яких рекомендацій щодо роботи з тваринами (місцевих, національних) дотримувались під час проведення цих процедур.

Основні вимоги до статей: новизна й обґрунтованість фактичного матеріалу, відтворюваність експериментальних даних за наведеними методами, оформлення рукопису відповідно до правил журналу «*Biotechnologia Acta*» для авторів.

Подання статті

Надсилати на електронну пошту редакції biotech@biochem.kiev.ua:

- Повний текст статті, включаючи резюме трьома мовами, таблиці, рисунки і підписи до них, розміщені в порядку згадування в тексті, а також список літератури. Цей файл слід назвати за прізвищем першого автора (family_name.doc).
- Кольорові ілюстрації.
- Інформацію про авторів: прізвище, ім'я, по батькові (повністю) кожного автора, їхні посади, наукові ступені та звання, телефон (із зазначенням коду міста), e-mail, місце роботи та поштова адреса установи англійською, українською та російською мовами. Ця інформація потрібна для коректного індексування статей у відповідних міжнародних базах даних. Будь ласка, зазначте автора для листування, вказавши його контактний (мобільний) телефон.
- Договір про передачу авторських прав, який набуває чинності лише після прийняття статті до друку. Жодна частина публікації не може бути використана з комерційною метою без дозволу редакції. У разі відхилення статті редколегією журналу договір автоматично втрачає силу. Підписання договору автором (авторами) означає, що він (вони) ознайомлені та згодні з умовами договору.

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Редакція залишає за собою право повертати неправильно оформлений рукопис без реєстрації та розгляду в разі відмови авторів дооформити його.

Датою отримання рукопису вважається дата надходження його останньої, правильно оформленої версії до редакції.

Матеріали слід подавати у форматі, що підтримується Microsoft Word, книжкової орієнтації, шрифтом Times New Roman, 14 кегль, полуторний міжрядковий інтервал.

Обсяг рукопису (у тому числі таблиці, список цитованої літератури, рисунки та підписи до них) не повинен перевищувати: для оглядових статей — 30 сторінок (60 тис. знаків), для експериментальних — 20 стор. (40 тис. знаків), для коротких повідомлень — 8 стор.; обсяг рисунків — 1/4 обсягу статті. Сторінки мають бути із суцільною нумерацією, включаючи таблиці, список літератури, підписи до рисунків, резюме тощо. Таблиці, рисунки і підписи до них слід розміщувати в порядку згадування їх у тексті. Вони повинні мати заголовок і порядковий номер. Примітки до таблиць розміщують безпосередньо під ними. Не можна дублювати одні й ті самі результати в таблицях і на рисунках. Якщо у статті містяться ілюстрації, опубліковані іншими авторами, автор рукопису зобов'язаний надати редакції документальне підтвердження дозволу на використання цих ілюстрацій від власника авторських прав.

Рисунки, що їх наведено в повному тексті, потрібно додатково подати в електронному вигляді у форматі *.jpg або *.tif. Для оглядів і експериментальних статей допустимо не більше 6 рисунків; для коротких повідомлень — не більше 4. Підписи до рисунків не включаються в сам рисунок і подаються окремо.

Рукопис статті обов'язково підлягає подвійному анонімному рецензуванню провідними спеціалістами у відповідній галузі.

Структура статті

Оформляючи статтю, слід дотримуватися такої послідовності:
- ***Титульна сторінка***
- ***Структуровані резюме*** (трьома мовами)
- ***Текст статті***
- Подяки
- Дані про фінансову підтримку
- ***Література.***

Титульна сторінка:
- УДК;
- заголовок статті;
- автори: прізвища та ініціали всіх авторів;
- назва та поштова адреса установи, в якій працює кожен автор;
- e-mail автора для листування.

Зоголовок статті має бути коротким (не більше 8–10 значущих слів), інформативним, містити ключові слова й точно відображати зміст статті. У перекладі назв статей англійською мовою не повинно бути жодних транслітерацій з української або російської мов, окрім власних імен, що не перекладаються, назв приладів, найменувань фірм. Це також стосується резюме й ключових слів.

Прізвища авторів статей подаються в одній з прийнятих міжнародних систем транслітерації. До опису потрібно включати всіх авторів. Якщо авторів декілька і вони працюють у різних установах, слід позначити ці установи арабськими цифрами (індексами).

Назва установи англійською мовою: всі значущі слова (крім артиклів і прийменників) — з великої літери.

Резюме:

Резюме обсягом до 1500 знаків (з пробілами) містить:
- заголовок статті;
- прізвища та ініціали всіх авторів;
- текст резюме експериментальних статей має бути структурованим і обов'язково висвітлювати мету, методи, результати, висновки.
- ключові слова (не більше десяти).

Крім того, в резюме необхідно:
- пояснити, як проводили дослідження, зазначивши, зокрема, які використовували моделі й організми, без методичних подробиць;
- коротко повідомити про найважливіші отримані результати та їх значення.

Резюме не повинні містити:
- посилання на літературу;
- скорочення (окрім загальноприйнятих).

Текст статті

В експериментальних статтях мають бути такі розділи: вступ (без заголовка); матеріали і методи; результати та обговорення; висновки.

У вступі обґрунтовується актуальність та формулюється мета дослідження.

Метод або методологію проведення роботи доцільно описувати в тому разі, якщо вони відзначаються новизною.

На загальновідомі методи достатньо дати посилання. Одиниці фізичних величин наводять за Міжнародною системою СІ. Амінокислоти позначають скорочено символами з трьох латинських букв або в однобуквеному форматі FASTA. Замість термінів «білок» і «фермент» використовувати «протеїн» та «ензим». Назви ензимів і їхні шифри наводять згідно з рекомендаціями Міжнародного біохімічного товариства (Enzyme Nomenclature. Інформація на сайті http://www.chem.qmul.ac.uk/iubmb/enzyme/). Необхідно зазначати назви фірм-виробників реактивів і матеріалів, що використовувались у дослідах, з посиланням на країну. Обов'язково вказують вид, породу, лінію, кількість тварин і методи знеболювання та евтаназії. Достовірність даних позначають так: * — $P < 0{,}05$.

Усі кількісні дані потребують статистичної обробки. Її результати слід подавати у відповідних таблицях і рисунках.

Латинські назви роду і виду організмів потрібно виділяти *курсивом*. При першому згадуванні наводять повну видову назву (бажано із зазначенням автора таксонів), а при повторному — найменування роду можна позначити скорочено однією буквою, якщо не йдеться про види, що належать до різних родів з однаковою першою літерою. Тоді використовують скорочення з декількох літер, наприклад *Staph. aureus*, *Str. lactis*. Латинські назви таксонів наводять відповідно до сучасної систематики. Слід використовувати тільки загальноприйняті скорочення назв мір, фізичних, хімічних і математичних величин і термінів, усі інші скорочення потрібно розшифровувати. Не варто наводити скорочення (крім загальновідомих) у таблицях і в підписах до рисунків.

Обговорення завершується формулюванням висновку, в якому потрібно дати конкретну відповідь на питання, поставлене у вступі. У разі використання ілюстрацій із цитованих публікацій обов'язковим є не тільки посилання на джерело ілюстрації, а й дозвіл їхніх авторів на публікацію (зазвичай такий запит робиться через Інтернет). Наприкінці статті висловлюється подяка приватним особам, співробітникам установ і фондів, які сприяли проведенню досліджень і підготовці статті, а також вказуються джерела фінансування роботи.

Результати роботи слід описувати гранично точно та інформативно з наведенням основних фактичних даних, виявлених взаємозв'язків і закономірностей, уникаючи їх повторення у таблицях. Слід вказати межі точності й надійності даних, а також ступінь їх обґрунтування.

Висновки, сформульовані наприкінці статті, можуть супроводжуватися рекомендаціями, оцінками та пропозиціями.

Література (References) наводиться в порядку цитування джерел у тексті і позначається цифрами у квадратних дужках. Використання гіперпосилань у списку літератури неприпустимо. Оскільки журнал прийнято для індексування міжнародними базами даних, список літератури слід подавати до редакції двома окремими блоками:

Блок 1 **(Література)** — список літератури мовою оригіналу.

Блок 2 **(References)** — той самий список літератури, в якому вже містяться посилання на іноземні джерела. Посилання кирилицею потрібно подавати за романським (латинським) алфавітом. Прізвища авторів, назви журналів подаються згідно з однією з міжнародних систем транслітерації. Назви статей у посиланнях обов'язково мають бути перекладені англійською мовою.

Автори несуть повну відповідальність за коректність наведення першоджерел. Слід цитувати тільки опубліковані й легкодоступні для читачів матеріали: статті, книги, матеріали конференцій, дисертації, патенти. На неопубліковані матеріали посилатися не можна. У переліку джерел літератури в експериментальній роботі бажано наводити не більше 20 назв, а в огляді — не більше 100. При цьому мають переважати посилання на роботи останніх років.

За правильність і повноту бібліографічних даних редакція відповідальності не несе.

Оглядові статті — це науковий аналіз певної проблеми, в якому дається критична оцінка та узагальнення відповідних досліджень з посиланням на офіційні джерела та власні дані авторів (не обов'язково, але бажано). На відміну від експериментальних робіт, в оглядових статтях не подаються нові неопубліковані дані.

Огляд слід закінчити висновком, що дасть змогу зрозуміти важливість розглянутої теми, та рекомендаціями щодо її подальшої розробки.

У списку літератури повинні переважати публікації останніх років без зайвого самоцитування.

Рекомендації щодо складання посилань за романським алфавітом в англомовній частині статті та пристатейній бібліографії, призначеній для зарубіжних БД:

1. Дотримуватись правил, що дають змогу легко ідентифікувати 2 основних елементи описів – авторів і джерело.
2. Назви статей не транслітерувати, а подавати тільки їх переклад.
3. Додержуватися однієї з поширених систем транслітерації прізвищ авторів і назв джерел.
4. У разі посилання на статті з російських журналів, що мають перекладну версію, більш доцільно посилатися саме на перекладну версію статті.

На сайті http://www.translit.ru/ можна скористатися, безкоштовно, програмою транслітерації тексту, записаного кирилицею, на латиницю.

Оформлення посилань у списку літератури

Для журналу «*Biotechnologia Acta*» прийнятною є така структура бібліографічного посилання.

Статті:

прізвища та ініціали авторів (транслітерація, *курсивом*); переклад назви статті англійською мовою (прямим шрифтом); *назва джерела* (транслітерація, *курсивом*); послідовно (прямим шрифтом) вихідні дані статті — рік, том, номер (у дужках), сторінки. Обов'язково зазначається мова оригіналу статті, якщо вона не є англійською.

Таке посилання є зрозумілим і зручним для пошуку в базах даних. Це — найповніший варіант бібліографічного опису. У зарубіжних БД проста транслітерація заголовка статті без його перекладу англійською мовою не має сенсу.

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Deming D., Dynarski S. The lengthening of childhood. (NBER Working Paper 14124). *Cambridge, MA: National Bureau of Economic Research*. Retrieved July 21, 2008, from http://www.nber.org/papers/w14124

Посилання на книги:

McCartney E. J. Optics of the atmosphere. *N. Y.: Willey*. 1977, 400 p.

Посилання на розділ книги:

прізвища та ініціали авторів (транслітерація, *курсивом*); повна назва роботи в перекладі англійською мовою (прямим шрифтом); прізвище відповідального редактора, *назва видавництва* (транслітерація, *курсивом*, без скорочень), рік видання і номер першої та останньої сторінок цитованої роботи, мова видання.

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Посилання на патенти:

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Автор. Назва патенту. Країна. *Патент х ххх ххх*(номер). Місяць, число, рік.

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Короткі повідомлення

Журнал «*Biotechnologia Acta*» публікує менші за обсягом статті, які мають безумовну новизну і значущість для біотехнології. Ці статті проходять прискорене рецензування і публікуються в короткі терміни. Загальний обсяг короткого повідомлення обмежений 10 машинописними сторінками, кількість рисунків і/або таблиць — не більше 3, а список використаних літературних джерел не повинен перевищувати 15.

Розділи короткого повідомлення аналогічні розділам оригінальної статті, але не виділяються заголовками і підзаголовками; результати можуть бути викладені разом з обговоренням.

Доцільність такої позачергової публікації має бути обґрунтована в листі, що надсилається Головному редакторові автором для кореспонденції. У разі прийняття така робота може бути опублікована протягом 3–4 місяців.

Примітка

У зверстаному варіанті статті авторам можна виправляти тільки помічені помилки без внесення структурних змін і доповнень.

Редакційна рада має право відмовити друкувати рукописи, що містять раніше опубліковані дані, а також матеріали, які не відповідають фаху журналу, або матеріали досліджень, що були проведені з порушенням етичних норм.

Рукописи надсилайте в редакцію за адресою:

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