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BIOLOGICAL DATABASES USING OBJECT-ORIENTED SYSTEM ANALYSIS

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The aim of the work was to develop electronic database of biological organisms (fishes, viruses) on the base of the methods that have been used before in mathematics, engineering, physics. The methods of object-oriented system analysis, construction of models of subject spaces (problem space, solution space), *ER*-diagrams, of constructing databases and others were applied. The examples of prototypes of modern databases with information about fishes developed over the past years in Western European and American countries were presented. An overview of applied methods, concepts and terminology from the areas of object-oriented system analysis, database designing was disclosed. In conclusions the description of constructed database and practical recommendations for the development of databases with the information about domestic biological organisms for electronic information systems are given.

Key words: object-oriented system analysis, biological objects, electronic information systems, fishes, viruses.

Electronic information systems with databases for biotechnology, environmental protection of bioobjects and other biological sciences. Electronic information technology (IT) penetrated deeply into our life — industry, technology, science; they have become a feature that characterizes our reality and level of development of the countries. Being an integrative part of the Europe and modern industrial world, Ukraine is also incorporated into the world scientific and technological progress and has all opportunities to develop progressively new technologies in its own territories. Over the last 30 years, computer technique and related new technologies — network, Internet technologies, and others — have become widespread even in the everyday life of the people. Consequently, the information and computer revolution in classical biology and medicine has happened too. To the solution of these problems the people came about 30 years ago. One of the most actual contemporary tasks is the creation of information systems (ISs) in medicine and biology, and the majority of these ISs operate with the data that have been ordered in electronic databases (DBs). In the last 20–25 years in medicine and in the last 15–20 years in biology, the number of new electronic ISs has been increased rapidly; they had their own peculiarities and specifics in comparison with similar systems in engineering, physics and other similar industries. The number of such ISs in biology and medicine is growing from day to day. Nowadays, at the beginning of the XXI century, the scientists in the field of information and computer technologies (ICTs) discussing further development of new software and computer technology specifically

for medicine and biology. In contemporary world there is an important idea to use such novel ICTs with DB for ordering of information about living organisms — for example fishes [1]; their accounting, descriptions and even for determination of such organisms on the base of these new technologies [2–20]. Sure, in base of electronic information systems (ISs) elaboration the real biological data have to be placed [1, 21–36]. Such primary versions of ISs in Ukraine have been done; technologies of their elaboration partially are described [37–49], and the data about different objects of fauna [21–36, 45, 46] and flora [50–52] may be ordered in them. For the success of such works it is necessary to use positive experience of prototypes development; either of practical or theoretical ways of these tasks solutions [2–20, 53–172]. Some of such prototypes were developed for monitoring of biodiversity changes in fauna or ecological changes also under anthropogenic pressure using ICTs [2–7, 9, 14–18, 46–48, 56, 59, 81, 148, 149, 153, 165, 169–172, 174–176, 178, 183, 186–188]. Some works of this article authors were defended by patents [32, 173–189]. Contemporary knowledge of ICTs technologies, electronic DBs construction were used for present work fulfillment [190–192].

A number of important reasons determine the necessity of the work on the creation of IS with the databases (DBs) in biology. Actually, the previous author's works [45, 46] are the continuation of the works done in present article, because relative databases developments [45] have to be based on the resuts of object-oriented system analysis from present publication. Primarly, the ICTs with databases were developed, first of all, for the needs of technology and such fields as mathematics, physics, statistics and economy (first of all, for energetic). These technologies began to be developed in biological sciences later because of the set of reasons: objects with which the biology deals with are much more complicated, main attention in contemporary societies is paid on high industry development, and so on. In general, ICT with databases comes in two ways to medicine and biology: through the acceptance of ISs that were developed earlier for other industries with further their transformation depending on the specifics of medicine and biology, and by creating of original types of these systems. Today, the samples of ISs with databases in biology are not numerical in comparison with technique, they do not satisfy all practical needs, and therefore almost all new works in these industries are valuable and find their application. In addition, the material in these items that was given our publications has the practical value because it can be the basis for the creation of new types of systems.

Because the specifics of biology ever were the works with complex objects, living systems, multifactorial influences on them, and etc. thus, an analysis of such specificity and its discussion is really relevant. It is also important that because of peculiarity and specifics of medical and biological objects, accumulation in these areas of great numbers of the results of experiments, observations, etc. — the use of modern information and computer technologies with databases will contribute to the next progress in these areas, since it will allow ones to analyze and to process a huge (and constantly growing!) amount of the data. With the use of previous technologies this was impossible indeed, and this led to the loss with time a significant part of obtained results (including the results of expensive high-precision experiments).

Importance of object-oriented system analysis in process of electronic databases construction. The construction and development of electronic databases (DB) of biological organisms (local DB (separated) or as a part of ISs) is an important task still. Such databases should, as far as possible, contain a collection of information about individual biological organisms, their communities and other related information. The scope of such databases usage is broad: solution of problems in biotechnology, molecular biology and genetics; in preserving of species diversity, protecting the environment from harmful influences; database as the electronic determinants of living organisms and many other applications [37–49]. In our previous works we have provided already the information on developing a database of biological organisms, for example, *Noctuidae* (*Lepidoptera*) and some other *Arthropodae* [37, 45, 46]. However, the methods of DB constructing which have been demonstrated in our publications [37–49, 173–176, 178, 183, 186–188], are standard for use when creating a database of all biological organisms, not just *Arthropodae* [2–20]. Therefore, in this publication we woud like to demonstrate the possibility of their use for other groups of living organisms: fishes and viruses — pathogens of fishes' diseases, in order to involve our fauna representatives as fully as possible in our works. In addition, due to the limited volume of our previous publications,

we were not able to pay necessary attention to the important set of works preceding the process of database development — to object-oriented system analysis of virtual space containing the objects that have to be included into the developed electronic database.

Object-oriented system analysis of the investigated space is extremely important for the reasons of the most rational design of the databases. Our Nature includes a huge number of different groups of organisms. Each of these organisms has its own characteristics, which must be included into the database— there are billions of records. In case of insufficiently qualified determining of such characteristics, establishment of wrong links between organisms, etc., the DBs become irrationally constructed, overloaded with unnecessary details and lack of important information. This leads to slowdown the information search, and even to impossibility of data mining in an imperfect database. Accordingly, the time of data mining in such imperfect databases increases. The situation is even more complicated in case of different databases combining, for example, if they were constructed at different universities. Consequently in present publication, basing on the authors' experience, some practical recommendations were given for the development of modern, perfect biological, Internet-based databases (primarily relational) taking into account the specifics of medical and biological objects, which confirms the relevance of the work performed.

Some examples of modern databases and information systems with information about the fishes. In this section, let's observe some examples of modern databases with information about fishes.

A. Global Information System On Fishes. A global biodiversity information system on fishes is called "FishBase" [16]. Its initial goal is to provide key facts on population dynamics for 200 major commercial fish species. Now DB of this IS grown to having a wide range of information on all fish species currently known in the world: taxonomy, biology, trophic ecology, life history, and uses, as well as historical data reaching back to 250 years.

At present, FishBase covers more than 33,000 fish species compiled from more than 52,000 references in partnership with more than 2,000 collaborators (>300,000 common names and >55,000 pictures). The breadth and depth of information in the database, combined with the analytical and graphical tools available in the web can satisfact different needs of diverse groups of users (scientists, researchers, policy makers, fisheries managers, donors, conservationists, teachers and students). Its various applications are aimed for sustainable fisheries management, biodiversity conservation and environmental protection. FishBase is result of the work of non-profit, non-governmental organization engaged in the development and management of global databases on aquatic organisms, including their distribution and ecology. FishBase provides the database and web interface for free for 25 years.

B. A database of fish biotransformation rates for organic chemicals. A method published by other authors [17] for estimating whole-body in vivo metabolic biotransformation rate constants (kM) is applied to a database of measured laboratory bioconcentration factors and total elimination rate constants for fish. The method uses a kinetic mass balance model to estimate rates of chemical uptake and elimination when measured values previously were not reported. More than 5 400 measurements for more than 1 000 organic chemicals were critically reviewed to compile a database of 1 535 kM estimates for 702 organic chemicals. Biotransformation rates range over six orders of magnitude across a diverse domain of chemical classes and structures. Screening-level uncertainty analyses provide guidance for the selection and interpretation of kM values. In general, variation in kM estimates from different routes of exposure (water vs diet) and between fish species is approximately equal to the calculation uncertainty in kM values. Examples are presented of structure-biotransformation relationships. Biotransformation rate estimates in the database are compared with estimates of biodegradation rates from existing quantitative structure-activity relationship models. Modest correlations were found, suggesting some consistency in biotransformation capabilities between fish and microorganisms. Additional analyses to further explore possible quantitative structure-biotransformation relationships for estimating kM from chemical structure were encouraged, and recommendations for improving the database were provided.

C. A global database on freshwater fish species occurrence in drainage basins. In their actual publication the authors stated that nowadays the growing interest is devoted to global-scale approaches in ecology and evolution that examine patterns and determinants of species diversity and the

threats resulting from global change [18]. These analyses obviously require global datasets of species distribution. Freshwater systems house a disproportionately high fraction of the global fish diversity considering the small proportion of the earth's surface that they occupy, and are one of the most threatened habitats on Earth. The authors provided complete species lists for 3119 drainage basins covering more than 80% of the Earth surface using 14 953 fish species inhabiting permanently or occasionally freshwater systems. The database results from an extensive survey of native and non-native freshwater fish species distribution based on 1 436 published papers, books, grey literature and web-based sources. Alone or in combination with further datasets on species biological and ecological characteristics and their evolutionary history, this database represents a highly valuable source of information for further studies on freshwater macro ecology, macroevolution, biogeography and conservation.

D. Databases of freshwater fish species distributions. Another report about the databases of freshwater fish species distributions was suggested in [18]. The authors discussed the paradox of fishes species that approximately 40% of all described fish species inhabiting freshwaters, while the remaining 60% are marine habitants. Describing global scale freshwater fish diversity patterns, understanding the environmental drivers and evolutionary processes shaping such diversity and revealing the major human-related threats were the major goals that motivated the compilation of presented database. They conducted an extensive survey of freshwater fish species distribution based on 1 436 published papers, books, grey literature, databases and web-based sources, resulting in species lists for 3 119 drainage basins covering more than 80% of the Earth surface. To date, these databases have been used in several studies that have increased our understanding of freshwater fish species distributions. These studies allowed to accurately map global patterns of native, endemic and non-native freshwater fish species richness and to reveal their environmental and human-related determinants. The databases were also used to evaluate non-native species influence on native communities' structure, to forecast climate change effects on species extinction processes and to analyse effects of current and future scenarios of species introductions on fish faunas homogenization processes. Recent studies also focused on analyzing the influence of past river connections on the present distribution of native fish species, on analyzing geographical and other differences in diversification rates and origin of actinopterygian fish families, and on evaluating human-related extinction drivers.

Methods of object analysis, constructing of models of subject spaces when creating a biological database about fishes and viruses that they infect. This section deals with the consideration and the characteristics of the methods of analysis of subject space and model construction [191–192]. To solve such problems, the object analysis methods [190] have been developed, which is described below.

The main purpose of objective analysis is to represent a subject space (SbS) as a set of objects with their properties and characteristics that are sufficient for their defining and identification, as well as for determining the behavior of objects within the framework of the chosen system of concepts and abstractions.

For example, in present work we would like to make the database about fishes and viruses that they infect; respectively, for our object analysis in framework of this task solution we have to select primary such main objects as "Fish" and "Virus". Sure, with time in process of task solution, other objects may be added to these first two.

All SbS concepts are essences. Each object is a unique element; it has at least one property or characteristic and a unique identifier in a set of objects.

Analysis of SbS is carried out using object-oriented methods and corresponding standards. The ultimate objective of the object-oriented analysis of SbS is the definition of the object model (OM) with the help of selected objects, the relations between them and their properties and characteristics.

A. Brief overview of object-oriented methods for analyzing and constructing of models. For today, more than 50 object-oriented methods of SbS analysis are known, which have been practically tested. In present work we used only 3 of them:

1. Object-Oriented System Analysis (OOAS). This is a method, which allows identifying the entities and objects of SbS, defining their properties and relationships, as well as building on their basis an information model, a model of objects state and processes of presentation of data flows (dataflow) [190].

2. Object Oriented Analysis (OOA). This is a method, which provides simulation of OM and formation of requirements for SbS by means of a notion "entity-relationship ER", specification of data streams and related processes [190-192]. The scheme — ER-diagram for abstract objects "Fish" and "Virus" is given on Fig. 1.

3. Object Oriented Analysis and Design (OOAD). This is methodology, which is based on ER-modeling of entities and relationships in SbS object model. OOAD provides a system definition and organization of the data using structured diagrams, diagrams "entity-reations" and the matrix of information management [190].

The study of object-oriented methods varieties shows that they have many common features (for example, *ER-modeling*, Dataflow), as well as their specific features. Each developer of the method of object-oriented analysis invented his own new necessary concepts, which semantically coincide often with similar concepts in other methods. Therefore, the terminology the very conceptual apparatus used in this sphere does not always coincide in different methods.

B. Basic concepts of the methods of object analysis of SbS. To the basic concepts of the methods of SbS object analysis would belong the following:

SbS object is an abstract image with behavior that depends on its characteristics and relationships with other SbS objects.

Entity is a semantically important object or type of object, that exists in SbS in reality or it is an abstract concept, whose information must be known and / or recorded.

Concept is the value of some abstract entity of SbS, it is denoted by a unique name or identifier. A group of such concepts is a parent concept, which is obviously determined by some set of common attributes. The concept, along with its attributes is presented graphically in OM or in text form.

Attribute is an abstraction that has all abstracted essence of concepts. Each attribute is denoted by a name unique within the concept description. The set of grouped attributes denotes the identifier of this group. A group of attributes can be combined into a class and to have a class identifier.

Relationship is an abstraction of a set of relations that take place between different kinds of SbS objects which are abstracted as concepts. Each relationship has its unique identifier. Relationships can be textual or graphical. To formalize relationships between concepts, the auxiliary attributes and links to identifiers of these relationships are added. Some relationships are formed as consequence of other relationships existence.

Class is a set of objects with the same properties, operations, relationships, and semantics. Any object belongs to a class. A class is represented by various methods (for example, by lists of objects, operations,

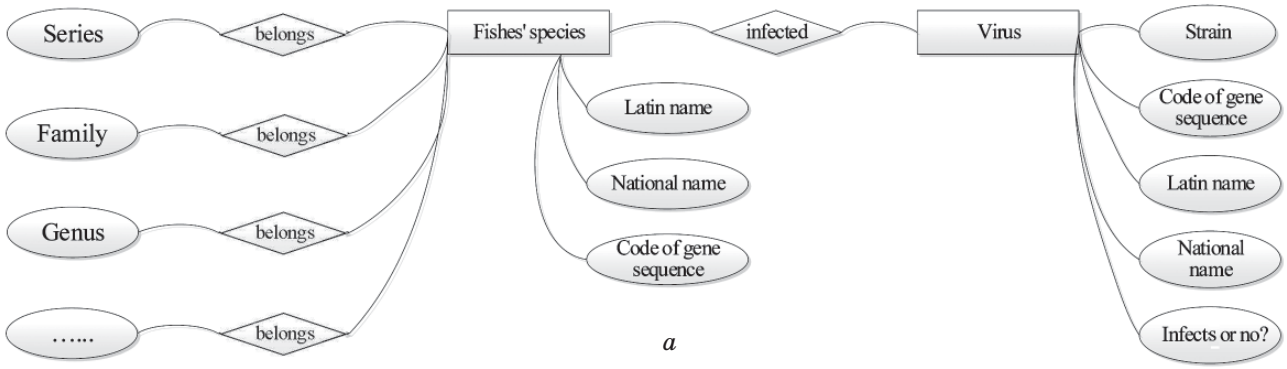

Fig. 1. **ER-diagram for the database "Fishes And Their Virus Diseases": analysis for two main objects "Fish" and "Virus":**
a — fragment of ER-diagram (explanations see in text);
b — symboic denotation of objects, relations and attributes at the scheme

states). Class is measured by the number of the samples, operations, etc.

Subject space (SbS) is what is analyzed in order to isolate a specific set of concepts (entities, objects) and relations between them. On the set of these concepts, the tasks are defined for the purpose of their automated solution. The *subject space* can be divided into the *problems' space* and the *solutions' space*. The *problems' space* includes the entities, the concepts of SbS, and the *solutions' space* is the set of software implementations of the tasks, including functional components that provide the solution of the tasks and functions of SbS represented in this space.

Allocation of SbS entities is carried out taking into account the differences determined by the relevant conceptual structures. SbS model is a set of precise definitions of concepts, objects and their characteristics, as well as a set of synonyms and classified logical relationships between these concepts.

Relations or links are established for model objects. There are static (permanent) relations that do not change or change rarely, and dynamic relations that have certain states and change during the operation of the system. The state of connections between objects can evolve over time.

The links between the objects can be following (Fig. 2):

• *one-to-one relationship* (1: 1) exists when one sample of an object of a certain class is associated with a single sample of another class, i.e. single instances of the classes are involved in communications;

• *one-to-many relationship* (1: N) exists when one samples of an object of a certain class is associated simultaneously with one or more samples of another class or of the same class;

• *many to many relationship* (M: N) exists when several samples of objects of two classes take part in relationships, i.e. one or more samples of another class is associated with one or more samples of the first class.

Hierarchical model of biological objects' organization and classification from the point of view of DB construction. Hierarchical model is extremely important model of objects' organization either in living Nature or in object-oriented methods of programming; in our models we have to refect obviously this hierarchical type of objects' organization. In our previous publications [37] we have written already that hierarchical models in informatics are enough similar to hierarchy of living organisms in nature. As example of DB construction let's study now the pair of organisms: the fish (carp) and virus that cause its disease. It is known that for hierarchical classification of organisms in living nature, Carl Linnaeus proposed his scheme on 1 761 [70, 37]. Let's represent according to his scheme such well-known fish as ordinary carp (*Cyprinus carpio* L.) that belongs to animals from *Eukaryota* Domain (Fig. 3).

Further in our investigation we would like to unite in linked databases the information about fishes (for example, the carp *Cyprinus carpio* L.) and viruses that are the reasons of fishes' diseases. For example, for *C. carpio* it is known that they have such virus diseases: (spring viremia of carp, infectious necrosis of hematopoietic tissue, infectious necrosis of pancreas, viral necrosis of erythrocytes, herpesvirus infections, iridovirus infections, and etc.), which are characterized by acute flow with high mortality of fishes that causes significant economic losses to world aquaculture.

At the next step we have to describe at one scheme these 2 objects (carp-virus) with all their important characteristics and relations between objects. Such scheme one could see on Fig. 4, that is "*ER*-diagram" modified for the fish "Carp" (compare with Fig. 1)

Functional role of ER-diagram is to represent in whole all *objects* from the database with all their *relations* and with all characteristics of the objects that are called "*attributes*". Sure, characteristics those are important from the point of view of the database representation! For example, such characteristic as "Maria likes to fry carps"

Fig. 2. **Power of reactions between the objects during the database elaboration:**
"M: N" — "many-to-many". In this example, this means:
"M species of fishes have been infected by N strains of viruses"

Domain	*Eukaryota*
Kingdom	*Metazoa*
Type	*Chordata*
Subtype	*Craniata*
Class	*Actinopterygii*
Infraclass	*Teleostei*
Series	*Cypriniformes*
Family	*Cyprinidae*
Genus	*Cyprinus*
Specie	*Cyprinus carpio* L.

Fig. 3. **Scheme of ordinary carp (*Cyprinus carpio* L.) hierarchic classification**

probably, is not so important for academic databases. But for such DB are too important the data (attributes) as gene sequences, specie, family, genus and other similar. For the representation of these 3 main notions of *ER*-diagram the special symbols are used (Figs. 1, 4): rectangle (object), rhombus (relation), oval (attribute). A rectangle (object) usually includes a noun — for example, the "fish". A rhombus (relation) is ever a verb — for example, "to infect". And an oval (attribute) ever includes characteristics: species (or other classification unit), color, dimensions, etc. Such diagram helps to analyze better all entities that we would like to include into the database and to represent them in their entirety. Without such analysis the construction of DB of a high quality would not be possible.

Since each species of fish may be infected by several viruses (N), and each virus can infect different species of fish (M), then the links between the objects have to be marked as "M:N", which actually means: "M species of fishes can be infected by N strains of viruses" (Fig. 2). As noted above, other variants are possible, like "M:1" — means "M species of fishes can be infected by the virus of one strain". Also "1:1" — means "one specie of fish can be infected by the single virus strain". Such abstraction allows us to design a high-quality database and to verify its accuracy in the visual diagram — "ER-diagram" in future.

Development of the database with information about fishes' on the base of "ER-diagram". The scheme "ER-diagram" we need for further development of the database with information about fishes on its basis. Let's study the representation of such database in the form of a set of tables, which are given on Fig. 5. It is easy to see that:

1. Objects of "ER-diagram" were turned into separate tables.

2. Attributes were turned into the fields of these tables (in our case — in the columns).

3. The relations between the objects were turned into relations between separate tables.

At the same time, the power of reations reflects the number of tables that need to be linked with the object; and the developer deside what number have to be. If the power of reation is 1, then the link is formed with one table, if "M" — with "M" numbers of tables.

Objects in our model can be characterized by the number of attributes. The lists of some attributes is given below. On Fig. 5 one can see the tables with the less number of attributes because of limited space of the page.

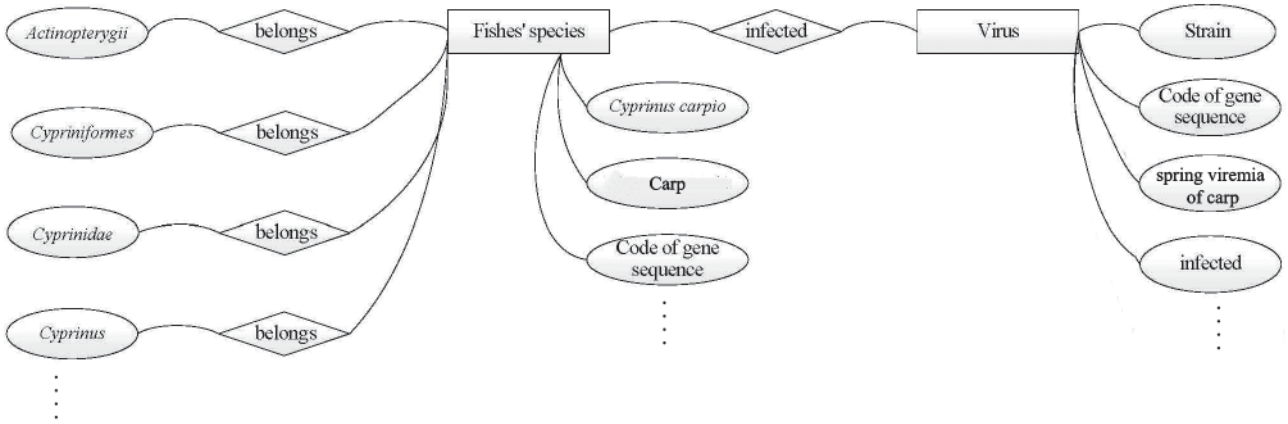

Fig. 4. **Fragment of ER-diagram "Fishes And Their Virus Diseases" on example of *Cyprinus carpio* L.**

Object "Fish"
Attributes:
Primary key
The name of the fish (in Latin, Ukrainian and Russian).
Class
Subclass
Series
Family
Genus
Species
Area of inhabitance.
Biotopes of inhabitance.
Objects of nutrition.
Data of fishing.
References to literary sources.
…….
Comments.
Other.

Object "Virus"
Attributes:
Primary key
The name of the virus (in Latin, Ukrainian and Russian).
Dimentions
Code of gene sequence (if it known)
Name of disease
Symptoms of disease
References to literary sources.
……..
Comments.
Other.

Relations between the tables using "keys." In order not to lose the information that is filled in the database of biological objects, the technique of "keys" is used.

"Keys" are necessary "tools" for relations establishing between the objects; they guarantee that the information from the database is accessable and it always may be obtained from there. Indeed, there is no sense to write thousands of data records into a structure if these data can not be used, if they are not accessible! Consequently, the "keys" are the elements of each table; they are related to each other through programmed links. By obtaining the information from one table, it is possible to obtaine the information from another table associated with it through a link that unites their keys. Usually, as a "key" serves a certain code (numbers or other symbols), and the links between such codes from individual tables in many modern software environments, even a young developer can make by themselves (Fig. 5).

At our tables (Fig. 5) there are 2 codes (or keys) have to be linked:

1) 1* (*Cyprinus carpio* L.) and
2) kB (Rhabdovirus carpio — Spring Viraemia of Carp Virus (SVCV)).

This means that when user in Internet will "click" on "*Cyprinus carpio* L." he will see consequently the information about (Rhabdovirus carpio (Spring Viraemia of Carp Virus (SVCV)) — virus that infects carp.

In addition, it is easy to follow in the diagram whether all entities are linked together. In this case it makes possible to access all records, so, the information from the database becomes fully available for everybody (the condition of the "integrity" of the data is fulfilled). Alternatively, if this condition is violated, the user will not be able to obtain the information from those parts of the database that are not connected by such links. However, using the methods described above, the programmer can easily recover connections based on the ER-diagram and linking the objects using keys. In the application of the abovedescribed methods, there are no differences between biological and technical databases and anybody for such purpose can study this technique using appropriate manual. The previous works of Klyuchko O.M. [45, 46] are the continuation of the works done in present article, because relative databases developments [45] have to be based on the resuts of object-oriented system analysis from present article.

Thus, according to the purpose of the work there were demonstrated that the methods for relation electronic databases construction, that were developed earlier for physics, engineering, etc. can be applied in other branches which traditionally do not refer to technical disciplines. Usually these were done by their acceptance in the new spheres of application, for example, in biology and medicine. In present publication, the following set of methods was used to develop databases with biological content: object-oriented system analysis (OOAS), object-oriented analysis (OOA) and OOAD (object-oriented analysis and design) — the latter methodology is based on ER-modeling of entities and relationships in the object model (ER-diagram).

Actually, the previous author's works [45, 46] are the continuation of the works done in present article, because relative databases developments [45] have to be based on the resuts of object-oriented system analysis from present publication. At the beginning of present article, a number of examples of electronic databases of biological objects that were developed in different countries of the

Table "Fish"

a

Primary key	Name of species (Latin)	Name of species (national)	Photo	Class	Subclass	Series	Family	Genus	...
1*	*Cyprinus carpio* L.	Короп звичайний	Foto 1: http://www2.dnr.cornell.edu/cek7/nyfish/Cyprinidae/common_carp.jpg	*Actinopterygii*	*Neopterygii*	*Cypriniformes*	*Cyprinidae*	*Cyprinus*	
2*	*Salmo labrax* Pallas	Лосось чорноморський (сьомга)	Foto 2: https://redbook-ua.org/media/images/main/a-327.png	*Actinopterygii*	*Neopterygii*	*Salmoniformes*	*Salmonidae*	*Salmo*	
3*	*Salmo trutta* L.	Лосось струмковий (форель струмкова)	Foto 3: https://ih1.redbubble.net/image.65006165.3846/flat,800x800,070,f.u2.jpg	*Actinopterygii*	*Neopterygii*	*Salmoniformes*	*Salmonidae*	*Salmo*	
...									

Table "Virus"

b

Primary key	Name (Latin)	Name (national)	Genom	Family	Dimentions	Disease it cause	Symptoms of disease	Image	Other
kA	VHS virus	Virus of haemorrhagic septicaemia	RNA	Rhabdoviridae	180–240× 60–75 nm	Viral haemorrhagic septicaemia, VHS	Darkening of the skin and fecundity of the fish. The sick fish slowly floats on the surface of the water, almost does not react to irritation. Gum is anemic, with striped hemorrhages. The hemorrhages are also found in the conjunctiva. darkening of the skin, anorexia, inhibition of fish. The disease runs in acute, chronic and nervous forms.	Image 1: https://en.wikipedia.org/wiki/Viral_hemorrhagic_septicemia#/media/File:VHS.png	
kB	Rhabdovirus carpio (Spring Viraemia of Carp Virus (SVCV))	Rhabdovirus carp	RNA	Rhabdoviridae	105–125×70–85 nm	Spring Viraemia of Carp Virus, SVCV	Darkening of the skin, anorexia, inhibition of fish. The disease runs in acute, chronic and nervous forms.	Image 2: https://nas.er. usgs.gov/XIMAGESERVERX/ 2012/20120424150715.JPG	
...									

Fig. **5. Tables of developed database with information about fishes:** *a, b* — the attributes became the fields of the tables. "Primary keys" are the tools for the formation of the relationships between the tables. Cyrilic words are in tables for domestic use in Ukraine. The link between codes 1* and kB means that carp is infected by "Spring viremia virus" and this will be shown in future database

world were given. Within the framework of the thesis on the correspondence of the hierarchical classification of living objects and the idea of hierarchical subordination of objects in some methods of computer sciences (including databases development), an example of the hierarchical classification of *Cyprinus carpio* L. carp was given. It was noted that similar type of subordination can be reflected in the attributes in process of relational biological database development, exactly this was done further by the authors.

This publication also provides a description of the concepts of objects, relationships, attributes, keys, and others. In addition, on the example of biological objects, the types of relations 1:1, 1:N, M:N were shown. On the basis of such theoretical generalizations an ER-diagram for two types of objects was developed: for the carp *Cyprinus carpio* L. and viruses causing diseases of this fish. At the end of the pubication it was shown how relational tables of biological databases are formed, and how they are interconnected with the "keys". The authors hope that present work will be useful for training students of the corresponding specialties, as well as will contribute to the further elaboration of domestic biological databases in Ukraine.

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БІОЛОГІЧНІ БАЗИ ДАНИХ ІЗ ЗАСТОСУВАННЯМ ОБ'ЄКТНО-ОРІЄНТОВАНОГО СИСТЕМНОГО АНАЛІЗУ

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Метою роботи було розробити електронну базу даних біологічних організмів (риб, вірусів) на основі методів, які дотепер застосовували у математиці, техніці, фізиці. Під час виконання роботи використовували методи об'єктного системного аналізу, побудови моделей предметних областей (простір задач, простір рішень), *ER*-діаграми, методи конструювання баз даних та інші. У статті розглянуто приклади-прототипи сучасних баз даних з інформацією про риб, що їх розроблено протягом останніх років у країнах Західної Європи та Америки. Наведено огляд застосованих методів, концепцій та термінології з областей об'єктного системного аналізу, конструювання баз даних. У висновках подано опис сконструйованої бази даних та практичні рекомендації з розроблення баз даних з інформацією щодо вітчизняних біологічних організмів для електронних інформаційних систем.

Ключові слова: об'єктно-орієнтований системний аналіз, біологічні об'єкти, електронні інформаційні системи, риби, віруси.

БИОЛОГИЧЕСКИЕ БАЗЫ ДАННЫХ С ИСПОЛЬЗОВАНИЕМ ОБЪЕКТНО-ОРИЕНТИРОВАННОГО СИСТЕМНОГО АНАЛИЗА

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Целью работы было разработать электронную базу данных биологических организмов (рыб, вирусов) на основе методов, которые до сих пор применялись в математике, технике, физике. При выполнении работы были применены методы объектного системного анализа, построения моделей предметных областей (пространство задач, пространство решений), *ER*-диаграммы, методы конструирования баз данных и другие. В статье рассмотрены примеры-прототипы современных баз данных с информацией о рыбах, разработанные в последние годы в странах Западной Европы и Америки. Приведен обзор применяемых методов, концепций и терминологии из областей объектного системного анализа, конструирования баз данных. В выводах приведено описание сконструированной базы данных и практические рекомендации по разработке баз данных с информацией об отечественных биологических организмах для электронных информационных систем.

Ключевые слова: объектно-ориентированный системный анализ, биологические объекты, электронные информационные системы, рыбы, вирусы.

cassette transporter (ABCA1), cholesterol 24-hydroxylase (cyp46), etc. [14].

Most of the mutant genes in AD patients encode multifunctional proteins active in many branched biochemical pathways. This presents some difficulties for the pharmacological therapy aimed to correct the expression of such genes. Thus, using specific miRNA (also known as miR) to regulate the expression of target genes is a promising direction of research [15, 16].

MiRs are small (18–25 nucleotides), evolutionally conservative, non-coding, single-stranded RNAs which are key in various biological processes through regulating expression of target genes by binding with 3′-non-translated loci of their mRNA [17, 18]. Each miR is proven to control up to several hundreds of genes, and one gene can be a target for more than one miR [19]. These regulatory RNAs can "silence" a gene through various ways. First, they inhibit the gene expression by interacting with mRNA: miR attach to mRNA and block the translation process. Another way to deactivate a gene is during transcription, when miR, as part of poliprotein complex, induces epigenetic modifications in the genome: methylation of DNA and histones, and deacetylation of histones. Protein synthesis can also be inhibited by the interaction of miR with repressor proteins that block translation [20]. However, in very rare circumstances (namely, arrested cell cycle), miR, conversely, activates translation [21]. Hence, miRs are more and more used in diagnostics and therapy of neurodegenerative, cardiovascular, cancer and other pathologies [22].

MiR-101 belong to the family of miRNAs, which participate in several cellular activities such as cell proliferation, differentiation, invasion, and angiogenesis [23]. Hypoxia-sensitive miR-101 stimulates angiogenesis and factors in regulation of the vascular remodeling [24]. Deregulation by miR-101 is observed during the development of malignant neoplasms, which indicates its suppressor function in a number of tumor varieties [25]. MiR-101 regulates several simultaneous postnatal programs of brain development, because the balanced excitement/ deceleration is necessary for the normal functioning of neural networks [26]. Transitory loss of miR-101 regulation on pyramidal neurons in dorsal hippocampus causes the hypersensitivity of the neural network and cognitive deficit, thus concentration of miR-101 in the postnatal period is critical for further functioning of neural chains. This miR inhibits NKCC1 chloride importer (gene *Slc12a2*) needed for initiating the maturing of GABA-ergic signaling system. That causes the reduced spontaneous synchronized activity and prevents dendrite overgrowth. Also, miR-101 is a part of program of development which activates the repression of motor protein 1A KIF1A (gene *Kif1a*) from the superfamily of kinesin and Ankyrin-2 (gene *Ank2*), to inhibit the excessive collection of pre-synaptic components and the decrease in the density of glutamatergical synapses. Targets of miR-101 also include mRNAs of the following genes: *Abca1*, *Ndrg2*, *Slc7a11*, *PMCA2*, *Rapgef1*, *Slc25a4*, *Camk2a*, *Clasp2*, *Dbs*, etc. [26]. It is shown that miR-101 is a key operator of mRNA's function for AβPP ($mRNA^{A\beta PP}$) by deactivating it and inhibiting the protein synthesis of the amyloid-β protein precursor and its amyloidogenic processing [27–28]. In [29–31], the cytokine activation is shown to directly affect the expression of gene *AβPP* and AβPP synthesis during chronic inflammation in central neural system (CNS), accompanying the process of amyloidosis.

In previous studies of experimental AD rat model, it was shown that a natural polyphenol curcumin in soluble and liposomal forms inhibited the cytokine response to the toxic action of β-amyloid aggregates in target departments of animal brain (neocortex and hippocampus) [32–33]. A possible biochemical mechanism for this is that curcumin suppresses the activation of IκB kinase (IKK), phosphorylation and degradation of IκBα (inhibitor of NFκB) and thus blocks the activation of nuclear transcription factor NFκB [34–35]. The anti-inflammatory effect of curcumin causes improvement of mnestic abilities and memory characteristics in animals with experimental AD. However, there is no evidence of direct and targeting inhibition effect of curcumin on excessive production of β-amyloid peptides.

The present work aimed to study the effect of liposomal miR-101 on the levels of β-amyloid peptide, and on the activation of cytokine system in brains of animals with experimental AD.

Materials and Methods

AD was modeled in aged male rats (14 months old) with intrahippocampal injections of aggregated Humanbeta Amyloid 1–40 protein (ChinaPeptidesCo., Ltd, China), as described previously in detail [26]. Commercial Aβ40 was dissolved in bidistillate to the final

concentration of 15 μmol/l, and incubated at 37 °C for 24 hr for aggregation. Large Aβ40 conglomerates were dispersed with ultrasound and sterilized directly before the injection. The suspension's volume was 10 μl per animal, infusion was carried out for 5 min. Stereotaxic coordinates of the area of injection in left hippocampus were determined using brain map in [40]. It corresponds to the distance from the intersection point of sagittal suture with bregma (zero point): 2 mm distally, 2 mm laterally and 3.5 mm in depth. Stereotaxic operations were performed on animals under general anesthesia with thiopental intraperitoneally (50 mg/kg). Intact animals served as control ($n = 6$).

Experimental AD rat model is generally accepted because it demonstrates not only the toxicity of Aβ aggregates (main mechanism of amyloidosis), but also the dementia symptoms characteristic for AD, such as worsening memory and violated mnestic abilities [36, 37]. Aβ40 was used instead of Aβ42 to model AD in rats because even though the latter β-amyloid peptide is thought to be a specific marker of amyloidosis, Aβ40 is synthesized in CNS by an order of magnitude more than Aβ42 [39]. Thus, Aβ40 aggregates are toxic for neural synapses. Also, Aβ40 and Aβ42 of rats do not aggregate, thus only Aβ40 Human aggregates were used in experimental AD models.

In 10 days after the model was established, miR-101-3p (OOO "NPF Sintol", Russia) was nasally administrated to experimental animals ($n = 7$) and empty liposomes were given to rats from the comparison group ($n = 6$). Liposomes were obtained from lipid films [41]. In total, 10 therapeutic sessions were concluded; in each an experimental animal was given $2.5 \cdot 10^{14}$ molecules of miR-101, in single 20 μl doses of liposome suspension. Another group of rats with AD model ($n = 6$) were not given anything.

In 10 days of nasal therapy (20^{th} day of experiment), all animals were decapitated. Neocortex, hippocampus and olfactory bulbs were removed in cold conditions, frozen and stored at −40 °C. Tissues of studied brain regions were homogenized in Tris buffer (50 mM tris-HCl, 150 mM NaCl, pH 7.5) and centrifuged at 14000 rpm for 5 min. Then, supernatant was collected. Supernatant samples of the aforementioned rat brain regions were used to determine concentrations of toxic endogenous form of Aβ42, tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and interleukin-10 (IL-10) in bioassay according to protocols of Rat Amyloid beta peptide 1-42 ELISA Kit (Bioassay Technology Laboratory, China) for β-amyloid peptide 42, and Rat ELISA Kits TNFα, IL-6 and IL-10 (Invitrogen BCM DIAGNOSTICS, USA) for cytokines. Concentrations were expressed in ng/mg of protein for Aβ42 and in pg/mg for cytokines. Absorption of samples was evaluated in microwell plate reader GBG Stat FAX 2100 (USA) at $\lambda = 450$ nm with wavelength correction at $\lambda = 630$ nm. Total protein content was measured according to Lowry [42].

Experimental protocols for rats were conducted in compliance with "General ethical principles of experiments on animals" (Kyiv, 2011).

The obtained results were statistically processed, average values and standard deviations were calculated. Statistical analysis of differences was done with Student's t-test for samples with normal distribution. Values were considered significant at $P < 0.05$.

Results and Discussion

1. *Anti-amyloidogenic effect of miR-101*

It was shown that the introduction of Aβ40 aggregates to rat hippocampus to model amyloidogenic processes in 20 days only in neocortex and hippocampus (significant increase in concentration of Aβ42 by 36% in neocortex and by 27% in hippocampus) while in olfactory bulbs, the concentration of Aβ42 did not change (Fig. 1). 10 days of nasal therapy with liposome miR-101, started in 10 days after establishing experimental AD model, normalized Aβ42 levels in target regions of rat brains, compared to empty liposomes. Thus, concentration of toxic endogenous Aβ42 decreased by 33% in neocortex and by 15% in hippocampus. No changes were seen in olfactory bulbs.

These results are in line with previous findings. According to [43–44], miR-101 negatively regulated AβPP expression and accumulation of Aβ in neocortex, and its function decreased in patients with AD. A few authors link that to single-nucleotide polymorphism in 3′UTR region of *AβPP* gene [45]. There is now a body of evidence that miR-101 regulates the level of AβPP in cell cultures, particularly in hippocampal neurons [46–47].

Considering that AβPP and Aβ are the main factors of Alzheimer's disease pathogenesis, we suggest inhibiting the expression of AβPP to mitigate the pathological processes underlying amyloidosis. Consequently, miR-101 may become a new tool for therapeutic modulation of AβPP levels. It is possible that

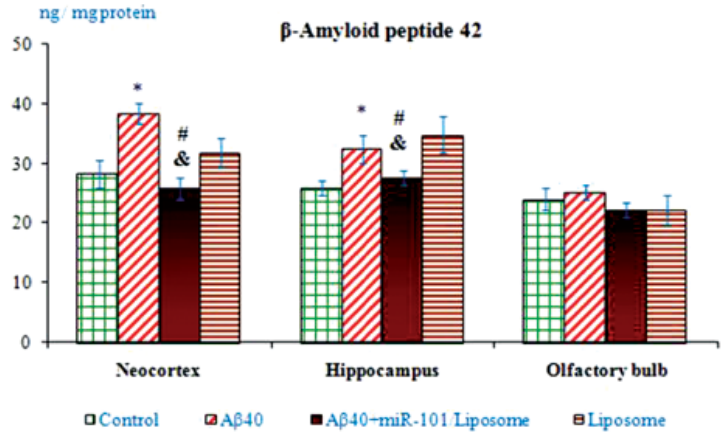

Fig. 1. **Level of Aβ42 in neocortex, hippocampus, and olfactory bulbs in rats with experimental model of Alzheimer's disease, nasally treated with liposome miR-101 for 10 days**
Hereinafter * — $P \le 0.05$ compared to control; # — $P \le 0.05$ compared to AD model (Aβ40 group); & — $P \le 0.05$ compared to therapy with empty liposomes (Liposome group)

either directly delivering miR-101 to CNS, or regulating its endogenous expression, should reduce AβPP levels in brains of patients. In [47] it was shown that miR-101 is expressed from two independent genomic loci contained in the intergenic regions on chromosome 1 and chromosome 9. The promoter elements regulating the transcription of miR-101 have not been sufficiently studied, and only now their detailed research is underway. Therefore, nasal therapy of miR-101 in liposomal form may be promising for the treatment of patients with Alzheimer's disease.

2. *Anti-inflammatory effect of miR-101*

Using an experimental AD model, it was shown that exogenous Aβ40 induces anti-inflammatory processes in neocortes and hippocampus (possible increase of total studied cytokines by 16–18% in neocortex and inflammatory cytokines TNFα and IL-6 by 14% in hippocampus). In olfactory bulbs, cytokine levels did not change significantly (Fig. 2, *A*, *B*, *C*).

Ten days of nasal administration of miR-101 in liposomal form decreased level of IL-6 by 23% in neocortex and by 19% in hippocampus, which was statistically significant compared to AD model and therapy with empty liposomes (Fig. 2, *A*). Significant decrease of TNFα levels by 12% under effect of miR-101 was seen only in animal hippocampus (Fig. 2, *B*). Unexpectedly, concentration of TNFα decreased in olfactory bulbs of rats with AD model after nasal treatment with empty liposomes (by 13%) and with liposomes containing miR-101 (by 10%). The levels of IL-10 did not change significantly under influence of miR-101 in any of the brain region in AD model rats (Fig. 2, *C*). Notably, treatment with liposomal miR-101 strongly affected the levels of IL-6 (Fig. 2). This can be caused by experimental conditions. At the 20th day of experiment, TNFα, formed and secreted early under treatment, becomes less prominent in the neural inflammation compared to second generation cytokine such as IL-6 [48].

Comparing our data to the previous findings on anti-inflammatory effect of curcumin in liposomes under similar experimental conditions [37], it should be noted that polyphenol has higher anti-cytokine potential than miR-101. Anti-cytokine potential of curcumin can be explained by the direct effect it has on the levels of cytokine genes induction, and its indirect influence on the Aβ level in animal CNS. MiR-101 targets the mRNA from which AβPP is translated.

Decreasing concentration of inflammatory cytokines (IL-6 and TNFα) under effect of miR-101 is, in our opinion, not a direct effect. It is related to the falling levels of the toxic endogenous Aβ42 in neocortex and hippocampus (brain regions that are responsible for memory and studying). However, a number of authors assume that miR-101 may have a possible direct influence by decreasing the induced levels of inflammatory cytokines [49], by increasing IL-6 production in response to transfection of cells with miR-101 [50], or in case of excessive expression in LPS-activated macrophages [51].

Thus, the feasibility of combining miR-101 and curcumin in a single liposomal

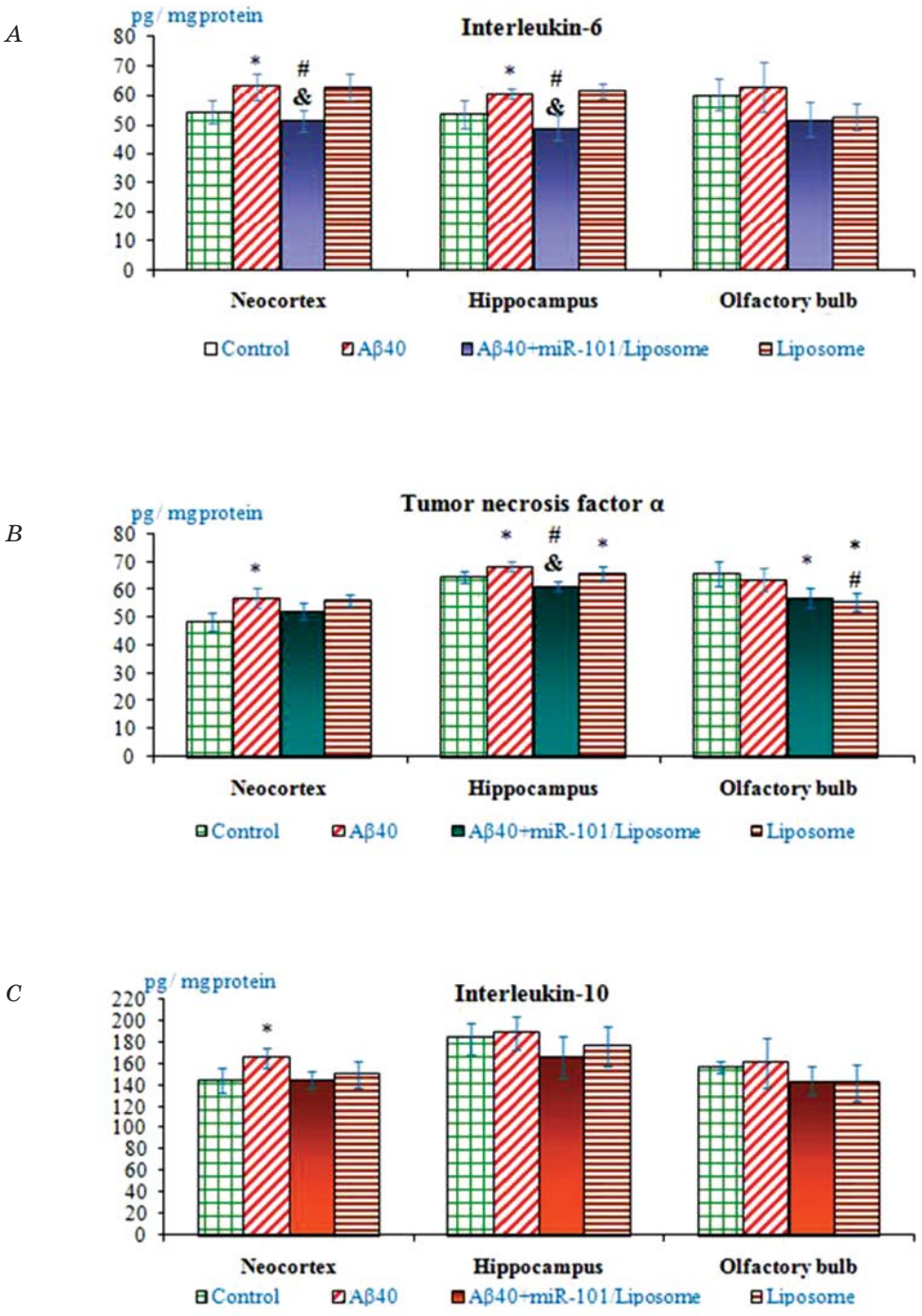

Fig. 2. **Concentration of IL-6 (*A*), TNFα (*B*) and IL-10 (*C*) in AD model rats treated nasally with miR-101 in liposomes for 10 days**

preparation should be considered to simultaneously eliminate the excess synthesis of AβPP with the formation of toxic aggregates of β-amyloid peptides and chronic neuroinflammation.

Thus, nasal therapy with miR-101 in liposomal form caused significant anti-amyloidogenic effect (normalization of Aβ42 levels in neocortex and hippocampus in rat brains with AD model).

Anti-inflammatory effect of miR-101 in liposomal form caused decrease in concentrations of inflammatory cytokines (IL-6 and TNFα in neocortex and TNFα in hippocampus of animals with experimental AD) due to decreased level of toxic endogenous Aβ42.

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АНТИАМІЛОЇДОГЕННА ДІЯ MiR-101 ЗА ЕКСПЕРИМЕНТАЛЬНОЇ ХВОРОБИ АЛЬЦГЕЙМЕРА

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Метою дослідження було визначення впливу miR-101 на рівень β-амілоїдного пептиду й активацію системи цитокінів у відділах головного мозку тварин за експериментальної моделі хвороби Альцгеймера. MiR-101 є ключовим оператором функції мРНК для протеїну попередника β-амілоїдного пептиду шляхом її деактивації і здатна пригнічувати його синтез та амілоїдогенний процесинг. Щурам-самцям пізнього зрілого віку інтрагіпокампально одноразово унілатерально вводили агрегати β-амілоїдного пептиду 40 у дозі 15 нмоль. Через 10 діб розпочинали назально вводити ліпосомальну форму miR-101 або пусті ліпосоми. Після 10 діб щоденної терапії у неокортексі, гіпокампі та нюхових цибулинах визначали рівень токсичної ендогенної форми β-амілоїдного пептиду 42 й активність цитокінової системи за показниками фактора некрозу пухлини α, інтерлейкіну-6, інтерлейкіну-10. Встановлено, що екзогенні агрегати β-амілоїдного пептиду 40 моделюють у щурів амілоїдогенний і прозапальний стан через 20 діб лише у неокортексі та гіпокампі (достовірне збільшення концентрації β-амілоїдного пептиду 42 на 36% і цитокінів на 16–18% в неокортексі та β-амілоїдного пептиду 42 — на 27% і прозапальних цитокінів фактора некрозу пухлин α, інтерлейкіну-6 — на 14% у гіпокампі), проте не в нюхових цибулинах. Десятиденний курс назальної терапії ліпосомальною miR-101 нормалізував рівень β-амілоїдного пептиду 42 та цитокінів: у неокортексі концентрація ендогенного токсичного β-амілоїдного пептиду 42 зменшилася на 33%, у гіпокампі — на 15%, а прозапальних цитокінів — на 11–20%. Таким чином, назальна терапія miR-101 у ліпосомах зумовила достовірний антиамілоїдогенний ефект у щурів з моделлю хвороби Альцгеймера, тоді як її антизапальна дія передусім сприяла зниженню концентрації β-амілоїдного пептиду 42.

Ключові слова: miR-101, β-амілоїдний пептид, амілоїдоз, хвороба Альцгеймера.

АНТИАМИЛОИДОГЕННОЕ ДЕЙСТВИЕ MiR-101 ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ БОЛЕЗНИ АЛЬЦГЕЙМЕРА

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Целью исследования было определение эффекта miR-101 на уровень β-амилоидного пептида и активацию системы цитокинов в отделах головного мозга животных с экспериментальной моделью болезни Альцгеймера. MiR-101 является ключевым оператором функции мРНК для протеина предшественника β-амилоидного пептида путем ее деактивации и способна подавлять его синтез и амилоидогенный процессинг. Крысам-самцам позднего зрелого возраста интрагиппокампально одноразово унилатерально вводили агрегаты β-амилоидного пептида 40 в дозе 15 нмоль. Через 10 суток начинали назально вводить липосомальную форму miR-101 или пустые липосомы. После 10 суток ежедневной терапии в неокортексе, гиппокампе и обонятельных луковицах определяли уровень токсической эндогенной формы β-амилоидного пептида 42 и активности цитокиновой системы по показателям фактора некроза опухоли α, интерлейкина-6, интерлейкина-10. Установлено, что экзогенные агрегаты β-амилоидного пептида 40 моделируют у крыс амилоидогенную и провоспалительную ситуацию через 20 суток только в неокортексе и гиппокампе (достоверное увеличение концентрации β-амилоидного пептида 42 на 36% и цитокинов на 16–18% в неокортексе и β-амилоидногопептида 42 — на 27% и провоспалительных цитокинов фактора некроза опухоли α, интерлейкина-6 — на 14% в гиппокампе), однако не в обонятельных луковицах. Десятидневный курс назальной терапии липосомальной miR-101 нормализовал уровень β-амилоидного пептида 42 и цитокинов: в неокортексе концентрация эндогенного токсического β-амилоидного пептида 42 уменьшилась на 33%, в гиппокампе — на 15%, а провоспалительных цитокинов — на 11–20%. Таким образом, назальная терапия miR-101 в липосомах обусловила достоверный антиамилоидогенный эффект у крыс с моделью болезни Альцгеймера, в то время как ее антивоспалительное действие прежде всего способствовало снижению концентрации β-амилоидного пептида 42.

Ключевые слова: miR-101, β-амилоидный пептид, амилоидоз, болезнь Альцгеймера.

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ILLUMINATION INFLUENCE ON *Chlorella sorokiniana* BIOMASS SYNTHESIS

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The aim of the work was to estimate the influence of illumination on the rate of *Chlorella sorokiniana* alga biomass synthesis; kinetic dependencies of the synthesis and unit rate of biomass growth at different illumination conditions. Verification of adequacy of kinetic dependencies has been implemented. The kinetic equations and values of the unit rate of biomass growth derived in studied illumination modes made it possible to calculate the time needed for the synthesis of the set amount of biomass and related growth medium consumption required for a set of cultivation conditions.

Key words: *Chlorella sorokiniana*, biomass growth, cultivation conditions, kinetic regularities.

Technologies employing vegetation biomass as a source of energy and valuable food components are currently considered as biotechnologies being implemented on a large scale. The search for productive types of biomass puts forward phototrophic organisms as a perspective source of energy and raw material for extracting phytocomponents [1–3]. When developing the processes of extracting the valuable phytocomponents from the biomass of phototrophic organisms it is important not only to consider the possibility of obtaining diverse target products from the biomass but also to organize a safe production with a minimal strain on environment [4–8].

These requirements are fairly fulfilled in the case of *Chlorella* alga biomass, which finds its vast scope of application as the source of biofuel of the third generation and raw material for obtaining valuable food components, including lipids and carotenoids.

In this regard, an urgent task is to select optimal biomass cultivation conditions that would not demand substantial energy-related and financial expenditures [9–13].

This work is focused on the studying of kinetic regularities of synthesis of alga *Chlorella sorokiniana* biomass in different illumination modes and deriving kinetic dependencies that characterize the influence of spectral structure of luminous flux on the biomass accumulation speed.

Materials and Methods

The initial concentration of *Chlorella sorokiniana* alga cells in suspension amounted to 4.2 mln cells per ml; temperature of the mixture is (23±1) °C; intensity of mixture aeration is 1.5 l per min (liters per minute); mixing mode is periodic (15 min once per day); mixing speed is 500 rps (revolutions per second).

Illumination conditions for the biomass suspension are the following:

- Mode 1. Fluorescent lamp, illumination intensity is 2200-2800 lx, T = 400 K, daylight (later referred as DFL);
- Mode 2. Infrared incandescent lamp, spectral area of the luminous flux — 3.5–5.0 μm, illumination is 14100 lx (later referred as IR);
- Mode 3. UV-radiation with a mercury gas-discharge lamp with a spectral area of the luminous flux of 320–400 nm during 3 hours on the first day of cultivation (later

referred as UV-A) and further illumination with a fluorescent lamp, illumination intensity 2200–2800 lx, T = 400 K, daylight (later as UV+DFL).

Dimensional parameters of the photobioreactor (Fig. 1) are the following: height — 380 mm, diameter — 50 mm, volume — 500 ml.

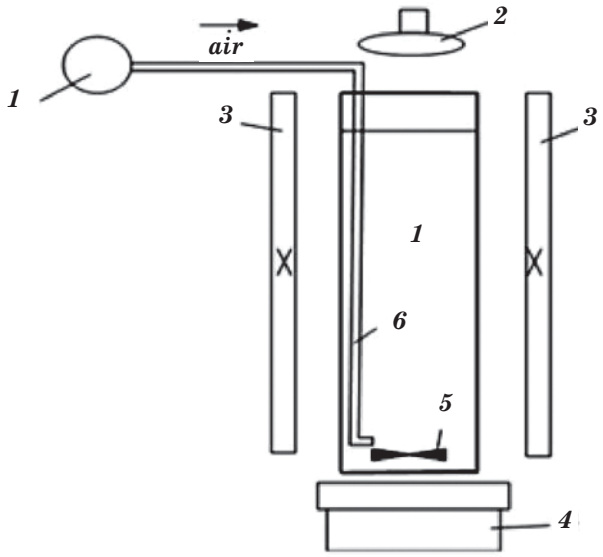

Fig. 1. **Laboratory photobioreactor for *Chloralla sorokiniana* microalga cultivation:**
1 — pump-aerator; *2* — source of radiation (IR or UV); *3* — daylight lamp; *4* — magnetic agitator; *5* — anchor of magnetic agitator; *6* — aeration tube

The concentration of the cell suspension of microalgae in the culture medium was determined using a calibration graph of dependence of the optical density of the suspension on cell concentration. The optical density of the suspension was determined at a wavelength of 750 nm. Culture medium was used as a reference solution. Sampling for spectrophotometry was carried out after mixing the suspension. The measurements were carried out in the range of optical density of 0.2–0.6, which corresponds to the linearity of the Beer-Lambert law. The cells were counted in in the hemocytometer in 5 large squares [14] in the absence of signs of cell flocculation. Dilution of the suspension was carried out at its high concentration. Dilution was taken into account when calculating the concentration.

Concentration of alga cells in the suspension was identified by measuring the optical density using a spectrophotometer with 750 nm wavelength and with a subsequent conversion to the quantity of cells in 1 ml of suspension in the hemocytometer . The values of optical density of the series of solutions were used for calibration graph construction.

Cultivation was implemented with the use of tap-water based growth medium containing a range of macro- and microelements shown in the table 1 below [15].

Regression analysis was used for mathematical processing of experimental data. Graphic dependences are presented after statistical processing using the least squares method implemented in Microsoft Excel. The degree of reliability of the experimental results is confirmed by their threefold repetition and reproducibility of data.

Results and Discussion

The derived calibration dependency is, as follows: $y = 26.729x - 0.591$ ($R'' = 0.9883$). It was used for identification of *C. sorokiniana* alga cells quantity at the wavelength $\lambda = 750$ nm.

Fig. 2 illustrates dynamics of *C. sorokiniana* cells' biomass synthesis in accordance with illumination conditions.

Analysis of experimental dependency of alga cells' biomass concentration on duration of the process demonstrated compliance between the curve manner and Verhulst logistic equation (1) [16]:

$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{K}\right), \qquad (1)$$

where x is concentration of cells' biomass, mln cells/ml at the current moment; K is population capacity, mln cells/ml; μ is unit speed of growth, days $^{-1}$.

Equation (2) is used to identify the unit speed of growth:

$$\ln X = \ln X_0 + \mu t, \qquad (2)$$

where X_0 and X are concentrations of cells' biomass, mln cells/ml at the initial and current moment, correspondingly.

It was established that the speed of alga biomass accumulation reaches its maximum at the concentration of cells of 21 mln cells/ml with a further decrease. Fig. 3 illustrates the relation between cell growth speed and their concentration.

Exponential phase of growth on the graph with vertical lnX and horizontal t axes represents a line with a tangent of inclination angle equal to the value of μ (Fig. 4).

Equation (3) is likewise appropriate to be used for calculation of the unit speed of growth:

Table 1. **The composition of the culture medium for microalgae for a laboratory reactor volume of 10 liters**

Culture medium A				
Substance	**Molecular weight, g/mol**	**Concentration in stock solution, mg/l**	**Concentration in culture medium, µg/l**	**Amount of substance per reactor, ml**
$ZnSO_4 \cdot 7H_2O$	287.53	0.1	100	10.0
$CuSO_4 \cdot 5H_2O$	249.66	0.1	10	1.0
$CoSO_4 \cdot 7H_2O$	281.06	0.1	100	10.0
$MnCl_2 \cdot 4H_2O$	197.91	0.1	500	50.0
H3BO3·WF	61.83	0.1	50	5.0
$Na2MoO_4 \cdot 2H_2O$	241.96	0.1	100	1.0
Culture medium B				
Substance	**Molecular weight, g/mol**	**Concentration in stock solution, mg/l**	**Concentration in culture medium, µg/l**	**Amount of substance per reactor, ml**
$FeCl_3 \cdot 6H_2O$	270.21	1.0	4,000	40.0
$Na_2EDTA \cdot 2H_2O$	372.24	1.0	6,000	60.0
Culture medium C				
Substance	**Molecular weight, g/mol**	**Concentration in culture medium, mg/l**	**Amount of substance per reactor, g**	
KNO_3	101.1	1,000	3,03	
KH_2PO_4	136.07	100	0,32	
$MgSO_4 \cdot 7H_2O$	246.48	240	2,4	

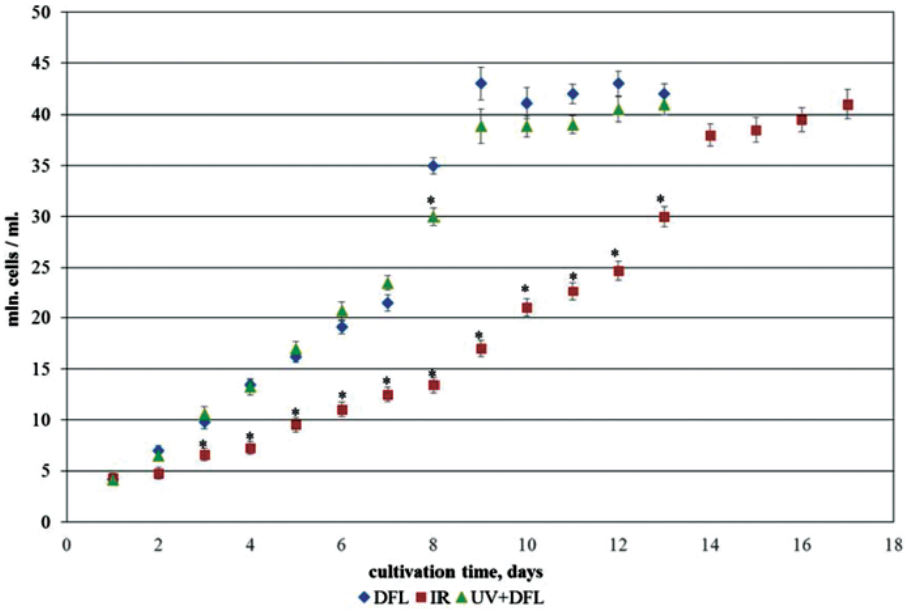

Fig. 2. **Dynamics of *C. sorokiniana* cells' biomass synthesis in different illumination modes:**
*$P < 0.05$ compared with the concentration of cells in the variant with the DFL lighting mode on the same day of cultivation

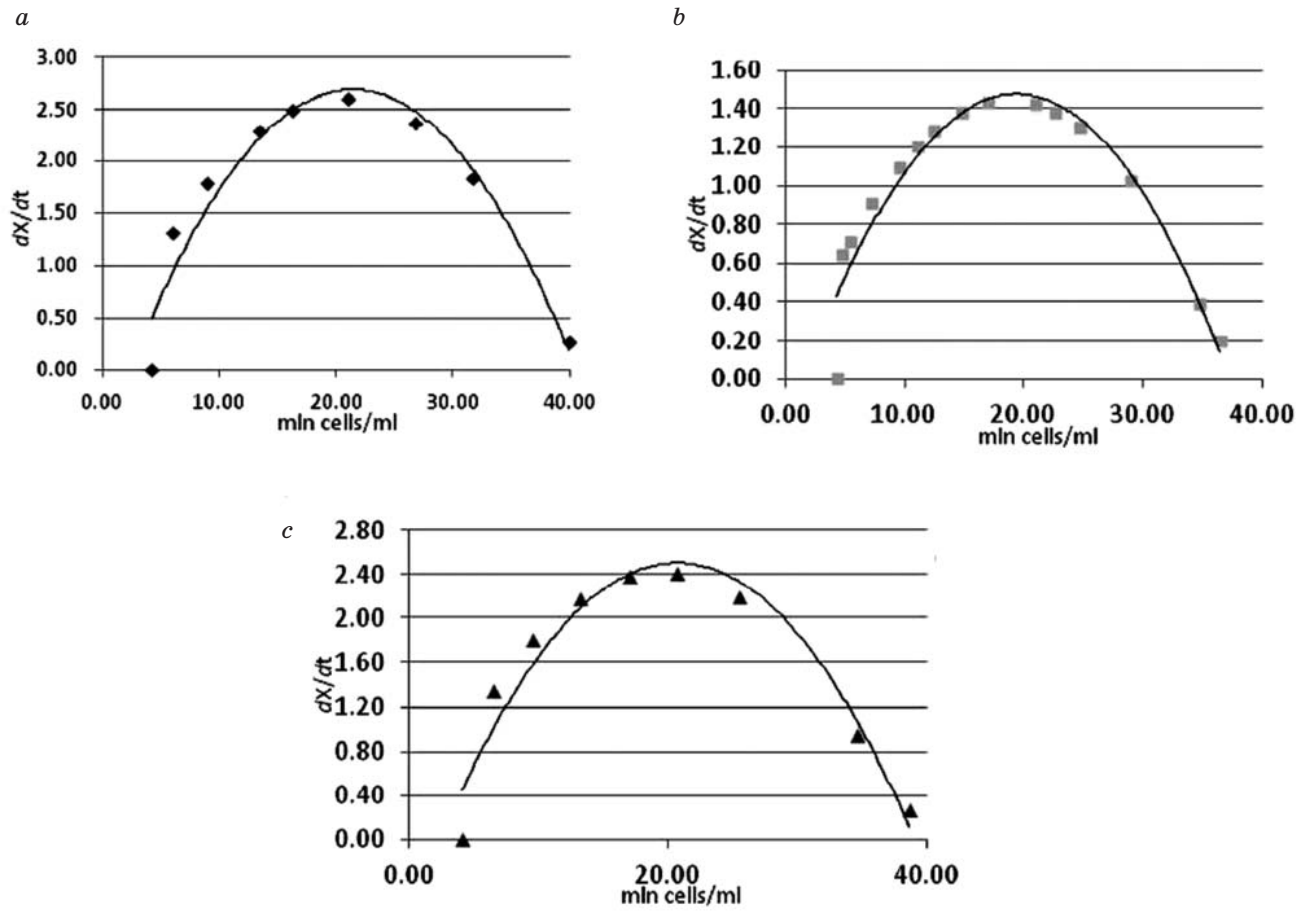

Fig. 3. **Dependency of growth speed of alga *C. sorokiniana* cells on their concentration (equation 1):**
a — DFL; *b* — IR; *c* — UV+DFL

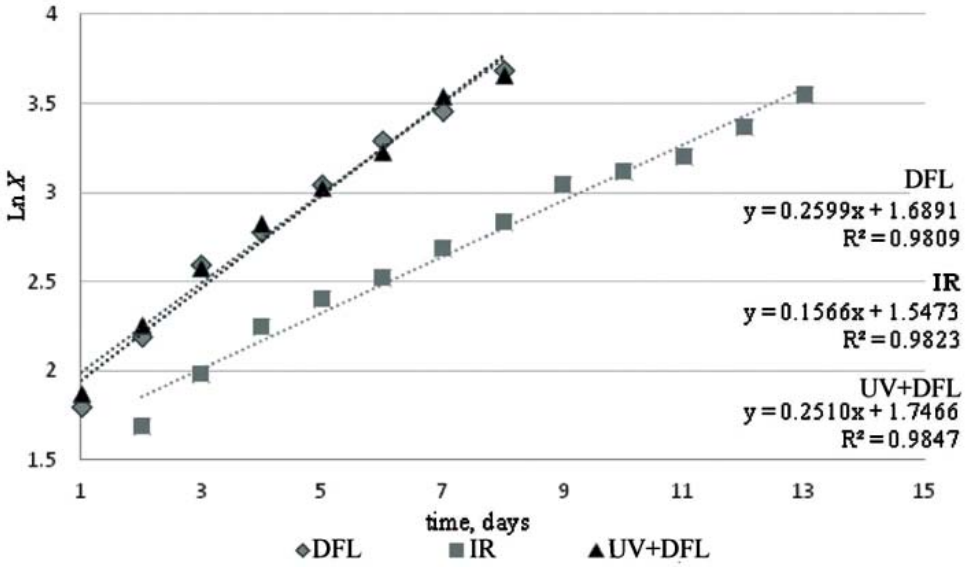

Fig. 4. **Exponential phase of growth of *C. sorokiniana* alga cells under various illumination condition modes in semi-log coordinates:**
the points represent experimental data; lines – calculated kinetic model

$$\mu = \frac{\ln(X_2/X_1)}{t_2 - t_1}. \quad (3)$$

With X_1 and X_2 as concentrations of cells' biomass, mln cells/ml at time t_1 and t_2, correspondingly.

Table 2 shows kinetic equations characterizing the speed of *C. sorokiniana* alga cells' biomass synthesis and unit speed of growth.

Thus, the maximum value of unit speed of growth of cells' biomass is reached at DFL illumination mode.

Table 2. **Kinetic equations of *C. sorokiniana* alga cells' biomass synthesis**

Cultivation conditions	Verhulst model and unit speed of growth, μ, days^{-1}
DFL, illumination intensity is 2200-2800 lx, T = 400 K (daylight)	$\frac{dx}{dt} = 0.26x\left(1 - \frac{x}{41}\right)$ μ = 0.26±0.01
IR, spectral area of luminous flux is 3.5–5.0 μm illumination intensity is 14100 lx	$\frac{dx}{dt} = 0.15x\left(1 - \frac{x}{38}\right)$ μ = 0.15±0.01
UV-radiation during 3 hours of the first day of cultivation using a mercury gas-discharge lamp with a spectral area of luminous flux 320–400 nm. DFL, illumination intensity 2200–2800 lx, T = 400 K (daylight)	$\frac{dx}{dt} = 0.25x\left(1 - \frac{x}{39}\right)$ μ = 0.25±0.01

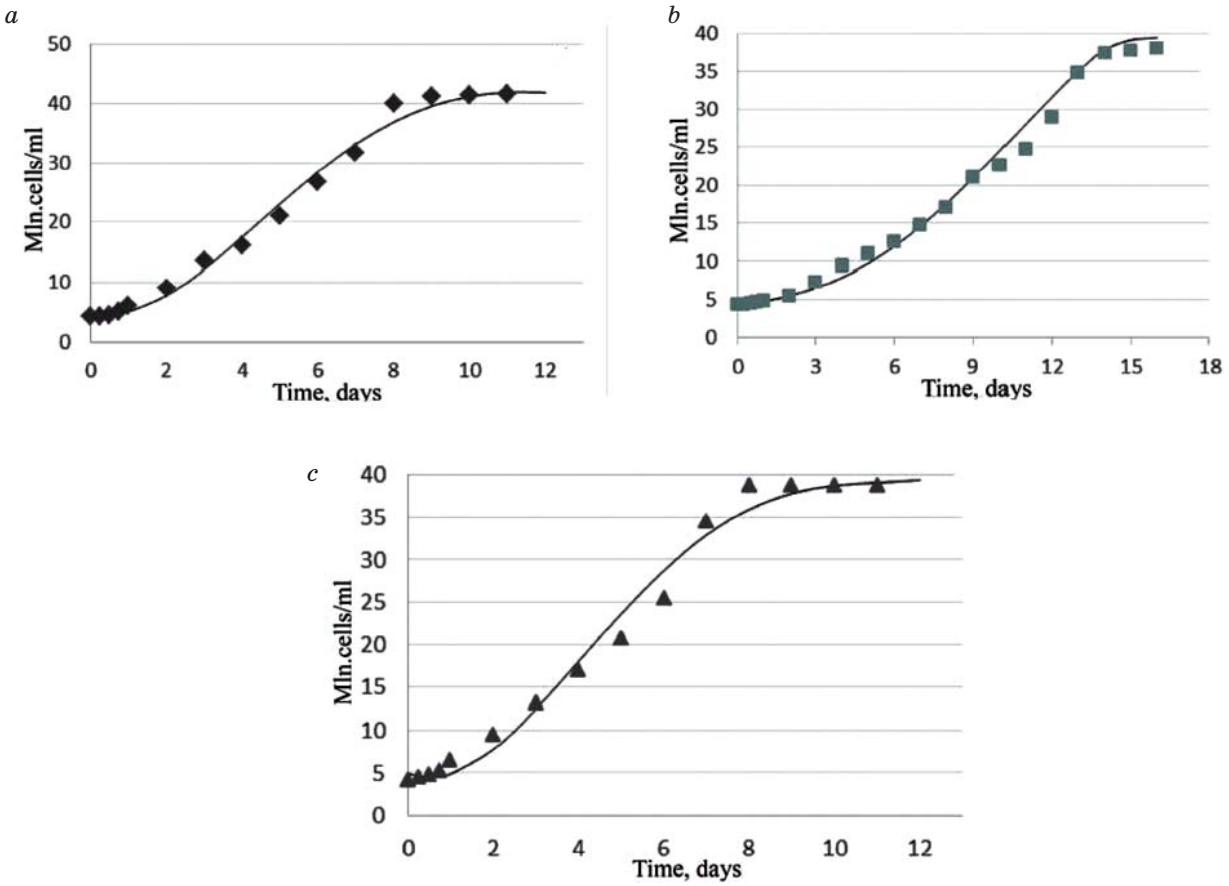

Fig. 5. **Kinetic dependencies of *C. sorokiniana* alga cells' biomass synthesis in various illumination modes: *a* — DFL; *b* — IR; *c* — UV+DFL**

Fig. 5 comprises results of adequacy verification for derived kinetic dependencies. The results of modelling of synthesis kinetics for *Chlorella* alga biomass complies with data obtained by the authors [17].

This way, the following observation has been established: the peak K population capacity in the set temperature conditions (23±1) °C amounts to 41 mln cells/ml during DFL illumination mode, 2200–2800 lx, (daylight) which is 1.7 times bigger than during IR illumination mode with radiation intensity of 14100 lux. It was identified that a short-time radiation during the initial stage of alga cells' biomass synthesis in a spectral diapason of 320–400 nm does not results in a significant increase of the synthesis speed.

The kinetic equations and values of unit speed of growth derived in the studied illumination modes provide the method of calculation of time required for the synthesis of a set amount of biomass and related growth medium consumption in the set cultivation conditions.

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ВПЛИВ ОСВІТЛЕНОСТІ НА СИНТЕЗ БІОМАСИ МІКРОВОДОРОСТЕЙ *Chlorella sorokiniana*

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Метою роботи було оцінити вплив освітленості на швидкість синтезу біомаси мікроводорості *Chlorella sorokiniana*, кінетичну залежність синтезу і питому швидкість росту біомаси за різних умов освітленості. Встановлено, що найбільше значення питомої швидкості росту клітин біомаси досягається в режимі освітленості 2200–2800 Лк; Т (К) 400 (денне світло). Проведено перевірку адекватності отриманих кінетичних залежностей. Кінетичні рівняння і значення питомої швидкості росту біомаси у вивчених режимах освітленості дадуть змогу розрахувати час, необхідний для синтезу заданої кількості біомаси і пов'язаних з нею витрат живильного середовища в заданих умовах культивування.

Ключові слова: *Chlorella sorokiniana*, зростання біомаси, умови культивування, кінетичні закономірності.

ВЛИЯНИЕ ОСВЕЩЕННОСТИ НА СИНТЕЗ БИОМАССЫ МИКРОВОДОРОСЛЕЙ *Chlorella sorokiniana*

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Целью работы было оценить влияние освещенности на скорость синтеза биомассы микроводоросли *Chlorella sorokiniana*, кинетическую зависимость синтеза и удельную скорость роста биомассы в различных условиях освещенности. Установлено, что наибольшее значение удельной скорости роста клеток биомассы достигается в режиме освещенности 2200–2800 Лк; Т(К) 400 (дневной свет). Проведена проверка адекватности полученных кинетических зависимостей. Кинетические уравнения и значения удельной скорости роста биомассы в изученных режимах освещенности позволят рассчитать время, необходимое для синтеза заданного количества биомассы и связанного с ней расхода питательной среды в заданных условиях культивирования.

Ключевые слова: *Chlorella sorokiniana*, рост биомассы, условия культивирования, кинетические закономерности.

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INFLUENCE OF SHORT-WAVELENTH ULTRAVIOLET LIGHT ON GENES EXPRESSION IN *Arabidopsis thaliana* PLANTS

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The aim of the work was to estimate the impact of the short wavelengths ultraviolet radiation (wavelength is 230 nm) on Arabidopsis thaliana. The stress response on some key flowering determination genes AP1, GI, LFY, FT, CO, and the repair gene RAD51 expression were investigated. The grown plants were applied by red (610–700 nm), violet (400–450 nm), neutral white (mixture wavelengths 380–750 nm), 20 W and high intensive white light (mixture wavelengths 380–750 nm) 40 W LED. The experimental group of plants was irradiated by short wavelengths ultraviolet on ontogenesis stage 5.1 by Boyes classification. The leaf length as growth parameter mark also was analyzed. The short wavelengths ultraviolet influence caused differences in photoperiodic pathway genes expression in plants grown under different illumination. Acceleration flowering phases under influence white intensive illumination and delay ones in case of violet and common white illumination were observed comparing with control groups. It was revealed that cryptochrome and phytochrome formation play an important role in plant development and stress resistance. It enables to understand the best way of plant cultivation in stressful condition.

Key words: illumination conditions, gene expression, short wavelengths ultraviolet, stress response.

The light illumination, nutrition and temperature strong are influence on plant development. Therefore the light illumination mode is one of the most important conditions for plants growth and development [1]. Light illumination conditions include light intensity, photoperiod and light spectrum. It is well known that the switch from vegetative growth to reproductive growth, i.e. flowering, is the critical event in a plant's life. Blooming is regulated either autonomously or by environmental factors which is regulated by the duration of the day and night periods, and spectra of the illumination of light, which is regulated by photosynthesis cell components, have been well studied. Additionally, it has become clear that stress also regulates flowering. The long wavelength ultraviolet B radiation can induce or accelerate blooming, or inhibit and delay it depend on plant species. This article focuses on the positive regulation of reproductive stage by stress. The induction or acceleration of blooming in response to stress that is known as stress-induced flowering — a new category of flowering response [2]. This research aims to clarify the concept and to summarize the full range of its characteristics of stress-induced flowering from a predominately physiological perspective. There are relevant quantities to flowering time gene regulatory network of plants grow and develop [3].

Nowadays genetic mechanisms of flowering regulation of Arabidopsis are known [4]. Flowering time regulation has been widely studied on the plant model species *Arabidopsis thaliana*. There are three main pathways which include the photoperiodic, vernalisation and autonomous branches. The photoperiodic pathway is the most important for arabidopsis because it is belongs on long day plant. Flowering time regulate by circadian clock and depend of day length [5]. The circadian clock genes are activated by the light spectrum. The light spectrum activates different photoreceptors in plant leaves. The impact of

light spectrum on plants development is studied during long time [6]. But today this environmental research problem has been relevant. The violet, blue, and red lights are important for plant growing and development [7] and they include the visible light spectrum within 380–730 nm. Different light spectrum excited signal transduction state and caused photomorphogenic changes. It also impacts on chlorophyll content in cells, dry mass accumulation and leaf surface square creating [8]. The visible light is absorbed mainly by chlorophyll a, b and carotenoids [1]. Blue (460 nm), orange (630 nm) and red light (660 nm) are playing a great role in photosynthesis [9], whereas violet (405 nm), and far-red influence to germination, vegetative growth, budding, and flowering processes [10, 1]. In experimental researches blue and red lights were necessary for investigation plant photosynthesis mechanisms, but violet and far-red usually were applied in secondary metabolite synthesis and photomorphogenesis studies [11].

Different spectrum is absorbed by several photoreceptors in leaves [7, 9]. Therefore several classes of photoreceptors have been described: phytochromes (PHYA-PHYE in Arabidopsis) generally absorb red and far-red light, but blue light is perceived by cryptochromes (CRY1 and CRY2), phototropins (Phot. 1 and Phot. 2), and Zeitlupes (ZKL, FKF1 and LKP2) [1].

In Arabidopsis the phytochromes involve in photoperiodic pathways [12, 13]. They interact on endogenous oscillators and activate expression of two floral genes *CONSTANTS* (*CO*) and *FLOWERING LOCUS T* (*FT*) in leaves [10]. The cryptochrome photoreceptors are present in organisms throughout the plant kingdom [7]. They enable absorbed the red light in plants. The red light in opposite of could down-regulate the gene *FT* expression and delay flowering [10].

A long wavelength ultraviolet (UV) radiation is a highly effective biological stress factor for plants. The UV-rays are similar to ionizing radiation regarding of biological action living cells [1]. Impact on plant UV-radiation is interesting to research for a time [14]. It is relevant to study during the last years too. The ozone layer gets thinner in combine with global warming. Therefore as a result it increases of atmospheric CO_2 and UV radiation [15, 16]. The investigation of the plant resistance to ambient factors now continues to be relevant.

UV light includes a long wavelength UV (wavelengths 320–400 nm), UVB (280–320 nm) and short wavelength UV (wavelengths below 280 nm) (Sastry at al. 2000). A long wavelength UV comprises more than 95% of the solar UV radiation. Most of UVB and all of UVC are removed by the ozone layer. The shorter wavelengths are less present in incident sunlight [17]. But if the ozone layer will decrease the level of short wavelength UV irradiation opposite will increase. In the environmental the short wavelength UV will become the most active and drastic stress factor.

The recent researches have shown, short- and medium-wavelength of UV light cause photo lesions in DNA conformation. The high doses of UV increase DNA dissociation and structural disintegration [18].

A long wavelength produce the DNA thionucleotides indirectly. Also UV induces DNA photo damage by generating reactive oxygen species. Proteins targeted for oxidation damage include DNA repair factors [16]. UVB radiation affects leaf growth in a wide range of some species without causing any other visible stress symptoms [19].

Increasing environmental UV radiation can delay flowering and decrease harvest production in many plants species [20].

The arm of our study was to investigate the illumination impact combining with the UV-radiation on the expression of *APETALA 1 (AP1)*, *GIGANTIA (GI)*, *FT*, *CO*, *RAD51* and *PCNA2*.

Materials and Methods

The plants of *Arabidopsis thaliana* (*ecotype Col*) were used in experiments. *A. thaliana* is a classical model object in molecular biology and genetics. This species is useful in lab and content a small genome [21]. Genetic mechanism of blooming term and growth phases' determination of Arabidopsis is widely studied [22]. We used light illumination with violet, red and white spectrum to growth plants. The plants grown were applied red (610–700 nm), violet (400–450 nm), neutral white (mixture wavelengths 380–750 nm), 20 W and high intensive white light (mixture wavelengths 380–750 nm) 40 W LED to grow plants. We irradiated plants by short wavelength UV. During vegetation growth and develop the irradiated plant with above-mentioned factors the length leaves was measured within twice per week.

The short wavelength UV irradiation

The short wavelength UV irradiation was done by 254 nm light generator with 30 W power. Each control and experimental group of plants

was implemented. Experimental groups were irradiated by short wavelength UV in shooting stage 5.9 [22]. We stressed plants with short wavelength UV irradiation in three different term modes 1, 2 and 5 minutes of UV exposure in the same distance from the generator.

Molecular studies

The RNA extraction isolated from leaves at 6.1 development stage at the starting of the flowering phase in according to Boyes (2001) classification. The RNA was isolated of each experimental and control groups after one week from UV irradiation. The total RNA was extracted by traditional phenol-chloroform method [23]. Quality of extraction RNA was checked with electrophoresis in 2% agarose gels. Concentration of extracted RNA was measured by spectrophotometer. The reverse transcription reaction was performed in order to obtain cDNA. In experiments, the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) kit was used.

In order to evaluate the genetic alterations caused by UV exposure we determined changes in the photoperiod pathway gene expression levels. In our experiment, we measured the expression of researched genes *AP1, GI, FT, CO, RAD51* and *PCNA2*. The qPCR equipment LightCycler® Nano Instrument by Roshe Diagnostics, Switzerland was used. Different programs and protocols were tested to set up real time qPCR conditions. We used *Thermo scientific SYBR Green master mix*. The quantitative qPCR primers on genomic DNA of *Arabidopsis* resulting in selection the working primers were tested too. An *ACTIN PROTEIN 2* (*ACT2*) and *PCNA2* on base preliminary experiments were chosen as a reference gene in our investigation. The standardization of real-time PCR primers was done in order to preliminary determines the efficiency of each primer.

Data analysis

Statistical analysis of vegetation data [24] was done by the help of StatPlus software. Relative expression of the genes statistically analyzed with double normalization on the base of reference gene and control group by the REST software [25].

Results and Discussion

Analysis of the plant's growth and vegetation development showed differences in grown with different light illumination [24].

Arabidopsis seedlings were started at 5.1 stage according to Boyes (2001) classification at 24 day-old age (Table 1) under the intensive white illumination at 24 °C temperature. The plants transferred into 6.3 phase (flowering) on 27 day-olds. The seedlings transferred into 8th phase on 31 day-olds and 9 phase (harvesting) on 36 days. The seedlings started 5.1 stage on 27 day-olds, the 6.1 phase started on 31 days, the 8 phase started on 36 days under red light at 24 °C. The plant seedlings started 5.9 stage on 31 day-olds and the 6.3 phase at 36 days-old under common white and violet light at 24 °C.

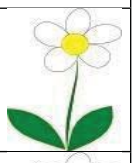
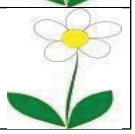
One-way Analysis of Variance (ANOVA) showed the significant differences between leaf length of different light spectrum growing plants (between groups SS = 1.04, within groups SS = 458.11, $F_{emp} > F_{crit}$, $P < 0.05$). See details in Fig. 1.

The leaf length of red light growing plants is different than common white light group ($t_{emp} > t_{crit}$, $P < 0.05$), as well as high intensive white light ($t_{emp} > t_{crit}$, $P < 0.05$) and violet light ($t_{emp} > t_{crit}$, $P < 0.05$) growing plants. The leaf length of the common white light growing plants is slightly different than white intensive light ($t_{emp} > t_{crit}$, $P < 0.05$) and violet light ($t_{emp} > t_{crit}$, $P < 0.05$) growing plants. The amount leaf length of the intensive white light growing plants is slightly higher than violet light growing plants ($t_{emp} > t_{crit}$, $P < 0.05$).

Comparative analysis of key photoperiodic pathway genes expression showed some differences between control and short wavelength UV irradiated groups ($P < 0,05$). The common white light illuminated plant group shown the changes in expression levels of key flowering determination genes after short wavelength UV treatment (Table 2). For example, **a) plants irradiated during 1 min by short wavelength UV**: The genes *RAD51* and *GI* are up-regulated in the experimental group in compare control plants by a mean factor of 2.936 and 1.494, comparatively. But the gene *CO* which take part in the circadian cycle is down-regulated for experimental plants with a mean factor of 0.648; **b) 3 min short wavelength UV**: The genes *RAD51* and *AP1* are up-regulated in an irradiated group of plants by a mean factor of 5.519 and of 31.685. The genes *CO* and *GI* are down-regulated in treatment group by a mean factor of 0.49 and 0.561; **c) 5 min UVC**: the genes *RAD51, AP1, CO* and *FT* are up-regulated in the experimental groups in compare of the control group by a mean factor of 46.869, 87.018, 137.253 and 6.15, comparatively.

We observed other features for activity some flowering, reparation, and proliferation genes of the violet illumination cultivated plants after that they were influenced UV-ray during several modes (Table 3). **a) 1 min UVC:**

Table 1. **Evaluation and demonstration the phenology phases of cultivated plants in different light conditions in depend of aged**

Light	Age, days	Phase		Age, days,	Phase		Age, days	Phase		Age, days	Phase	
Red	24	3.8		27	5.1		31	6.1		36	8	
White intensive		5.1			6.3			8			9	
Violet		3.6			3.8			5.9			6.3	
White common		3.6			3.8			5.9			6.3	

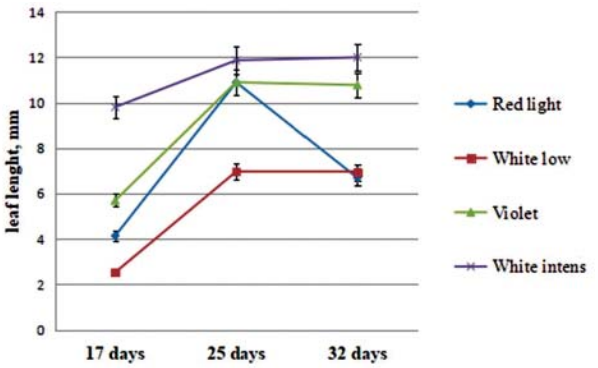


Fig. 1. **Dynamic grown the leaves length (mm) plants in depend of vegetation terms (days)**

AP1, *GI* and *FT* expression are down-regulated in experimental groups in compare of control group plants by a mean factor of 0.029, 0.444 and 0.074; **b) 3 min UVC:** *AP1* is up-regulated in experimental group plants by a mean factor of 4.966, **c) 5 min UVC:** *FT* is up-regulated in experimental group (in comparison to control group) by a mean factor of 1.748. But *CO* is down-regulated in the experimental group by a mean factor of 0.401.

The expression of key photoperiodic pathway genes after short wavelength UV in red light growing plants was described in Table 4. **a) 1 min UVC:** *AP1* is up-regulated in experimental group by a mean factor of 2.782 and genes *GI*, *CO*, *FT*, and *RAD51* are down-regulated in the experimental groups by mean factors of 0.171, 0.134, 0.025 and 0.450, comparatively; **b) 3 min UVC:** *AP1* and *FT* are down-regulated in experimental groups by a mean factor of 0.586 and 0.445 in in comparison to the control group. The gene *CO* is up-regulated by a mean factor of 2.644; **c) 5 min UVC:** *FT* and *RAD51* are up-regulated in the experimental group by a mean factor of 5.214 and 1.914, comparatively. The similar effect we observed for violet illumination plus UV-radiation.

The phenology data revealed about necessary of the full spectrum of solar light to normal activation of circadian clock genes. It is known that PHYA-PHYE accepts the visible red light. We suggest that phytochromes involve in flowering time regulation in the non-full spectrum of light. CRY1 i CRY2 accept the blue light [26]. However, decreasing of red light in illumination caused blooming time delay to compare white light growing plants. It also was explained in recent studies [1].

Our results shown the trend of flowering genes expression depends on red, violet and white light spectrum. We observed that *AP1*, *GI*, *CO* and *RAD51* increase their activity after stress. The response of *CO* and *FT* genes to stress factor did not observed.

We believe that changes of genes activity depend on light illumination conditions. However increasing of *RAD51* gene expression has been shown the activity of reparation processes in plant cells [27]. The expression levels of *RAD51* have differences in samples group that were grown in white, violet and red illumination. The differences can cause by cryptochromes or phytochromes.

In addition, we did not show the significant changes of photoperiodic pathway genes

Table 2. **Relative expression analyzes results of plants cultivated under common white light and treatment by 1, 3 and 5 min of UVC treatment**

Gene	Expression	Std. Error	95% C.I.	P(H1)	Result ($P < 0.05$)
1 min					
*PCNA2***	1				
RAD51	2.936	1.939–4.492	1.490–5.841	0	UP*
AP1	12.255	4.570–33.291	2.358–70.686	0.062	UP
CO	0.648	0.586–0.716	0.577–0.728	0	DOWN*
GI	1.494	1.273–1.757	1.161–1.925	0	UP*
FT	1.233	0.665–2.286	0.424–3.793	0.667	UP
3 min					
*PCNA2***	1				
RAD51	5.519	3.430–8.431	3.118–9.986	0.049	UP
AP1	31.685	16.502–73.653	9.321–108.277	0	UP
CO	0.49	0.383–0.564	0.377–0.572	0.034	DOWN*
Gi	0.561	0.473–0.686	0.406–0.764	0.022	DOWN*
FT	0.998	0.625–1.933	0.426–2.255	0.918	DOWN
5 min					
*PCNA2***	1				
RAD51	46.869	30.683–72.202	24.564–90.040	0	UP
AP1	87.018	39.391–192.333	37.248–203.375	0	UP
CO	137.253	110.717–179.260	109.005–182.074	0	UP
GI	1.678	0.351–8.065	0.288–9.822	0.611	UP
FT	6.15	3.322–11.095	3.078–12.423	0.026	UP*

* Statistically significant

**Reference gene = 1

Hereinafter: the expression level values compare with reference gene expression =1.The expression level values are calculated in base of row quantitative PCR data of control and experimental groups. The methodology shown the differences between control and treated groups as control — 1 min UV, control — 3 min UV, control — 5 min UV. It is not necessary to present the row control and experimental data. The hypothesis test P(H1) represents the probability of the difference between the sample and control groups.

expression after short wavelength UV in plants which cultivated in violet light, at 24 °C. We guess that the red and violet light growing plants have different expression because of the photoreceptors involved in short wavelength UV response. For example, the same short wavelength UV-doses cause different level of *AP1* expression in different groups (Fig. 2–4). This phenomenon could be explained by the involvement of cryptochromes in flowering regulation.

As known *RAD51* gene involved in repair processes after UV and ionizing radiation. Red light growing causes to increase *RAD51* activity (Table 4). At the same time increasing *RAD51* activity in violet and white light growing plants was observed only on 5 min short wavelength UV. It can be related to the light wavelength of illumination. We believe that shorter wavelength can suppress repair processes in plant cells.

The previous data showed that short wavelength UV influences on plant biomass formation, photosynthesis and leaf size of agriculture plants [14]. Our results also demonstrated that short wavelength UV also drastic influences on repair and bloom processes. Other authors in the recent studies report similar data. They have shown that different light conditions effect on stress resistance in plants [28].

However, the question of relation photoreceptors of the plant due to photoperiodic pathway genes expression is

Table 3. **Relative expression analyzes results of plants cultivated under violet light and treatment by 1, 3 and 5 min of UVC treatment**

Gene	Expression	Std. Error	95% C.I.	P(H1)	Result ($P < 0.05$)
1 min					
ACT2**	2.286				
PCNA2**	0.438				
RAD51	4.57	1.080–19.487	0.872–24.120	0.174	UP*
CO	0.264	0.213–0.326	0.206–0.338	0.075	DOWN*
GI	0.444	0.343–0.577	0.299–0.663	0	DOWN
FT	0.074	0.047–0.117	0.044–0.125	0.041	DOWN
AP1	0.029	0.020–0.038	0.018–0.041	0	DOWN
3 min					
ACT2**	0.242				
PCNA2**	4.137				
RAD51	4.455	1.017–19.538	0.929–21.385	0.268	UP
CO	0.467	0.402–0.543	0.388–0.562	0.077	DOWN
GI	1.236	1.045–1.462	0.930–1.647	0.183	UP
FT	0.949	0.755–1.192	0.707–1.274	0.772	DOWN
AP1	4.966	3.155–7.823	2.939–8.397	0.037	UP*
5 min					
ACT2**	0.214				
PCNA2**	4.672				
RAD51	4.462	1.030–19.387	0.904–22.069	0.283	UP
CO	0.401	0.340–0.471	0.329–0.488	0.042	DOWN*
GI	0.792	0.700–0.896	0.640–0.982	0.135	DOWN
FT	1.748	1.579–1.936	1.480–2.065	0.022	UP*
AP1	4.18	3.893–4.489	3.687–4.743	0.057	UP

* Statistically significant; **Reference gene.

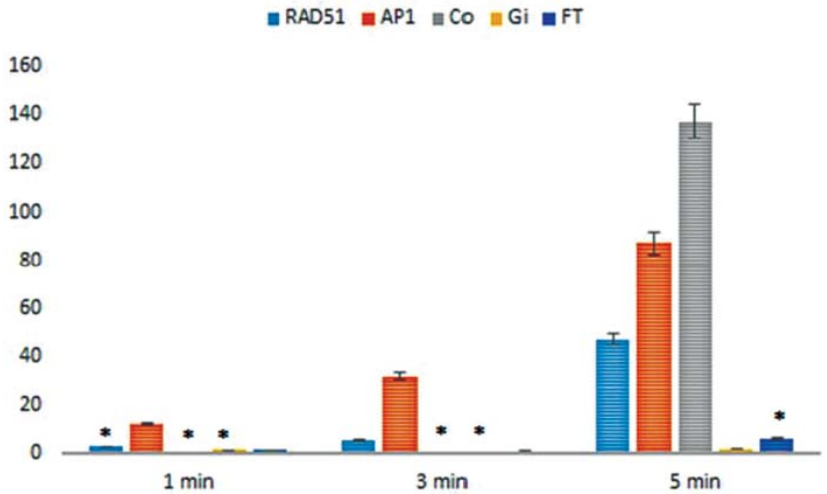


Fig. 2. **Dynamic of flowering genes expression of plants grown under illumination common white light in depend of UV-treatment term:**

Hereinafter: the expression level values compare with reference gene expression =1.The expression level values are calculated in base of row quantitative PCR data of control and experimental groups. The methodology shown the differences between control and treated groups as control — 1 min UV, control — 3 min UV, control — 5 min UV. It is not necessary to present the row control and experimental data. The data are comparing with control group. * Statistically significant

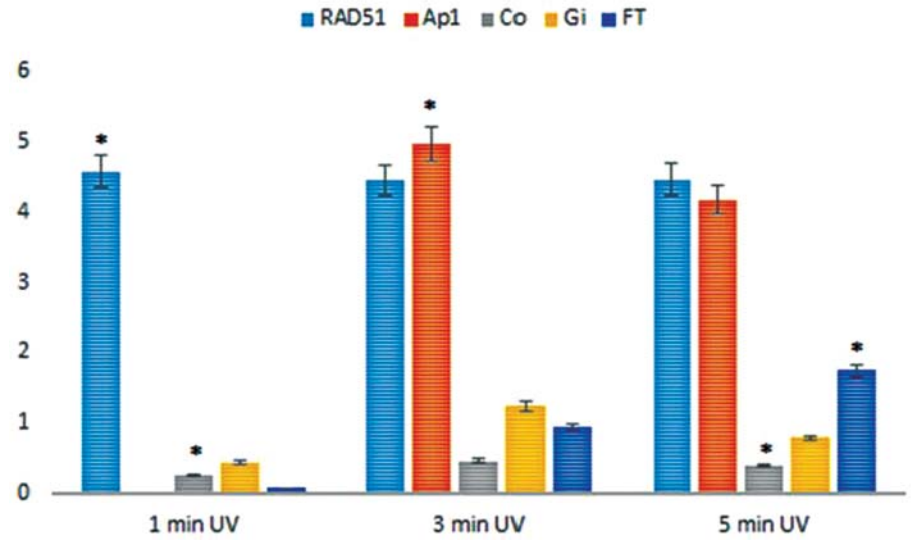


Fig. 3 . **Dynamic of flowering genes expression of plants grown under violet light in depend of UV-treatment term**

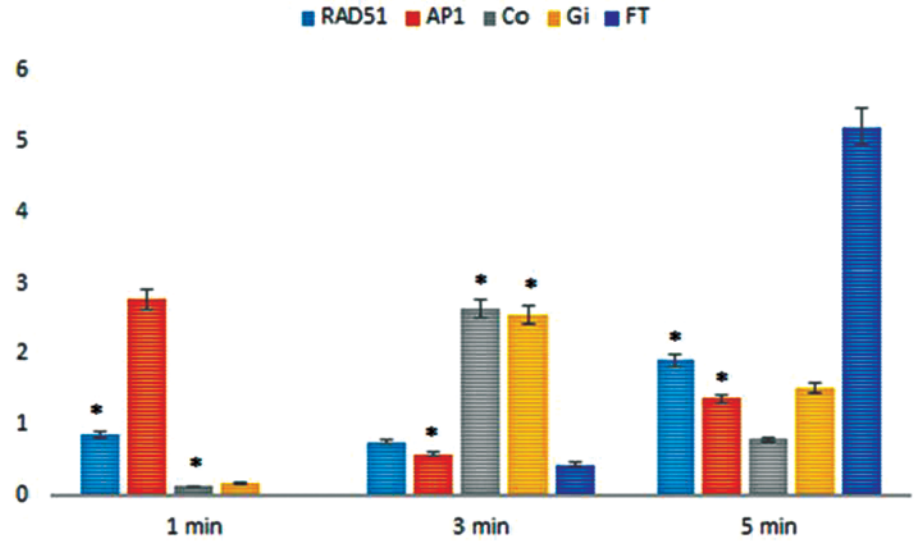


Fig. 4. **Dynamic of flowering genes expression of plants grown under red light in depend of UV-treatment term**

relevant. This phenomenon needs more dip studies of transcription factors, which are included in flowering regulation. The question of cultivation conditions impact on plant stress response is interesting for science and agriculture. The drought, salinity, oxidation stress are interested in scientists.

These researches will help to produce stress resistant sorts of agriculture plants, which can be planted in climate change conditions or unfavorable places of the planet [29].

Thus, our experimental data revealed that *Arabidopsis thaliana* plant cultivation under illumination of violet, red and orange spectra of light could drastically influence on photoperiodic pathway genes expression.

Post-irradiated with short wavelength UV-irradiation of plants grown under red light illumination caused downregulation expression of genes related to circadian clock *CO* and *GI* and repair genes *RAD51*.

Our data demonstrate that the plant cryptochrome and phytochrome formation and development condition play an important role in UV-radiation resistant and on the response of main photoperiodic pathway and repair genes expression.

Table 4. **Relative expression analyzes results of plants cultivated in red light and treatment by 1, 3 and 5 min of UVC treatment**

Gene	Expression	Std. Error	95% C.I.	P(H1)	Result ($P < 0.05$)
1 min					
ACT2**	1.929				
PCNA2**	0.518				
RAD51	0.868	0.813–0.924	0.800–0.967	0.049	DOWN*
CO	2.782	2.419–3.309	2.329–3.436	0	UP*
GI	0.134	0.104–0.166	0.101–0.179	0.034	DOWN
FT	0.171	0.157–0.191	0.151–0.194	0	DOWN
AP1	0.025	0.024–0.026	0.023–0.027	0	DOWN
3 min					
ACT2**	0.76				
PCNA2**	1.316				
RAD51	0.759	0.629–0.876	0.591–0.993	0.153	DOWN
CO	0.586	0.484–0.686	0.452–0.797	0	DOWN*
GI	2.644	2.486–2.890	2.434–2.955	0	UP*
FT	2.552	2.386–2.780	2.236–2.897	0.097	DOWN
AP1	0.445	0.372–0.533	0.347–0.572	0	DOWN*
5 min					
ACT2**	0.76				
PCNA2**	1.316				
RAD51	1.914	1.755–2.041	1.713–2.183	0	UP*
CO	1.368	0.915–1.978	0.783–2.432	0.195	UP
GI	0.794	0.547–1.117	0.531–1.203	0.345	DOWN
FT	1.518	1.334–1.760	1.283–1.786	0.054	UP
AP1	5.214	3.962–6.861	3.824–7.108	0	UP*

* Statistically significant; **Reference gene.

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ВПЛИВ КОРОТКОХВИЛЬОВОГО УЛЬТРАФІОЛЕТОВОГО ВИПРОМІНЮВАННЯ НА ЕКСПРЕСІЮ ГЕНІВ У *Arabidopsis thaliana*

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Метою дослідження було вивчення впливу опромінення короткохвильовим ультрафіолетом (довжина хвилі 230 нм) рослин *Arabidopsis thaliana*. Досліджено стресову реакцію на деякі ключові гени фотоперіодичного механізму детермінації цвітіння: AP1, GI, FT, CO та репарації RAD51. Для вирощування рослин застосовували червоне (довжина хвилі 610–750 нм), фіолетове (довжина хвилі 400–450 нм), нейтральне видиме (змішані хвилі з довжиною 380–750 нм) освітлення з потужністю LED ламп 20 Вт та 40 Вт.

Після цього експериментальну групу рослин опромінювали короткохвильовим ультрафіолетом (довжина хвилі 230 нм) на стадії онтогенезу 5.1 за класифікацією Бойса (2001). Як маркер вегетаційного росту було проаналізовано довжину листа. Виявлено, що опромінення короткохвильовим ультрафіолетом спричинювало відмінності у профілях експресії генів фотоперіодичного механізму регуляції у рослин, вирощених за різного освітлення. Спостерігалося прискорення фази цвітіння за вирощування в інтенсивному білому освітленні та запізнення за фіолетового та помірного білого освітлення порівняно з контрольною групою. Таким чином було виявлено, що криптохроми і фітохроми відіграють важливу роль у формуванні стресостійкості рослин. Дані дослідження є важливими для біотехнології та сільського господарства і дадуть змогу визначити найбільш оптимальні способи вирощування рослин в умовах стресу.

Ключові слова: умови освітлення, експресія генів, ультрафіолет короткохвильового діапазону, відповідь на стрес.

ВЛИЯНИЕ КОРОТКОВОЛНОВОГО УЛЬТРАФИОЛЕТОВОГО ИЗЛУЧЕНИЯ НА ЭКСПРЕССИЮ ГЕНОВ У Arabidopsis thaliana

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Целью исследования было изучение влияния облучения коротковолновым ультрафиолетом (длина волны 230 нм) растений *Arabidopsis thaliana*. Исследована стрессовая реакция на некоторые ключевые гены фотопериодического механизма: A*P1, GI, FT, CO* и *RAD*51.

Для выращивания растений применяли красный (длина волны 610–750 нм), фиолетовый (длина волны 400–450 нм), нейтральный белый (смешаные волны с длиной 380–750 нм) с интенсивностью LED-ламп 20 Вт и 40 Вт. Экспериментальную группу растений облучали коротковолновым ультрафиолетом (длина волны 230 нм) на стадии онтогенеза 5.1 по классификации Бойса (2001). В качестве маркера вегетационного роста также была проанализирована длина листа. Облучение коротковолновым ультрафиолетом вызывало различия в профилях экспрессии генов фотопериодического механизма регуляции цветения у растений, выращенных при разном освещении. Наблюдалась раннее начало фазы цветения при выращивании в интенсивном белом освещении и позднее при фиолетовом и обычном белом освещении по сравнению с контрольной группой. Таким образом было выявлено, что криптохромы и фитохромы играют важную роль в формировании стрессоустойчивости растений. Данные исследования важны для биотехнологии и сельского хозяйства, что поможет определить наиболее оптимальные способы выращивания растений в условиях стресса.

Ключевые слова: условия освещения, экспрессия генов, коротковолновой ультрафиолет, ответ на стресс.

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EXTRACTS OF EDIBLE PLANTS STIMULATORS FOR BENEFICIAL MICROORGANISMS

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The aim of the work is to determine the content of biologically active compounds in berries and fruits collected in ecologically clean areas, to find out their potential usage for the creation of targeted pharmabiotics, and the ability of such extracts to stimulate the growth of probiotic lactic acid bacterial strains and other representatives of commensal intestinal microbiota.

The content of biologically active compounds was determined by thin-layer chromatography. The plant-specific effect of methanol extracts from berries and fruits on the selected strains *L. acidophilus, L. catenaformis, L. casei, L. fermentum, E. coli* 058, *E. faecalis* (gut commensals), *B. subtilis* 090 (component of biopreparation), which are perspective for the creation of modern pharmabiotics, according to the results of its cultivation in the specified extracts had been shown.

It was also found that tested berry extracts were characterized by a higher content of polyphenols, compared to anthocyanins. Alycha' extract mainly inhibited the growth of most of studied bacterial strains, except *B. subtilis* 090. Extracts of red currant, sweet cherry, and jostaberry stimulated the growth of *L. catenaformis*, while extracts of sweet cherry and jostaberry, in addition to that of the above-mentioned lactobacilli strains, also stimulated the growth of *L. casei* and *L. fermentum*. Blueberry and plum extracts activated the growth of all lactobacilli strains. The ability to stimulate the growth of *B. subtilis* 090 was noted only for the extracts of alycha, jostaberry, and plum.

Key words: berries, fruits, flavanoids, anthocyanins, lactobacteria, pharmabiotics.

It is known that peptides, enzymes, and amino acids are used as separate medications and as prebiotic components in the composition of synbiotics [1]. Today the most frequently used plant extracts are characterized by various preventive and medicinal effects [2]. The objective of our paper was the screening (selection) of berries grown in the mountainous regions of Zakarpattia, which would be characterized by high biologically active compounds (BAC) contents and the ability to stimulate the growth of lactic bacteria strains of different origin, and thus could be perspective components for modern pharmabiotics.

Natural biologically active compounds of antimicrobial action include plant antibiotics, phytoncides, essential oils, balsams, resins, tannins, organic acids, alkaloids, and glycosides [3]. Usually, the BAC content varies depending on the plant species and the region of cultivation [4].

Therefore, our main task was to select berries and fruits, the extracts of which were rich in BAC and had antibacterial properties against opportunistic pathogens, as was shown earlier [5, 6], and at the same time stimulated the growth of beneficial microorganisms, which was the objective of this paper.

Agroclimatic conditions of Zakarpattia Region are favorable for the cultivation of berries rich in BAC. Gardening is well-developed in the region, which, in turn, provides the opportunity to obtain a large number of raw materials for BAC extraction, namely from alycha, cherry, jostaberry, red and black currant. Fruits of these plants are sources of antioxidants, including anthocyanins and polyphenols, which possess antibacterial properties [7].

The quantitative composition of the studied berries is underinvestigated. Determination of the total content of polyphenolic compounds in

plant raw material allows pre-evaluating the prospects of their use as a source of BAC for future use in the production of pharmabiotics.

So, the objective of our study was to determine the BAC content in the berries and fruits selected for the study and to find out their potential ability to stimulate the growth of selected microorganisms.

Materials and Methods

Methanol extraction

We determined the quantitative content of polyphenols and anthocyanins in methanol extracts by thin-layer chromatography (TLCH) [8].

We prepared the extract from 50 g of each kind of berries and used 100 ml of 80% methanol for extraction. In our subsequent experiments, we used the methanol liquid extract, not containing precipitate [9].

Determination of antibacterial properties of fruit extracts

Using vacuum evaporation, we obtained methanol-free extracts of the following berries: *Ribes rubrum* (red currant), *Prunus avium* (sweet cherry), *Prunus* × *domestica* (plum), *Ribes* × *nidigrolaria* (jostaberry), *Vaccinium myrtillus* (blueberry), *Ribes nigrum* (black currant), and *Prunus cerasifera* (alycha).

We studied the ability of the selected berries and fruits to stimulate *L. acidophilus, L. catenaformis, L. casei, L. fermentum* (LAB), *E. coli 058, E. faecalis B. subtilis 090* by culturing them in extracts obtained from these berries and fruits [10]. The initial concentration of the selected LAB strains, *E. coli 058, E. faecalis B. subtilis 090* was 3×10^8 CFU/ml (N0). The obtained data was expressed as the logarithm of the number of surviving bacteria (Nt) to the initial number of bacteria (N0) – lg (Nt/N0) for a certain culturing time (14 h and 24 h).

LAB strains used in the study were isolated by us from various sources, in particular, *L. acidophilus* — from the intestine of a healthy person, *L. catenaformis, L. casei, L. fermentum* — from fermented food [11]; they were identified (MALDI with subsequent sequence analysis) and registered with the Depositary of High-Value Cultures of the Microorganism Collection of IMVNASU as promising strains for the development of modern pharmabiotics. Additionally, the effects of berry and fruit extracts on the representatives of the commensal intestinal microbiota were studied: *E. coli 058, E. faecalis*, and *B. subtilis 090*– an integral component of the biological preparation [12].

Statistical processing of the results of experiments was carried out using a software OriginLab 2017 version 94E.

Results and Discussion

Alycha is a relatively unpretentious, frost- and drought-resistant plant [13]. Polyphenols play an important role among the BAC that the alycha fruits are rich in. Plant phenols represent a large group of substances classified as “secondary metabolites”, being of particular interest due to their antioxidant properties. Phenolic compounds are also characterized as antimicrobial, antitumor, and antiviral agents [14, 15]. Jostaberry is also a source of biologically active substances, but to date, the content of BAC in this fruit is almost unstudied. This is also applicable to the unknown content of BAC in the fruits of sweet cherry and currant, but the availability of such data is extremely relevant.

When choosing berries and fruits, we also considered their availability on the market, use in the food industry, and their well-known ability to suppress opportunistic pathogens.

The second stage of the study was obtaining methanolic extracts of alycha, plum, red and black currants, blueberry, sweet cherry, and jostaberry, followed by distillation of the solvent, and a quantitative determination of BAC content by means of thin-layer chromatography. In liquid extracts of berries, the content of biologically active substances was determined, that belonged to the class of anthocyanins and polyphenols, and their quantitative content in each investigated extract was measured.

Black currant and jostaberry fruits were characterized by the same qualitative composition of BAC (delphinidin-3-glucoside, delphinidin-3-rutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside), but different quantitative content of anthocyanidins and polyphenols. The total content of polyphenols in the fruit of black currant was half as much as the total content of polyphenols in jostaberry fruit. These extracts stimulated the growth of *L. casei*, and jostaberry extract additionally stimulated the growth of *L. fermentum* and *L. catenaformis* strains (Table 2).

The black currant extract (*Ribes nigrum*) contained the following biologically active substances: delphinidin-3-glucoside (20.4%), delphinidin-3-rutinoside (45%), cyanidin-3-

Table 1. **The results of anthocyanins content**

Serial Number	Isolated BAC	Tested Extracts					
		Ribes rubrum	*Prunus avium*	*Prunus domestica*	*Ribes × nidigrolaria*	*Vaccinium myrtillus*	*Ribes nigrum*
1	Cyanidin-3-monoboside, μg/ml	26.8	–	–	–	–	–
2	Cyanidin-3-xylosylrutinoside, μg/ml	118.5	–	–	–	–	–
3	Cyanidin-3-rutinoside, μg/ml	33.6	78.0	9.4	310.1	–	846.2
4	Cyanidin-3-glucoside, μg/ml	–	0.58	1.63	106.5	529.9	165.0
5	Cyanidin-3-arabinoside, μg/ml	–	–	–	–	298.5	–
6	Cyaniding-3-galactoside, μg/ml	–	–	–	–	429.5	–
7	Delphinidin-3-glucoside, μg/ml	–	–	–	56.9	697.8	590.1
8	Delphinidin-3-rutinoside, μg/ml	–	–	–	86.5	–	1285.1
9	Delphinidin-3-galactoside, μg/ml	–	–	–	–	651.8	–
10	Delphinidin-3-arabinoside, μg/ml	–	–	–	–	446.9	–
11	Petunidin-3-glucoside, μg/ml	–	–	–	–	472.5	–
12	Peonidin-3-rutinoside, μg/ml	–	–	7.8	–	–	–
13	Petunidin-3-galactoside, μg/ml	–	–	–	–	186.5	–
14	Peonidin-3-galactoside, μg/ml	–	–	–	–	40.7	–
15	Petunidin-3-arabinoside, μg/ml	–	–	–	–	113.9	–
16	Peonidin-3-glucoside, μg/ml	–	–	–	–	210.1	–
17	Malvidin-3-galactoside, μg/ml	–	–	–	–	149.6	–
18	Malvidin-3-glucoside, μg/ml	–	–	–	–	461.5	–
19	Malvidin-3-arabinoside, μg/ml	–	–	–	–	89.7	–

glucoside (5.6%), and cyanidin-3-rutinoside (29%). The total content of polyphenols was 5042 μg/ml. The black currant extract stimulated the growth of *L. casei* (Table 2).

The red currant fruit (*Ribes rubrum*) contained the following anthocyanidins: cyanidin-3-sambubioside (15%), cyanidin-3-xylosylrutinoside (66%), and cyanidin-3-rutinoside (19%). The total content of polyphenols was 3962 μg/ml. This extract was characterized by pro-bacterial properties in relation to *L. catenaformis* (Table 2).

The fruit of sweet cherry had the ability to stimulate the growth of *L. casei, L. catenaformis* and *L. fermentum*. However, in contrast to the extracts of black currant and jostaberry, only two types of anthocyanidins were identified in the fruit of sweet cherry:

Table 2. **Biological influence of fruit and berry extracts on the growth of lacto acid bacteria, in dynamics**

	L. acidophilus		***L. catenaformis***		***L. casei***		***L. fermentum***	
N0 (CFU/ml)	3×10^8		3×10^8		3×10^8		3×10^8	
Time of cultivation, hours	14	24	14	24	14	24	14	24
Tested Extracts	lgNt/N0	lgNt/N0	lgNt/N0	lgNt/N0	lgNt/N0	lgNt/N0	lgNt/ N0	lgNt/N0
Prunus cerasifera	−5.5 ±0.5*	−5.5 ±0.5*	−4.5±1.4*	−5.5 ±0.79*	−5.5 ±1.7*	−5.5 ±0.19*	0.67± 0.25*	−5.5 ±0.17*
Ribes × nidigrolaria	−3.7 ±1.1	−3.2 ±1.1	−3.5 ±0.5	−0.1 ±0.5	−2.8 ±0.35	0±0.3	−2.8 ±0.2	0.52 ±0.3
Prunus avium	−0.78 ±0.79	−1.7 ±0.5	−3.4±1.1	−0.78 ±0.26	−3.7 ±0.3	0±0.29	−2.5 ±0.42	−0.78 ±0.4
Ribes nigrum	−3.5 ±0.5	−4.1 ±0.76	−4.1 ±0.5	−0.1 ±0.5	−0.1 ±0.35	0.52±0.28	−2.8 ±0.38	−2.5 ±0.57
Ribes rubrum	−3.9 ±0.5	−4.1 ±1.2	−0.78 ±0.26	0.2±0.5	−4.8 ±0.5	−4.8 ±0.17	−0.78 ±0.38	−5.5 ±0.45
Vaccinium myrtillus	−2.8 ±0.83	0.34±0.5	−3.5 ±0.29	−0.78 ±0.29	−2.8 ±0.35	0.52±0.5	−2.8 ±1.0	0.52± 0.92
Prunus domestica	−1.5 ±0.2	0.34±0.5	−0.48 ±0.26	−0.78 ±0.29	−0.78 ±0.5	−0.48 ±0.5	−0.48 ±0.78	−0.78± 0.38

Note. Significant differences with the *Prunus cerasifera* extracts on growth of lacto bacteria by *(P*$<$ 0.05).

cyanidin-3-glucoside (0.7%), and cyanidin-3-rutinoside (99.3%), while the total content of polyphenols was only 525 μg/ml.

Plum fruits (*Prunus domestica*) contained three types of anthocyanins in their composition: cyanidin glycoside (8.6%), cyanidin-3-rutinoside (50%), and peonidin-3-rutinoside (41.4%). The total content of polyphenols was 668 μg/ml. As to the effect of the plum extract on strains of lactobacilli, we observed the stimulation of growth of all tested LAB and *B. subtilis 090* strains (Table 2).

Analyzing the obtained data of the quantitative study of extracts composition (Table 1), we could note that the number of compounds of anthocyanins and polyphenols is significantly lower in the extracts of the *Pr nus* genus than in the extracts of the *Ribes* genus, but according to the experimental data obtained, plum and sweet cherry extracts were better in stimulating the growth of probiotic strains of microorganisms. Analyzing the obtained chromatogram of the plum extract and comparing it with literature sources, based on chromatographic analysis of extracts of different varieties of plums, we observed that the substances of the first priority here were cyanidin-3-xyloside and cyanidin-3-glycoside in various amounts [16, 17]. However, while examining our extract of *Prunus domestica*, we see that the peak on the chromatogram corresponds to the cyanidin-3-glycoside substance. This can be explained by the fact that, depending on the geographical location, variety, and the year of life of the plant, the quantitative composition of compounds of the anthocyanins may change [3].

Blueberries (*Vaccinium myrtillus*) contained the largest gross anthocyanin content in their composition. The extract contained 15 compounds of anthocyanins: delphinidin-3-galactoside (13.7%), delphinidin-3-glucoside (15%), cyanidin-3-galactoside (8.9%), delphinidin-3-arabinoside (9%), cyanidin-3-glucoside (11%), petunidin-3-galactoside (4%), cyanidin-3-arabinoside (6.4%), petunidin-3-glucoside (9.8%), peonidin-3-galactoside (1.0%), petunidin-3-arabinoside (2.4%), peonidin-3-glucoside (4.3%), malvidin-3-galactoside (3.1%), malvidin-3-glucoside (9.6%), and malvidin-3-arabinoside (1.8%) (Table 1). The total content of these compounds exceeded 5000 μg/ml, and the amount of polyphenols was 8945 μg/ml. We noticed the ability of the blueberries to stimulate the growth of all LAB strains we selected.

Table 3. **Biological influence of fruit and berry extracts on growth of *E. coli* 058, *E. faecalis*, *B. subtilis* 090, in dynamics**

	E. coli		***E. faecalis***		***B. subtilis* 090**	
N0 (CFU/ml)	3×10^8		3×10^8		3×10^8	
Time of cultivation, hours	14	24	14	24	14	24
Tested Extracts	lg (Nt/N0)	lg (Nt/N0)	lg (Nt/N0)	lg (Nt/N0)	lg (Nt/N0)	lg (Nt/N0)
Prunus cerasifera	−6.7 ±0.29	–	−6.7±0.5	–	−3.7 ±0.78	−0.48 ±0.83
Ribes × *nidigrolaria*	−6.7 ±0.29	−6.7* ±0.79	−6.7±0.5	−6.7±0.5	−0.78±0.5	−0.78±0.2
Prunus avium	−6.2±1.1	−6.7* ±0.29	−6.5±0.5	–	0.91±0.85	−5.5±0.74
Ribes nigrum	−4.8±0.5	–	−6.7±0.5	–	−3.7±0.3	−3.7±0.57
Ribes rubrum	−6.7±0.5	−6.7 ±0.5*	−4.8±0.29	−4.8±0.5	−0.78 ±0.87	−5.5±1.14
Prunus domestica	−4.8±0.79	−6.7 ±0.79*	−4.8±0.29	−4.8 ±0.79	−0.78±1.4	0.93±0.44
Vaccinium myrtillus	0.59±0.2	−3.8 ±0.5*	−4.8±0.29	−6.7 ±0.29	−5.5±0.93	−6.7±0.83

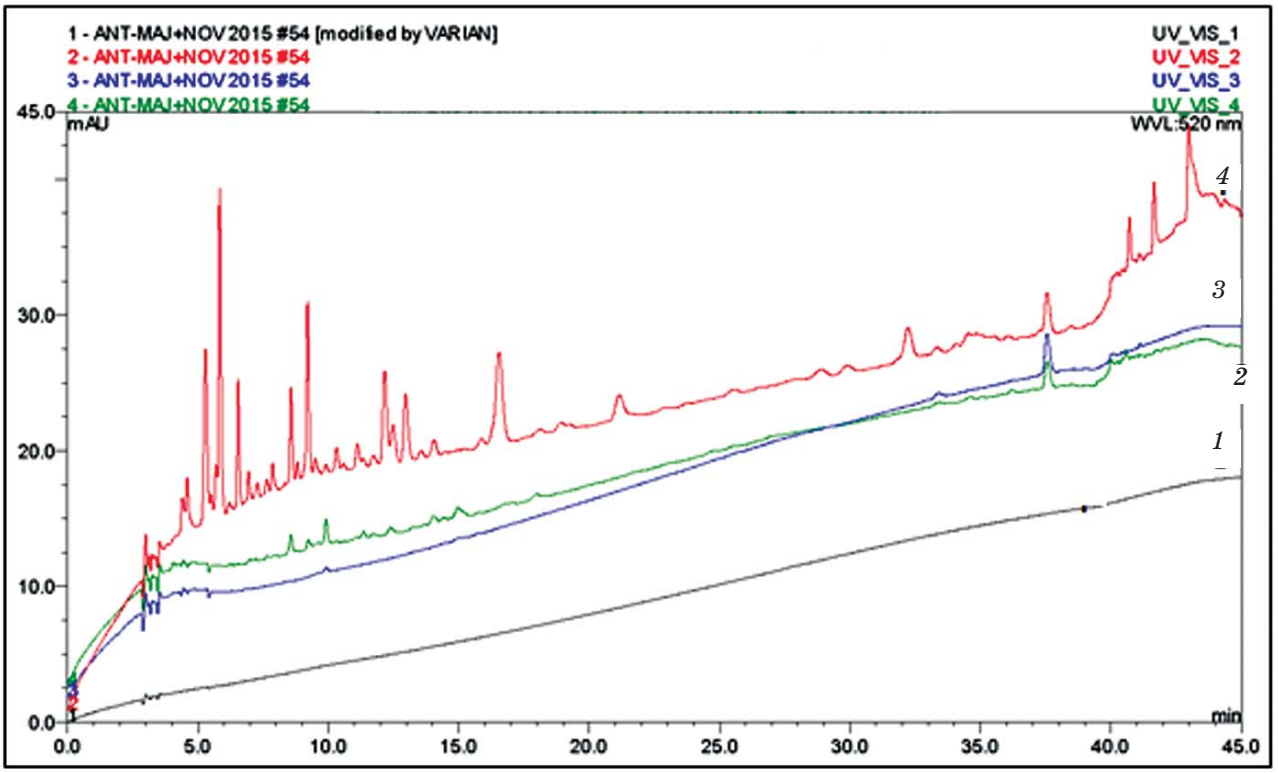
Note. Significant differences with the of fruit and berry extracts on growth (24 hours of cultivation) of *E. coli 058* by *(*P* < 0.05).

According to the data obtained, the fruits of alycha did not contain anthocyanins in their composition. This is confirmed by the fact that due to the presence of anthocyanins in the fruits, the fruits were of red to violet color, and alycha fruit was yellow. Analyzing the obtained chromatogram (Figure), we see that it has a very small amount of flavonoids and a small amount of coumarin. We also observed here the content of polar phenols and, according to the analysis of literature data, they could be phenolic acids, for example, catechin [18]. The total content of polyphenols was 219 μg/ml. The alycha extract inhibited the growth of all beneficial microorganisms we selected, and therefore when using it as a prebiotic component of pharmabiotics, such undesirable effect on the LAB tested by us should be taken into account (Table 2, 3).

Analyzing the quantitative and qualitative composition of BAC, included in the extracts tested by us, it can be concluded that the content of polyphenols significantly exceeded the content of anthocyanins, with no anthocyanins in the alycha extract detected whatsoever.

We determined the contents of anthocyanins and polyphenols in extracts of fruits and berries, and compared them with a database of BAC content in plant extracts ePlant LIBRA [19]. According to the analysis of the content of BAC in the following extracts: *Ribes rubrum, Ribes nigrum, Ribes* × *nidigrolaria, Vaccium myrtillus, Prunus avium*, which were included in the above mentioned database, the cyanidin-3-rutinoside compound was not identified [20, 21], but it was found in the extracts of the fruits and berries that we studied; there was also a difference in a quantitative content of anthocyanins and polyphenols. Comparing the BAC content of the *Prunus domestica*, and *Prunus cerasifera* extracts studied by us, with the ePlant LIBRA database, we noticed a difference, however, not in the qualitative composition of extracts, but in their quantitative indices [22].

While investigating the probacterial properties of extracts in relation to beneficial strains of microorganisms, we found that extracts of red and black currants, jostaberry, plum, and blueberry possessed such properties. In other words, these are the extracts, that contain anthocyanins and polyphenols in their composition, and therefore, we can conclude that the prebiotic properties are the properties of anthocyanins. This hypothesis is confirmed by the experimental data we obtained: since the extract of alycha had no anthocyanins in its composition, it had an inhibitory action on the strains of lactic bacteria.



The Chromatogram of the alycha extract:
UV-VIS *1* — 520 nm (anthocyanins); UV-VIS *2* — 280 nm (phenols); UV-VIS *3* — 360 nm (flavanoids); UV-VIS *4* — 320 nm (coumarin)

So, comparing the results of our research with literature data, we can argue that namely the compounds of anthocyanins in the extracts of the berries we have studied have probacterial properties. The previously-published papers show that anthocyanidins, namely cyanidin-3-glucoside, inhibit the growth of only *E. coli* and do not inhibit the growth of probiotic strains of microorganisms and other representatives of human commensal microbiota [23, 24]. But, analyzing the experimental data we obtained, we can conclude that it is the compound of cyanidin-3-rutinoside that not only does not inhibit the growth of lactic bacteria, but rather contributes to their growth. This is confirmed by the fact, that the fruits of sweet cherry and plum stimulated the growth of the beneficial microorganisms we selected.

Thus, comparing the experimental data obtained with our previous studies, we can suggest that there are compounds of anthocyanins in the extracts of the studied berries that have probacterial properties.

The data obtained testify to possible further testing of berry extracts for their use as new generation pharmabiotics. After all, biologically active compounds that are part of the extracts, not only do not inhibit the growth of beneficial (probiotic and commensal) microorganisms, but rather stimulate it.

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ЕКСТРАКТИ ЇСТІВНИХ РОСЛИН ЯК СТИМУЛЯТОРИ РОСТУ КОРИСНИХ МІКРООРГАНІЗМІВ

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Метою роботи було визначення вмісту біологічно активних речовин у ягодах і плодах, зібраних в екологічно чистих зонах, для з'ясування перспективності їх використання для створення фармабіотиків спрямованої дії та здатності одержаних з них екстрактів стимулювати ріст пробіотичних штамів лактобактерій і представників коменсальної кишкової мікробіоти.

Вміст біологічно активних речовин визначали методом тонкошарової хроматографії. Вплив метанольних екстрактів ягід та плодів на відібрані нами штами: *L. acidophilus*, *L. catenaformis*, *L. casei*, *L. fermentum*, *E. coli 058*, *E. faecalis* (коменсали кишечнику), *B. subtilis 090* (складова біопрепарату), які є перспективними для створення сучасних фармабіотиків, визначали за результатами їх культивування в зазначених екстрактах.

Виявлено, що досліджувані ягідні екстракти характеризувалися більш значним вмістом поліфенолів порівняно з антоціанами. Екстракт аличі пригнічував ріст усіх тестованих нами штамів, окрім *B. subtilis 090*. Екстракти червоної смородини, черешні та йошти сприяли росту *L. catenaformis*, а екстракти черешні і йошти на додаток до вищезазначених штамів лактобактерій додатково стимулювали ріст *L. casei* та *L. fermentum*. Екстракти чорниці та сливи активували ріст усіх штамів лактобактерій, а чорниці, чорної та червоної смородини пригнічували ріст відібраних нами коменсальних мікроорганізмів. Здатність стимулювати ріст *B. subtilis 090* було відзначено лише в екстрактах аличі, йошти та сливи.

Ключові слова: ягоди, плоди, флаваноїди, антоціани, лактобактерії, фармабіотики.

ЭКСТРАКТЫ СЪЕДОБНЫХ РАСТЕНИЙ КАК СТИМУЛЯТОРЫ РОСТА ПОЛЕЗНЫХ МИКРООРГАНИЗМОВ

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Целью работы было определение содержания биологически активных веществ в ягодах и плодах, собранных в экологически чистых зонах, для выяснения перспективности их использования для создании фармабиотиков направленного действия и способности полученных из них экстрактов стимулировать рост пробиотических штаммов лактобактерий и представителей комменсальной кишечной микробиоты.

Содержание биологически активных веществ определяли методом тонкослойной хроматографии. Влияние метанольных экстрактов ягод и плодов на отобранные нами штаммы: *L. acidophilus, L. catenaformis, L. casei, L. fermentum, E. coli 058, E. faecalis* (комменсалы кишечника), *B. subtilis 090* (составляющая биопрепарата), которые являются перспективными для создания современных фармабиотиков, устанавливали по результатам их культивирования в указанных экстрактах.

Выявлено, что исследуемые ягодные экстракты характеризовались более значительным содержанием полифенолов по сравнению с антоцианами. Экстракт алычи подавлял рост всех тестируемых нами штаммов, кроме *B. subtilis 090*. Экстракты красной смородины, черешни и йошты способствовали росту *L. catenaformis*, а экстракты черешни и йошты в дополнение к вышеупомянутым штаммам лактобактерий дополнительно стимулировали рост *L. casei* и *L. fermentum*. Экстракты черники и сливы активировали рост всех штаммов лактобактерий, а черники, черной и красной смородины подавляли рост отобранных нами комменсальных микроорганизмов. Способность стимулировать рост *B. subtilis 090* была отмечена только в экстрактах алычи, йошты и сливы.

Ключевые слова: ягоды, плоды, флаваноиды, антоцианы, лактобактерии, фармабиотики.

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MULTIPLEX-TOUCHDOWN PCR FOR RAPID SIMULTANEOUS DETECTION OF *Rhizoctonia cerealis* AND *Rhizoctonia solani*

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The aim of the study was to develop rapid and sensitive assay for the simultaneous detection of *R. cerealis* and *R. solani*. Pure cultures of fungi were grown on a potato dextrose agar for 5 days at 28 °C, and mycelium was harvested and used for DNA extraction. Total DNA was extracted using a commercial test-systems. Molecular identification of phytopathogenic fungi was performed using a multiplex-touchdown PCR with further electrophoretic separation of amplification products in agarose gel. The specific sequence characterized amplified region primers RtubR4/RtubF4 for *R. cerealis* and ITS1/GMRS–3 for *R. solani* were tested for their specificity and useability in PCR multiplex capacity. The specificity of the multiplex-touchdown PCR was tested using DNA from wide range of fungal species and non-target DNA from healthy wheat. The used primer pairs provided only specific fragments for *R. cerealis* and *R. solani*. No PCR products were obtained during amplification with the negative control or non-target DNA templates from other species. Coupled to this we have optimized the temperature regime for the multiplex PCR protocol. Taken together, our protocol convincingly demonstrated the simultaneous ability to detect *Rhizoctonia cerealis* and *Rhizoctonia solani* and can be used for the diagnosis of compound *Rhizoctonia* root rot.

Key words: phytopathogenic fungi, *Rhizoctonia*, multiplex PCR.

Considerable changes came in the pathogenic complex of grain-crops in Ukraine and outside the country during the last decades [1–3]. Intensive technologies mastering resulted in distorting the ecological balance in agrocoenoses, entailed the origin of hearths and growth of distribution of those disease of wheat, that did not have the special value before. The occurrence of these plant diseases is a result of reducing of soil suppression due to the depletion of microbiota [4, 5]. These diseases include collar rot, Fusarium head blight, winter wheat leaf blotch and root rot. Root rot is a group of cereals diseases that affect roots, root portion of the stem, an underground intestine and planting site. Pathogens with necrotrophic phase in life cycle such as soil-borne necrotrophic basidiomycetes including *Rhizoctonia* are responsible for these plant diseases [6, 7]. The sharp eyespot caused by necrotrophic fungus *Rhizoctonia cerealis* van der Hoeven (teleomorph: *Ceratobasidium cereale* D. Murray & L.L. Burpee) is a stem-base wheat disease that is widely distributed in wheat-growing regions worldwide. In Ukraine, sharp eyespot is spread in the steppe and in the south regions of forest-steppe zone [8]. This disease is able to strike more than 230 types of yearling and perennial plants from 66 families, among those the most valuable agricultural crops such as a potato, wheat, barley, rice and other [9]. Sharp eyespot is associated with yield losses due to interference with nutrient and water uptake caused by stem weakening [10, 11]. In the field, rhizoctonia diseases of wheat are frequently caused by at least two soil-borne pathogens fungi, including *R. cerealis* and *R. solani* [12]. *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris*

[Frank] Donk) is another soil-borne necrotrophic fungus causing several wheat diseases, such as collar rot and wet root rot [13, 14]. The severity of disease caused by these pathogens is increased during the last several decades due to global climatic changes, earlier sowing, the use of fungicides etc. Moderate and severe disease can reduce wheat yield substantially and causes deterioration of its quality [15]. In order, to precisely determine yield losses due to these fungi, correct identification of the causal agents is required. In addition, early, rapid, and specific identification of plant pathogens is essential for effective plant disease management. Specific disease diagnosis is also required for timely and proper control measure.

In field conditions, root rot type identification in a correct and timely manner is quite problematic. Visual diagnostics is complicated because of the fact that all pathogens are necrotrophic, and therefore the main symptom that they cause is necrosis. In addition, visible symptoms show up at advanced stage of the disease that is why providing the timeliness of realization of treatments is problematic. Molecular diagnostic technologies based on PCR have been developed for rapid and precise detection of numerous phytopathogenic fungi. However, these methodologies are developed for detecting a single pathogen including those for the identification of *R. cerealis* [15] and *R. solani* [16]. As mentioned above, under field conditions, Rhizoctonia diseases occur primarily as mixed infections. Therefore, the multistep PCR methodology seems to be too time- and labor-consuming, as well as too expensive for practical use. Multiplex PCR technology is more advanced. This approach can simultaneously amplify several primers, thereby decreasing the detection cost and overcoming the drawbacks of single PCR detection. This methodological approach is successfully used for the simultaneous detection of several phytopathogenic fungi that cause compound infections [17]. This study aimed to develop a rapid and sensitive assay for the simultaneous detection of *R. cerealis* and *R. solani*. For this purpose we used touchdown PCR. Touchdown PCR provides a simple and rapid means to optimize PCRs, increasing specificity and sensitivity, without the necessity for optimizations and/or the redesigning of primers [18].

Materials and Methods

All isolates used in the study are presented in Table 1. Isolates were obtained from the collection of the Laboratory of Mycology and Phytopathology, All-Russian Institute for Plant Protection.

Pure culture of all isolates have been growing on a potato dextrose agar (PDA) for 5 days at 28 °C, and mycelium was harvested and used for genome extraction. The total fungal genomic DNA was extracted using the Agrosorb NK kit (LLC Agrogen Novo, Ukraine). The quantity and purity of extracted DNA were measured using a spectrophotometer NanoDrop 1000 (Thermo Fisher Scientific, USA). Purity DNA preparations have expected at A260/A280 ratio and DNA concentration is estimated by measuring the absorbance at 260 nm.

For assaying primer specificity, DNA was extracted from healthy plants of wheat (negative control) and from other fungal species (*Alternaria solani, Fusarium culmorum, F. graminearum, F. oxysporum, F. poae, F. cerealis, F. sporotrichioides, F. verticilloides, F. proliferatum, F. avenaceum, F. solani, F. subglutinans, F. tricinctum, F. langsethiae*).

To confirm the correct identification of the *Rhizoctonia* isolates the PCR was performed using the specific Sequence Characterized Amplified Region (SCAR) primers RtubR4/RtubF4 for *R. cerealis* and ITS1/GMRS–3 for *R. solani* (Table 2) [19, 20]. PCR reactions were carried out on a T100™ Thermal Cycler (Bio-Rad Laboratories Ltd., USA) under thermal cycling parameters as follows: initial denaturation at 95 °C for 7 min; then, 39 cycles of denaturation at 94 °C for 30 s, primer annealing at 56–65 °C for 30 s and primer extension at 72 °C for 45 s; finally, final primer extension at 72 °C for 5 minutes. The reaction was conducted in a 10-µl reaction mixture consisting of 3.4 µl ddH2O, 1,7 µl 5x PCR-buffer blue 15 µM Mg^{2+} (AmpliSens, Russia), 0.3 µl 5x PCR-buffer 0 µM Mg^{2+} (AmpliSens, Russia), 1 µl dNTP (1.76 µM of each nucleotide), 1 µl primer mix (0.25 µM of each primer), 0.1 µl Taq polymerase (5 U) (Thermo Scientific, USA), 2.5 µl template DNA. Sterile distilled water was used as a negative control to test for the presence of contamination in PCR reagents.

PCR amplicons were electrophoresed on 2.0% agarose gel stained with ethidium bromide and visualized by UV light (260 nm) and photographed using Bio-Rad Gel DocTM XR+ gel documentation system (Bio-Rad Laboratories Ltd., USA).

Table 1. **Isolates of R.cerealis, R.solani and other fungal species used in the study**

Species	Isolate code	Year and region of Collection	Host Plant	Source
Rhizoctonia cerealis	-2	2016, Kyiv region, Ukraine	Wheat	-
R. solany	-	2015, Kyiv region, Ukraine	Potato	-
Alternaria solani	P 043-021	2006, Primorsky region, Russia	Potato	MF1
Fusarium culmorum	G 102100	2007, Kirov region, Russia	Barley	MF
F. graminearum	G 58570	2014, Krasnodar region, Russia	Wheat	MF
F. oxysporum	G 58767	2015, Leningrad region, Russia	Cucumber	MF
F. poae	G 102702	2007, Kirov region, Russia	Oats	MF
F. cerealis	G 58694	2003, Heilongjiang province, China	Wheat	MF
F. sporotrichioides	G 163101	2011, Krasnodar region, Russia	Wheat	MF
F. tricinctum	G 58368	2010, Krasnodar region, Russia	Wheat	MF
F. verticillioides	G 59039	2016, Almaty region, Kazakhstan	Corn	MF
F. proliferatum	G 58380	2010, Krasnodar region, Russia	Wheat	MF
F. langsethiae	G 192201	2013, Lipetsk region, Russia	Wheat	MF
F. avenaceum	G 59019	2015, Latvia	Wheat	MF
F. solani	G 90602	2007, Leningrad region, Russia	Barley	MF
F. subglutinans	G 59045	2016, Kostanay region, Kazakhstan	Peas	MF

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Table 2. **Primers used in the study**

Pathogen	Primer	Sequence (5′–3′)	Direction	Source
R. solani	GMRS-3	AGT GGA ACC AAG CAT AAC ACT	Reverse	Johanson et. al., 1998
	ITS1	TCC GTA GGT GAA CCT GCG G	Forward	
R. cerealis	RtubR4	GCT AGT GCG GTC AAT GTA TAG	Reverse	Guo et al., 2012
	RtubF4	CCT AAA TGA GTC TGG AGT AAG TC	Forward	

Results and Discussion

PCR method allows detecting the presence of a certain sequence of pathogenic nucleic acid in samples and due to high sensitivity, determines single copies of its genomes. For today, a PCR-analysis is one of the most widespread technologies of laboratory diagnostics that develops dynamically.

The diagnostic PCR assay involves several critical steps, such as DNA extraction from specimens, PCR amplification, and detection of amplicons. The amount of total DNA in a PCR has a marked effect on the outcome of the analysis procedure. Sample overloading would result in packed DNA in the confined space of the reaction vessel and can lead to false priming and even poor DNA synthesis due to the obstructed diffusion of large *Taq* polymerase molecules [21]. Therefore, the final DNA concentration was adjusted to 2.5 ng/µl by sterile deionized water.

For assaying primer specificity DNA was isolated from different fungal species. As a negative control, the non-target DNA, healthy wheat DNA diluted with distilled water (ddH2O) was used.

The primer pair RtubR4/ RtubF4 provided only a single specific fragment of 138 bp for *R. cerealis*. No PCR products were obtained during amplification with the negative control or non-target DNA templates from other species (Fig. 1, *a*).

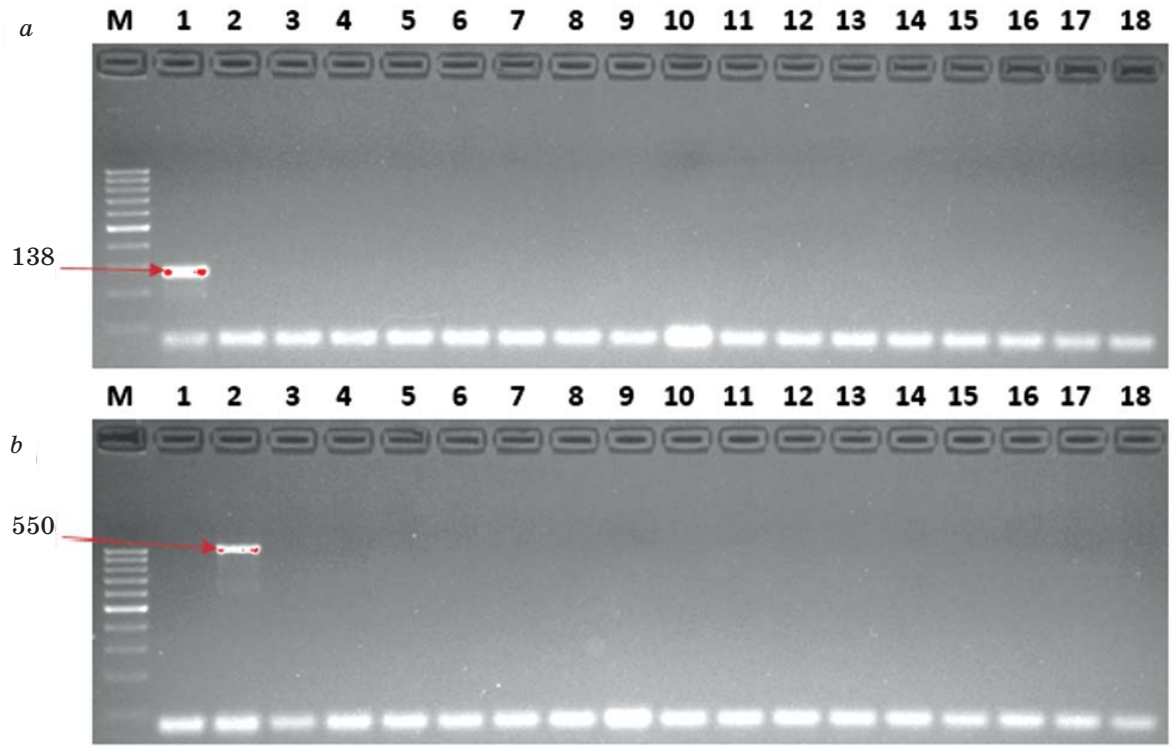

Fig. 1. **Agarose gel electrophoresis of the amplification products of target and non-target DNA using species-specific primer sets:**
a — RtubR4/RtubF4; ***b*** — ITS1/GMRS–3; M: GeneRuler 50 bp DNA Ladder (IsoGen, Russia).
Lane 1 — *Rhizoctonia cerealis;* lane 2 — *R. solani;* lane 3 — healthy plant; lane 4 — ddH_2O; lane 5 — *Alternaria solani;* lane 6 — *Fusarium culmorum;* lane 7 — *F. graminearum;* lane 8 — *F. oxysporum;* lane 9 — *F. poae;* lane 10 — *F. cerealis;* lane 11 — *F. sporotrichioides;* lane 12 — *F. verticilloides;* lane 13 — *F. proliferatum,* lane 14 — *F. avenaceum;* lane 15 — *F. solani;* lane 16 — *F. subglutinans;* lane 17 — *F. tricinctum;* lane 18 — *F. langsethiae*

The PCR reaction with ITS1/GMRS-3 primers confirmed the occurrence of *R. solani*, giving the expected product of amplification 550 bp. Moreover, the specific PCR fragment was amplified from the DNA of *R. solani* isolates, but not from the DNA of other fungal isolates (Fig. 1, *b*).

Multiplex PCR is a valuable tool in many biological studies, but it is a multifaceted procedure that has to be planned and optimised thoroughly to achieve robust and meaningful results [22]. The development of an efficient multiplex PCR usually requires strategic planning and multiple attempts to optimize reaction conditions. For a successful multiplex PCR assay, the relative concentration of the primers, concentration of the PCR buffer, balance between the magnesium chloride and deoxynucleotide concentrations, cycling temperatures, and amount of template DNA and Taq DNA polymerase are important. An optimal combination of annealing temperature and buffer concentration is essential in multiplex PCR to obtain highly specific amplification products [23, 24].

Optimization of the reaction conditions in our experiments included: optimal annealing temperature screening for each pair of primers, determining the optimal composition of the reaction mixture and the number of primers.

Testing different temperature regimes, it was found that oligonucleotide systems respond specifically to DNA from phytopathogenic fungi at the gradient from 59 °C to 62 °C (Fig. 2, *d–g*). In this case, the annealing of the primers in the multiplex reaction did not occur at the temperature gradient from 63 °C to 65 °C (Fig. 2, *a–c*). It also should be noted, that the multiplex system worked at all temperature regimes, but only for the DNA from single pathogen, not for DNA from both fungi.

As the temperature rise, the reaction specificity increases, and, other way around, temperature decrease leads to reaction specificity reduction, but at the same time, increases the PCR products yield. Taking into account, we decided to use the Touchdown PCR method.

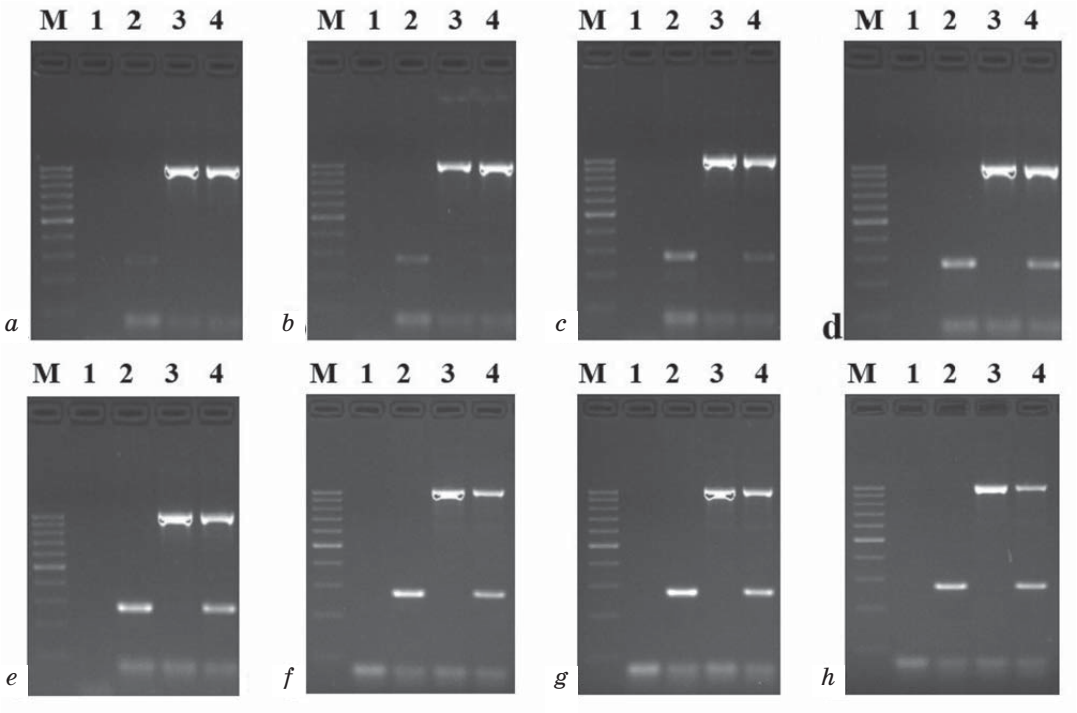

Fig. 2. **Effect of different annealing temperature on multiplex PCR detection of *Rhizoctonia* species using species-specific primer sets RtubR4/RtubF4 & ITS1/GMRS–3:**
a — annealing temperature 65 °C; *b* — annealing temperature 64 °C; *c* — annealing temperature 63 °C; *d* — annealing temperature 62 °C; *e* — annealing temperature 61 °C; *f* — annealing temperature 60 °C; *g* — annealing temperature 59 °C; *h* — Touchdown (TD) program: the program gradually lowers the hybridization temperature by 1 °C per-cycle increments from 65 °C to 56 °C; M: GeneRuler 50 bp DNA Ladder (IsoGen, Russia). Lane 1 — healthy plant; lane 2 — *Rhizoctonia cerealis;* lane 3 — *R. solani;* lane 4 — DNA mixture of *R. cerealis* & *R. solani*

With this method, the influence of non-specific hybridization of primers on the formation of the product was reduced. Touchdown PCR uses an initial annealing temperature above the projected melting temperature of the primers being used, then progressively transitions to a lower, more permissive annealing temperature over the course of successive cycles. The first cycles in our experiments were carried out at a temperature higher than the annealing temperature, and then the subsequent 10 cycles were performed at the reduced annealing temperature by 1 °C. At that temperature, the oligonucleotide system passed through the band of optimal specificity of the primers to the DNA (Fig. 2, *h*).

Thus, due to the lack of rapid effective methods of detection of causative agents of the diseases affecting the root system, the root portion of the stem and seeds (grain) of wheat, data on their distribution in Ukraine is mostly fragmentary. Based on the inherent difficulties and inaccuracies associated with an attempt to distinguish fungal organisms based on their growth pattern and morphological characteristics, we have optimized multiplex PCR that can detect these fungal pathogens simultaneously.

As a result of research, the PCR conditions (composition of the reaction mixture, amplification conditions) were optimized for multiplex PCR analysis. It has been established that used combination of primers under given conditions does not show cross-reactivity with other phytopathogenic fungi, and can be used for the diagnosis of *Rhizoctonia* root rot, which will allow detecting of two pathogens at ones and reduce the cost of reagents and timing by half.

Optimized multiplex PCR system for identifying *Rhizoctonia cerealis* and *Rhizoctonia solani* from the plant material successfully tested in Syngenta diagnostic centers and allowed us to conduct qualitative analyzes of pathogens among grain crops.

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Conflicts of Interest: Authors declare no conflict of interest.

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МУЛЬТИПЛЕКСНА ТАЧДАУН ПЛР ДЛЯ ШВИДКОГО ОДНОЧАСНОГО ВИЯВЛЕННЯ *Rhizoctonia cerealis* І *Rhizoctonia solani*

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Метою роботи було розробити швидкий і чутливий метод одночасної ідентифікації *R. cerealis* та *R. solani* упродовж однієї реакції. Чисті культури фітопатогенних грибів культивували 5 днів на картопляно-глюкозному агарі за 28 °С, після чого відбирали міцелій та виділяли ДНК за допомогою комерційних тест-наборів. Молекулярну ідентифікацію проводили за допомогою ПЛР з подальшим електрофоретичним розділенням продуктів ампліфікації в агарозному гелі. Специфічні пари праймерів для ділянки з відомою послідовністю: RtubR4/RtubF4 для *R. cerealis* та ITS1/GMRS-3 для *R. solani* тестували щодо їхньої специфічності та можливості застосування у мультиплексному варіанті ПЛР. Для перевірки специфічності праймерів здійснювали аналіз ПЛР з іншими фітопатогенними грибами та з ДНК, виділеною зі здорових рослин пшениці. Встановлено, що обрані нами пари праймерів продукували єдині специфічні фрагменти *R. cerealis* і *R. solani* та не виявляли перехресної специфічності щодо інших фітопатогенних грибів. За допомогою градієнта температур встановлено оптимальні значення температур відпалу праймерів. Таким чином, розроблений нами протокол мультиплексної ПЛР придатний для одночасної ідентифікації *Rhizoctonia cerealis* та *Rhizoctonia solani* протягом однієї реакції і може бути використаний для діагностики поліетіологічних ризоктоніозів.

Ключові слова: фітопатогенні гриби, ризоктонії, мультиплексна ПЛР.

МУЛЬТИПЛЕКСНАЯ ТАЧДАУН ПЦР ДЛЯ БЫСТРОГО ОДНОВРЕМЕННОГО ВИЯВЛЕНИЯ *Rhizoctonia cerealis* И *Rhizoctonia solani*

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Целью работы было разработать быстрый и чувствительный метод для одновременной идентификации *R. cerealis* и *R. solani* на протяжении одной реакции. Чистые культуры фитопатогенных грибов культивировали 5 дней на картофельно-глюкозном агаре при 28 °С, после чего отбирали мицелий и выделяли ДНК с помощью коммерческих тест-систем. Молекулярную идентификацию осуществляли с помощью ПЦР с последующим электрофоретическим разделением продуктов амплификации в агарозном геле. Специфические пары праймеров для области с известной последовательностью: RtubR4/RtubF4 для *R. cerealis* и *ITS1/GMRS–3* для *R. solani* тестировали относительно их специфичности и возможности использования в мультиплексном варианте ПЦР. Для проверки специфичности праймеров проводили анализ ПЦР с другими фитопатогенными грибами и с ДНК, выделенной из здоровых растений пшеницы. Установлено, что выбранные нами пары праймеров продуцировали единственные специфичные фрагменты *R. cerealis* и *R. solani* и не проявляли перекрестной специфичности относительно других фитопатогенных грибов. С помощью градиента температур установлены оптимальные значения температур отжига праймеров. Разработанный нами протокол мультиплексной ПЦР пригоден для одновременной идентификации *Rhizoctonia cerealis* и *Rhizoctonia solani* на протяжении одной реакции и может быть использован для диагностики полиэтиологических ризоктониозов.

Ключевые слова: фитопатогенные грибы, ризоктонии, мультиплексная ПЦР.