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APPLICATION OF CONTENT MANAGEMENT SYSTEM BY EXAMPLE OF MUNICIPAL CLINIC

Abstract. The paper investigates the issues of ready management systems application at creating and managing a site on the Internet. The paper highlights the advantages and features of the Content Management System (CMS) application for site development. In addition, the CMS functioning and its technological structure are described in details, the standard structure of the site is shown, the existing ready solutions are described, the available models of data presentation and its classification are shown. The main problems that could appear during filling the content, site editing are highlighted, and possible ways of its elimination are shown. As an example, the municipal clinic #17 site created using the CMS was analyzed.

Key words: content management systems, information technologies, site building, municipal polyclinic.

Introduction. The adoption of contemporary information technologies in medicine is not a typical thing, but this brings the health care system to the next level as the immediate access to the information and its exchange decreases significantly the time expended to solve a problem, and the time is often a crucial factor for saving the people lives.

The creation of an own site can become a key factor for further development of a medical organization. At the same time the development of sites for municipal clinics is a quite difficult task.

Currently, there are different systems to manage the content. Among others, there is the Content Management System (CMS) that is considered as the breakthrough technology to create the projects of any complexity. The distinctive features of the CMS are high reliability and quality of the performance. In addition, the CMS popularity is gained by its open-source feature. And, at the same time, the system capabilities are the same as of paid contents.

The CMS allows creating the sites without knowledge of html, css and other web-programming features. The CMS also allows quick adding, removing, editing and formatting the content that is important for significant facilitation of the site management process.

The opportunity to add text files and multimedia materials allows enhancing the attractiveness of the site for new users. Another advantage of the CMS application is automated generation of the administrator panel that is connected with all fields of the site operations showing the system convenience and usability.

The application of the CMS for a web-site creating for medical organizations allows improving the level of services quality, makes the medical aid more accessible, allows satisfying the population with the rendered medical service, provides maximum transparency and patient awareness with the results of diagnostics, treatment, health assessment, methods of preventive care and monitoring of its health state. This work describes the application of the CMS for the site creation for the municipal polyclinic #17.

1.1. Technological base of the CMS. For the detailed analysis of individual capabilities of the CMS to create and support the medical organization sites and to understand the difference it is necessary to provide a definition for the CMS and its functions.

The Content Management System is software allowing any user to publish and change by himself the information published in the site without addressing to the site developers. To use the system it is not necessary to have any special knowledge and technologies. However, the training of the personnel is obligatory in the part of learning the system operation, not more [1].

The CMS is usually consists of back-office (infrastructure system) and front-office (user interface). Back-office provides the functionality and information store, and includes the applications servers and port solution. Namely the back-office implements all functional operations and site management, and the front-office is responsible for user features. Front-office has the web-interface allowing editing documents using standard office packages such as text editor, electron table, presentation, mail system etc. [2, 3].

The already in-built design templates of the CMS allow users creating the design of pages and change them minimizing the dependence on developers. The CMS administrator, using a computer mouse, can place different information blocks within the set page, determine its size, color and other features of the site. Another significant advantage of the CMS is Drag&Drop function to manage the site content. To set a new position for a document in the structure or to make a note in the list, it is just necessary to "catch" this position with the mouse and "drag" to a new place the same as it is done with files in Microsoft Windows explorer.

1.2. The structure of the site based on the CMS. The CMS system has two databanks: relational DBMS and file system. The first databank is designed for data published in the site, the second – for presentation elements (templates, images etc.). In addition to the external presentation of the site, there are two specialized workstations [4].

The first workstation is designed for the site developers. Here the developers can set the structure of site and content, change design and site view, set the templates for the information presentation. Usually, these operations are semi-automated. The site setting is conducted using the CMS means, and another part of information is placed manually.

The second workstation allows the site owners and a company staff to place the information in the site themselves. The customer managers operate through the specialized workstation.

Today, the most organizations, independently on its size, structure and type of activity, have their own web-site. The local Ethernet is used by a tiny part of companies. At the same time, the contemporary web-sites of the Companies are based on static pages and as a result the process of the information placement is not visible for users. But this type of operation requires managers to have knowledge and competencies in the field of programming and HTML as the risk of errors is high [5].

The CMS system also allows an employee to renew information quickly without addressing the site developers. The information is renewed itself, so there are no expenses for a developer or the site maintenance. Thus, the cost of support decreases.

The CMS provides a lot of services – search, forum, poll etc., i.e. for the creation of a site based on the CMS the tested ready modules can be used. In comparison with a static site, the CMS splits data and its presentation allowing changing the external view of the site with lower expenses.

When the site is created using the CMS the further information is renewed by non-technical specialists. This became possible owing to the definite web-interface allowing placing information, use definite visual instruments for editing, import information from Office documents. Web-interface is a specialized workstation accessible via the Internet Explorer [6].

One of the key moments is the opportunity of visual editing of the text. The system has WYSIWYG (What You See Is What You Get) – an editor allowing for placing a text and formatting a document without special technical knowledge [7]. Along with a text, it is also possible to place different images – graphs, diagrams etc.; the system has an opportunity to transfer data from the Office applications to the site.

The Microsoft Internet Explorer has a managing element allowing for visual editing. Developers access this managing element and then the results of its work are used. As the system uses the in-built capabilities of the Microsoft Internet Explorer (MSIE), to publish the information it is necessary to use the Internet Explorer. But this is only for developers and owners of the site who replenish and edit the content. As for the site users they can use any Internet browser [8].

During the site content replenishment, the managers encounter with a problem of text placing together with images, i.e. inside the text, not in its beginning or end. The WYSIWYG editor described above does not have this capability. It allows placing a link to the image at any place and requires indicating the server address (URL) where the image is placed. In result, the image automatically appears in the server at the discretion of editor.

To solve this problem it is necessary to modify this software so an image could be placed into the common library. Another way is image link to a definite document, and further during the editing process choose necessary image to include.

The difference of the methods is in quantitative application of images. The first method allows repeated application of the same images, but if its quantity is large it becomes difficult to find necessary one, and deleting unnecessary data is complicated. The second method does not allow using the same image in several documents; otherwise it is necessary to upload an image several times for each document. But the advantage of the second method is more facilitated process of selection and automated deleting of images together with the document.

Thus, the Content Managing Systems allow managers having no technical knowledge and competence to publish information in the site and implement the simple operations on text editing. Text highlighting with italics on the site page is made the same as in Microsoft Word and this capability exists in all systems.

1.3. Existing CMS solutions. At the present time the CMS is one of the most competitive applications in its field. There are several tens of thousands of such applications in the world that can be divided into the following classes:

– closed-source systems;

– open-source systems.

The closed-source applications are mainly used by large enterprises and organizations. The most famous applications among them are Microsoft Content Management Server, Documentum, Plumtree Portal, IBM WebSphere Portal [9].

Due to high price of the project adoption on the base of these solutions, the field of its application is quite narrow and is limited to creation of the Internet solutions for large organizations.

The open source systems have such features as accessibility, source code availability, localization opportunity. At the same time there are the following problems of its exploitation:

1. Lack of technical support – all problems appearing during the process of such systems application are solved by a developer himself.
2. Narrow application field – most often the product was a side result of own tasks solving.

For example, the developers created a site-community for communication. And further, the solution on which the site operates is proposed as the CMS.

It is clear that such solution can solve well the tasks on creating the similar sites, but can be absolutely unpractical to solve other tasks (online commerce, b2b, etc.).

The most famous examples of such systems are OpenCMS, PhpNuke, PostNuke, Portal Starter Kit etc.

1.4. CMS classification. Over the years the Content managing systems improved significantly. The CMS can be classified by the fields of its application [10]:

Portals. Portals are used for information resources; its main goal is to facilitate maximally the publication of papers and news. They can consist of individual modules. Among the most famous representatives of this class are AngelineCMS, Bes-cms, CoolPHP, CPG-Nuke, вебZE, Xaraya, xNuke, XOOPS and other.

Engines without SQL. This branch in the CMS elaboration is developed poorly as application of files instead of database tables for storage causes a lot of problems which solving is very difficult. Its advantage is accessibility for content modification and opportunity to be placed at free of charge hosting. This idea was used by the following companies: Cute News, DeeLight CMS, Progressive, SAPID.

Blog. A “blog” originates from English “weblog”. Russian term – “network diary” – this is a site containing author’s private notes. And the notes are links to the sites interesting to the author, and comments to them. The blog can consist not only of links, but online diary of a user. There following CMS can be related to this category: b2evolution, bBlog, BLOG:CMS, MyPHPblog, Nucleus, pLog, pMachine Free, Serendipity, Textpattern, WordPress, XHP.

Forum – is the instrument for communication on the site. The forum messages are similar to e-mail messages – each has an author, topic and content. However, to send a message within a forum, no additional utility is required – it is just necessary to fill a proper form in the site.

A distinctive feature of the forum is that its messages are combined in threads. When one user answers the message of another one, his answer will be “tied” to the original message. The famous forums are FUDforum, openBB, Phorum, phpBB, PunBB, W-Agora, XMB, Zorum, ExBB, IPB, vBulletin.

Stores. A store is any site at which any goods can be ordered. “Goods” is absolutely everything including the access time to the Internet, minutes of cell connection. The virtual stores created using the CMS: MyMarket, osc2nuke, osCommerce, Zen Cart.

Groupware is the software allowing arranging the operation of a company, relations with clients and customers in the Internet network. Usually, this is completely or partially closed part of the site allowing tracking the implementation time of the set tasks, distribution of roles and time standards. It is also possible to raise the issues for discussion for making decisions by higher management. The following CMS are known: dotProject, eGroupWare, MoreGroupware, phpCollab, PHProjekt.

Training (e-Learning) is a distant form of education through the Internet. The distant learning has being used for a long time in educational systems of a lot of world countries. Its main strategic goal is fast renew of knowledge and effective application of the information. There are a lot of such systems: ATutor, Claroline, LogiCampus, Moodle, Segue, Site@School.

Knowledge Base provides an opportunity to collect the experience of numerous developers. Each database has its own distinctive structure; therefore currently there are no definite common solutions. The most famous knowledge base is RFC [Request For Comment] – request for comment that describes a work with a protocol etc.

Billing. Billing is the software that allows providers and resellers to work with clients’ bills. This kind of the CMS represents an integral part of a large system on recording the services consumed by users. The task of the CMS is to render the information on the provided services, add new services, change current parameters, accept payment etc. Often such systems are developed by own means. The billing-panel RuWEB has a lot of service plans allowing paying only for those hosting parameters (traffic, hard disk space, MySQL, PHP, Perl…) that are used to its full extent.

Administrator hosting panel. This class has such products as Direct Admin and Control Panel.

1.5. Models of CMS data presentation. The frame-based data model uses such definitions as class and object. Classes determine the data structure and represent a set of attributes (text string, round number, image etc.).

The class representatives (objects) have definite structure and can include other objects forming undefined hierarchical structure. The objects can copy the properties, content and behavior of the objects they contain. The objects examples are documents, images, folders and users accounts. The content class does not keep real data – such information is stored in objects (class templates). After determination of one class it is possible to create a lot of its representatives (content objects).

The CMS-systems usually keep data in the relational database. In this case the frame-based data model is reflected on the relational database model. The links between the objects are created, for example, using such tables as id, from_object, from_object_version, to_object [11].

Usually, the systems based on the object-oriented data model are more functional, flexible, but, at the same time, more complex.

The frame-based data model in CMS divides the content into individual modules by the type of content. The data structure depends on the module and all work with content is focused inside the module. The modules do not depend on other systems and are responsible for the work with documents of this type. The documents are described using definite set of parameters – the types of documents are strictly fixed. The functionality can be enlarged by adding a new module, replacement or editing of the existing code. Usually, the documents of different modules and the documents of the same module are not interconnected. The content of standard set of content (modules) types consists of links, articles, files, news, sections and forums. The obvious limitation of data module did not affect the system popularity owing to the simplicity of its application.

These are such systems as PHPNuke, Joomla! and Xoops. The common shortage of the module CMS-systems is a strict fixation of content structure within the module. At the same time to enlarge its functionality it is possible to use external modules. The distinctive advantage of the frame-based systems is obtaining of almost ready to use portal over a short period of time [12].

1.6. Parameters and elements of the web-site of municipal clinic #17. The clinic web-site of MUS on REM "Municipal clinic #17" of the Healthcare Department of Almaty is located on http://gp17.kz.

MUS "Municipal clinic #17" is legal successor of the municipal hospital #2 (hereafter MH #2). Municipal clinic #17 is the polyclinic of mixed type, rendering services for 76500 persons, among them: adults – 55106, children – 21394, kids under 5 years – 8380, women of fertile age – 20238 [13].

The polyclinic uses innovation technologies to render services for population. "Electronic Queuing Systems" are installed at the reception and all departments of the polyclinic where the queues are possible: fluorography, mammography, X-ray, treatment rooms. The time-table of doctors' reception is actualized in the electronic board "Time-table of reception", the changes are automatically copied into the time-table of the clinic web-site.

There are also other online informational services, such as "Prescription of medicines", "Sick notes", "Fluorography card register", "Immunoprophylaxis" that systematize and facilitate significantly the work of medical staff.

Digitization of the healthcare system of Kazakhstan is one of the priority tasks of the project "Modernization of the healthcare system" under the implementation of the President's Address "Third modernization of Kazakhstan: global competitiveness". The topicality of digitization issues is connected with increasing demand of citizens for qualitative medical services [14]. According to the schedule approved on 04.04.2018 by the Mayor Deputy of Almaty, since May the municipal clinic #17 started to transfer to "Damumed" Program. Since June 1, 2018 the polyclinic operates under this Program to full extent. Every citizen registered to municipal clinic #17 has the electron passport of health. Currently, the electron cards are filled with patients' data [13].

The application allows people booking an appointment with a doctor from home, receive the results of analysis, and call the ambulance. All actions of a patient in a clinic are shown in the personal profile of a user and in passport of health, and are accessible at every step of aid rendering.

The home page of the municipal clinic #17 is presented in several blocks: the upper part shows the site name represented by a graphic base and text with organization name.

The web-site has the blocks of right menu and main menu. The download of the homepage takes just a few seconds. There is no Flash screen in the site that is the advantage for the informational site. Colors, types and graphics are in uniform style. The main text is black on grey background. The colors of pages design are in balance.

On the left, closer to the middle part of the home page is the news of the clinic. On the right, under the menu of the home page is healthcare news. In the page bottom, under the blocks of menu and news of the clinic there is a clinic contacts block and different videos.

The web-site also has Damumed representing the integrated medical information system. For more convenient use of the site, there is a private profile option allowing receiving electron service for the registered user and members of his family. According to the National standards requirements the site has visually version.

All pages of the site were developed in uniform style. The considered web-site meets the contemporary requirements of target audience. To find necessary information no more than 3 hyperlinks pass are required. The home page is not overloaded with abundant information. Graphic design is minimal and does not hinder the information apprehension. The quality of graphics is good. The colors are pleasant for eyes. The text is easy readable, and does not merge into the background. The site supports scaling and can operate with any resolution starting from 800x600 pixels and higher. The lower angle of any page always has useful links, roadmap and contact information of the organization.

The web-site content corresponds to its purpose. The web-site has all regulatory acts regarding the clinic activity, time-table, regulations and rules of the polyclinic. All information necessary for a user-patient is easily accessible and found. In addition, the polyclinic site shows news and other medical announcements. The information in the site has no syntax or grammar errors, text abundance is not observed. Information is brief, clear and logically correct.

Thus, the considered site is easy for use, understandable, and its content is structured in details.

Conclusion. The conducted research allows making the following conclusion:

1. Any CMS in its base view is just a frame that can be added with additional functions using plugin

modules. These are also mini-utilities that are integrated with the system and implement necessary functions.

2. The application of the CMS for the site creation solves the following tasks:

– reduces the time for the site development owing to ready solutions, to create a site it is only necessary to connect and make settings;

– further exploitation of the site (filling, editing, deleting) does not require a programmer, the site can be managed by the site owner;

– the site operates on a stable system that is constantly renewed and adapts to new realities.

3. The web-site of the municipal polyclinic #17 developed on the base of the CMS application shows the information on the clinic, allows looking through the list of services, provides detailed information on the rendered services, allows booking an appointment with a doctor, receive the results of analysis online, call ambulance etc. Owing to the information technologies the citizens have continuous access to the polyclinic services, the load on medical personnel decreased significantly, and some other issues hampering the operation of other clinics in the country were eliminated.

4. The web-site of the municipal polyclinic #17 allows improving the level of quality, accessibility of medical aid, satisfaction of population with rendered services, ensures maximum “transparency” and awareness of a patient with the results of diagnostics, treatment, health examination, methods of preventive treatment, and monitoring of his health state dynamics.

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САЙТ МАЗМҰНЫН БАСҚАРУ ЖҮЙЕСІН ҚАЛАЛЫҚ ЕМХАНА МЫСАЛЫНДА ҚОЛДАНУ

Аннотация. Мақалада интернет желісінде сайт құру және басқару кезінде дайын басқару жүйелерін пайдалану мәселелері қарастырылған. Мақалада сайтты әзірлеуге арналған Content Management System (CMS) қосымшасының артықшылықтары мен ерекшеліктері көрсетілген. Бұдан басқа, CMS функционалы, оның технологиялық негізі толық сипатталып, стандартты сайт құрылымы мен қолданыстағы дайын шешімдер, деректерді көрсетудің қолданыстағы үлгілері және олардың жіктелуі көрсетілген. Сондай-ақ сайтты толықтыру, түзету барысында туындауы мүмкін негізгі мәселелер және оларды жоюдың ықтимал жолдары аталған. Мысал ретінде CMS көмегімен құрылған №17 қалалық емхананың сайтына талдау жүргізілген.

Түйін сөздер: контентті басқару жүйесі, ақпараттық технологиялар, сайт құру, қалалық емхана.

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ПРИМЕНЕНИЕ СИСТЕМЫ УПРАВЛЕНИЯ СОДЕРЖИМЫМ САЙТА НА ПРИМЕРЕ ГОРОДСКОЙ ПОЛИКЛИНИКИ

Аннотация. В статье исследуются вопросы применения готовых систем управления при создании и управлении сайтом в сети интернет. В работе отмечены преимущества и особенности применения Content Management System (CMS) при разработке сайтов. Кроме того, подробно описан функционал CMS, его технологическая основа, приведена стандартная структура сайта, описаны существующие готовые решения, приведены существующие модели представления данных, показана их классификация. Также выделены основные проблемы, которые могут возникнуть в процессе наполнения, редактирования сайта, и возможные пути их устранения. В качестве примера, проведен анализ сайта городской поликлиники №17, которая создана с использованием CMS.

Ключевые слова: системы управления содержимым сайта, информационные технологии, сайтостроение, городская поликлиника.

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KRAS MUTATION FREQUENCY AND SPECTRUM IN COLORECTAL CANCER: CORRELATION WITH THE TUMOR LOCALIZATION IN KAZAKHSTANI POPULATION

Abstract. *Aim.* Activating mutation in KRAS oncogene is one of the most significant events in colorectal cancer (CRC) molecular pathogenesis. Along with the success of complex treatment, understanding the CRC genomics due to the extensive use of molecular genetic studies promotes an optimum choice of therapy variants. The aim of this study was to define the frequency and spectrum of KRAS gene mutations in CRC patients depending on the tumor localization for choosing the treatment tactics and predicting the course of the disease.

Method. This retrospective study included 332 CRC patients treated in the Republic of Kazakhstan from 2010 to 2014. Their tumor material was formalin-fixed and waxed and morphologically assessed. KRAS mutation status was established by PCR study.

Results. The mutations were most frequent with rectal cancer (n=82, 55%), followed by left-sided colon cancer (n=43, 28.9%), and right-sided colon cancer (n=24, 16.1%). The mutations were most frequent in codon 12, in particular, G12D – 32.9%, G12V – 24.2%, and G13D – 19.5%.

Conclusion.

1. The obtained results on KRAS mutation frequency correspond to the data published by other researchers.
2. KRAS mutations are more frequent in left-sided colon cancer compared to right-sided colon cancer ($P = 0.001$).
3. There is an upward trend in KRAS mutation frequency with tumor localization in the distal parts of the colon, especially in the rectum.
4. The mutations were most frequent in codon 12, in particular, G12D, G12V, and G13D. G12V mutations were frequent in tumor localization in the rectum.

Introduction. Colorectal cancer (CRC) is a heterogeneous group of tumors which differ in both the mechanisms of carcinogenesis and, therefore, molecular changes, and the prognosis of the disease and the specifics of treatment. Today, choosing tactics for treating patients with metastatic colorectal cancer shall take into account not only clinical factors like tumor spread, patient functional status but also the molecular profile of the disease.

The frequency and spectrum of KRAS gene mutations and their correlation with clinical and morphological features of patients with CRC are widely studied in the literature. Several studies (see the analysis of 551 cases of CRC at diagnosis by Palomba et al.) did not reveal any significant correlation of KRAS gene mutation frequency with the patient’s age, gender, tumor localization and depth of invasion, the degree of malignancy, and the presence of regional or distant metastases [1, 2]. Still, there is evidence that KRAS gene mutates more often in rectal tumors than in tumors in the overlying colon. Some researchers have revealed the relation of the KRAS gene mutation in codon 13 with the stage of the tumor process [3-6].

Thus, the prognostic value of KRAS gene mutation in CRC tumor has not been fully proven in the literature and requires further research.

Purpose of this study was to define the frequency and spectrum of KRAS gene mutations in CRC patients depending on the tumor localization for choosing the treatment tactics and predicting the course of the disease.

Method. *Patients and data extraction.* This retrospective study included 332 patients diagnosed with CRC and registered at the Regional Cancer Centers of the Republic of Kazakhstan in 2010-2014. Their KRAS status was established by PCR study of their post-surgery or biopsy samples.

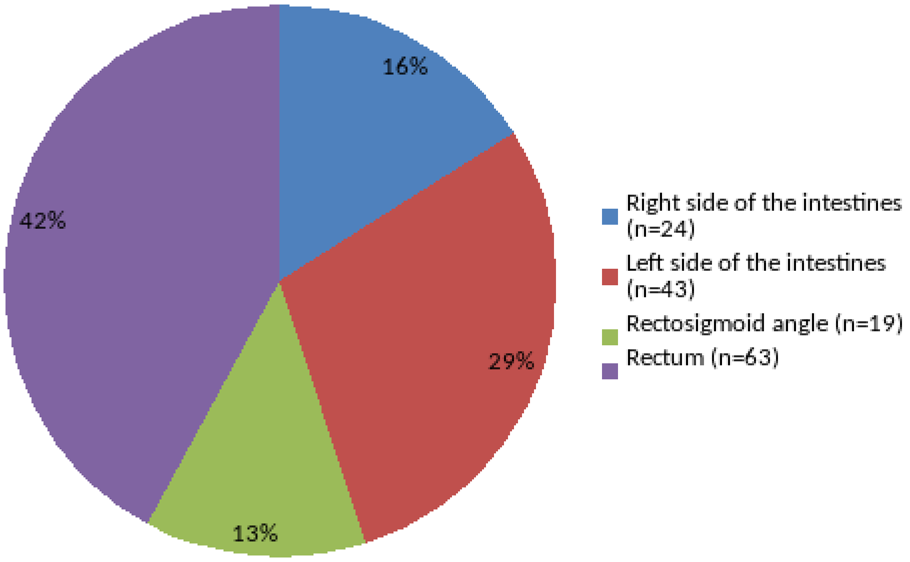
In the study, women (n=182, 54.8%) prevailed over men (n=150, 45.2%). Most of the patients (88%) were aged 44 and above; only 12% were below 44 years. The average age was 56.4 ± 10.5 years (25 to 79 years).

All patients underwent a complete clinical examination, X-ray, CT, ultrasound, MRI of the chest, abdomen, pelvic organs; their CRC diagnosis was confirmed morphologically.

DNA extraction and KRAS mutational analysis. Molecular genetic study of KRAS status was conducted at the Laboratory of Pathomorphology and Molecular Genetics of the Kazakh Institute of Oncology and Radiology. The quality of the obtained materials was assessed morphologically. Depending on the percentage of tumor cells in the sample, the samples were subjected to 3-5 macro dissections and dewaxing for DNA extraction. The samples containing less than 20% of tumor cells were microdissected along the slide zone previously marked by the morphologist to avoid false-negative results. The DNA was extracted using the FFPE DNA extraction kits (QIAGEN, Inc. Valencia, CA). The concentration of the extracted DNA was determined using NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA); DNA quality was assessed using real-time control PCR comparing the results with control DNA. The mutations in KRAS codons 12 and 13 in exon 2 were detected by allele-specific PCR method using BioLink kits.

The statistical processing of data was made using a PC with installed IBM SPSS Statistics 20 package (trial version). Pearson's linear correlation coefficient (r_p) was used to identify the relationship between the variables.

Results. KRAS gene status was determined in tumors localized in different parts of the colon. Of the 332 CRC patients included in the study, 48 (14.5%) patients had right-sided colon cancer (RCC) with tumor localization in the right side of the intestines (cecum, ascending colon, hepatic angle, or transverse colon) vs. 99 (29.8%) cases of left-sided colon cancer (LCC) with tumor localization in the left side of the intestines (splenic angle, descending colon, or sigmoid colon). The remaining 185 (55.7%) patients had a tumor in the rectum, including its rectosigmoid part (figure).



KRAS mutation frequency depending on the tumor localization in CRC (n=332)

Legend:
Blue – Right side of the intestines (n=24)
Red – Left side of the intestines (n=43)
Green – Rectosigmoid angle (n=19)
Violet – Rectum (n=63)

Of all the CRC patients included in the study (n=332), 149 (44.9%) had mutant-type KRAS (mt-KRAS), and 183 (55.1%) had wild-type KRAS (wt-KRAS). Among patients with RCC, the number of mt-KRAS and wt-KRAs cases was the same (n=24, 50%). Among patients with lesions of the left sections of the colon, including the rectum, 125 (44%) patients had mt-KRAS against 159 (56%) with wt-KRAS. KRAS mutations depending on the tumor localization were most frequent with rectal cancer (n=82, 55%) followed by LCC (n=43, 28.9%), and RCC (n=24, 16.1%) (table 1).

Table 1 – KRAS gene status and mutation frequency depending on the tumor localization in colorectal cancer

KRAS status	Right-sided colon cancer (n=48, 100%)	Left-sided colon cancer (n=99, 100%)	Rectal cancer (n=185, 100%)
Wild-type KRAS (n=183)	24 (50±3.1%)	56 (56.6±4.9%)	103 (55.7±3.6%)
Mutation KRAS (n=149)	24 (50±3.0%)	43 (43.4±4.9%)	82 (44.3±3.6%)

For colon tumors (n=67), the KRAS mutation frequency with LCC (n=43, 64.2%±5.8%) was higher than with RCC (n=24, 35.8±5.8%); the difference was statistically significant ($P = 0.001$).

In our study, 120 out of 149 (80.5%) patients with mt-KRAS had mutations *in codon* 12, of which G12D (32.9%) and G12V (24.2%) were the most common. G12D, G12V mutations were especially frequent in rectal cancer (28 out of 49 and 25 out of 36, respectively). G12S and G12C were less frequent (up to 10%). G13D was observed only in 29 (19.5%) of cases (Table 2).

No increase in mutation frequency with the tumor localization in the intestine was observed. The correlation coefficient for the pair "Right intestine – KRAS mutations" was $r_p = -0.042$, $P = 0.06$. The inverse relationship between these variables was very weak (table 2).

Table 2 – Correlation of KRAS gene mutations with tumor localization in colorectal cancer

KRAS mutations	Location												Mutations in codons
	Right-sided colon cancer				Left-sided colon cancer				Rectal cancer				
	n	%	r_p	ρ - value	n	%	r_p	ρ - value	n	%	r_p	ρ - value	
G12A	5	20.8	-0.025	0.05	7	16.3	0.013	0.06	8	9.7	0.006	0.06	20 (13.4%)
G12C	2	8.3	-0.034	0.06	1	2.3	-0.002	0.06	4	4.9	0.026	0.06	7 (4.7%)
G12D	9	37.5	-0.027	0.06	12	27.9	-0.011	0.06	28	34.1	0.029	0.06	49 (32.9%)
G12S	0	0	-0.053	0.06	5	11.6	0.023	0.05	3	3.7	0.016	0.06	8 (5.4%)
G12V	3	12.5	-0.068	0.06	8	18.6	-0.008	0.06	25	30.5	0.056	0.06	36 (24.2%)
G13D	5	20.8	-0.038	0.06	10	23.3	0.016	0.05	14	17.1	0.012	0.06	29 (19.5%)
Total mutations	24 (16.1%)	100	-0.042	0.06	43 (28.9%)	100	0.006	0.06	82 (55.0%)	100	0.025	0.06	149

Discussion and Conclusion. Of the 332 CRC patients included in the study, 149 (44.9%) had mt-KRAS, and 183 (55.1%) had wt-KRAS. Our results correspond to the results obtained in extensive multicenter studies which confirm the 30-50% mutation frequency of KRAS in colon tumors [1, 3, 5-7]. Thus, our results on the frequency of KRAS gene mutation are consistent with data published by other researchers.

The literature sources reported about 97-99% of KRAS gene mutations in codons 12 and 13 compared to 1-3% in other codons. Therefore, we focused our research on these codons as they mutated most often. A mutation was detected at any location of the tumor in the colon, but its frequency varied. We compared the KRAS mutation frequency in rectal cancer with other colon sections, including its right and

left sides. In our study, KRAS mutations in rectal cancer (n=82, 55.0%) were more frequent than in colon cancers of other localizations (n=67, 44.9%). Also, we revealed a statistically significant prevalence of KRAS gene mutations in LCC (n=43, 28.9%) vs. RCC (n=24, the least frequency – 16.1%) ($P = 0.001$). The mutation frequency was also growing with tumor localization in the lower section of the colon, and especially in the rectum (table 1).

The mutations were most frequent in codon 12, in particular, G12D – 32.9%, G12V – 24.2%, and G13D – 19.5%. However, in our study, a high G12V mutation rate was observed in rectal cancer (25 mutations out of 36) (table 2).

The obtained data on mutations in codons 12 and 13 suggests different etiology of carcinogenesis in different parts of the colon.

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ИЗУЧЕНИЕ ЧАСТОТЫ И СПЕКТРА МУТАЦИИ ГЕНА KRAS У БОЛЬНЫХ С КОЛОРЕКТАЛЬНЫМ РАКОМ (КРР) В ЗАВИСИМОСТИ ОТ ЛОКАЛИЗАЦИЕЙ ОПУХОЛИ В МАСШТАБЕ РЕСПУБЛИКИ КАЗАХСТАН

Аннотация. Одним из наиболее значимых событий в молекулярном патогенезе КРР является активирующая мутация в онкогене KRAS. Наряду с успехами комплексного лечения, понимание геномики КРР, благодаря широкому использованию молекулярно-генетических исследований, предоставило возможность оптимального выбора терапевтических опций.

Целью настоящего исследования было определение частоты и спектра мутаций гена KRAS у больных КРР в зависимости от локализации опухоли для определения выбора тактики лечения и прогнозирования течения заболевания.

Материалы и методы. Нами было изучен опухолевый материал 332 пациентов с диагнозом КРР, фиксированный в формалине, заключенный в парафин, проходивших лечение в онкологических диспансерах, онкологических центрах и в Казахском научно-исследовательском институте онкологии и радиологии (КазНИИОиР) за период с 2010 по 2014 годы. После морфологической оценки качества исследуемого материала в лаборатории молекулярной генетики было проведено молекулярно-генетическое исследование по определению мутации гена KRAS методом ПЦР.

Результаты. По полученным нами результатам, мы можем судить, чтонаибольшее количество мутаций гена KRAS было обнаружено при поражении прямой кишки – у 82(55%) из 149 пациентов. Далее по частоте встречаемости мутации занимали левые отделы толстой кишки – у 43(28,9%) пациентов. При поражении правых отделов толстой кишки мутация гена KRAS встречалась в 24(16,1%) случае. Наиболее частые мутации были в 12 кодоне, а именно G12D - 32,9%, G12V - 24,2% и G13D -19,5 %.

Выводы.

1. Полученные нами результаты по частоте встречаемости мутации генаKRAS согласуются с данными, опубликованными в литературных источниках другими исследователями.

2. Мутаций гена KRAS встречается чаще при первичной локализации опухоли в левых отделах по сравнению с правой локализации, (p= 0,001).

3. Прослеживается тенденция к увеличению числа мутации генаKRASс
возрастанием частоты поражения дистальных отделов толстой кишки и
особенно прямой кишки.

4. Наиболее частые мутации были в 12 кодоне, а именно G12D, G12V и G13D. Высокая частота мутацииG12V наблюдалась при локализации опухоли в прямой кишке (25 мутации из 36).

Ключевые слова: колоректальный рак; молекулярно-генетические исследования; мутация KRAS; дикий тип.

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CREATION OF A PROBIOTIC OF A BROAD SPECTRUM OF ACTIVITY ON THE BASIS OF LACTIC ACID AND PROPIONI ACID BACTERIA

Abstract. Information about development of complex probiotics on the basis of lactic acid and propionic acid bacteria of a broad spectrum of activity for veterinary science are provided.During creation of probiotics considered antagonism of the bacteria to clinical strains of causative agents of diseases also ability to biosynthesis of biologically active agents - hydrolytic enzymes, vitamins, irreplaceable amino acids, resistance to low values pH, bile, sublimation drying was considered.It is shown that the created drugs havethe immunostimulating action, increase proliferation of T-lymphocytes, granulocytes, monocytes, increase synthesis of immunoglobulins of class A, increase nonspecific immunity by increasing in lizotsimny activity of serum of blood and also increase concentration of hemoglobin in blood of animals.

The effectiveness of probiotics against causative agents of the mixed intestinal infection,coccidiosis, helminthiases, pasteurellosis of farm animals and birds, of beesdiseases, mastitis at the lactating cows is proved. The presented data indicate the prospects of creationof complex probiotics witch a broad spectrum of activity for prevention and treatment of diseases of farm animals and birds.

Keywords: lactic acid and propionic acid bacteria, probiotics, antagonism, spectrum of action, intestinal infections, pasteurellosis, mastitis.

Infections are the most frequent cause of death of young growth of farm animals and birds, decrease in their productivity. The complexity of treatment of infectious diseases consists in emergence at pathogenic microorganisms of polyresistance to antibiotics. Besides, application of antibiotics, unbalanced feeding and other negative factors lead to significant changes in the microorganismsof the gastrointestinal tract of animals,and residual amounts of therapeutic drugs in animal products negatively influence the human body [1-3]. This proves the need for wider use in the treatment of infectious diseases of probiotics on the basisof the symbionts of the gastrointestinal tract, harmless to humans and animals.

Probiotics in veterinary medicine are used to correct the intestinal biocenosis, immune, hormonal and enzyme systems of young animals. Besides, a probiotics has relevance not only for animal breeding, but also for public health.They reduce the risk of morbidity of people and increase the environmental safety of agricultural products [4-6].

The basis for application of probiotics in veterinary medicine are the positive effects caused by them in an organism of animals. The main effects of probiotics include improved digestion, immunostimulatory effects and increased animal productivity.

Improvement of the digestive processes occurs due to colonization of the intestine with microorganisms of probiotics, which are antagonists of opportunistic enterobacteria, produce biologically active substances. This increases the synthesis of microbial protein and vitamins, increases the absorption of nutrients [7-9].

Literature data indicate the enormous role of the normal intestinal microflora as a factor of nonspecific resistance, which is realized not only through microbial antagonism, but also nonspecific activation of macrophage phagocytic and cytostatic activity, stimulation of lymphoid tissue, and impact on immunocompetent T and B cells [10].

Now the large number of the probiotics for animal breeding consisting of lactic acidand bifidum bacteria, being the main protective group of the microorganisms of intestines harmless to the person and animals are known [11-14].

However the known probiotics did not become an alternative to antibiotics as have insufficiently broad antimicrobic spectrum of action and is used mainly for restoration of normal intestinal microflora.

In this regard, the most important task for receiving effective probiotics against gastrointestinal diseases is the directed selection of strains on their antagonistic activity. Preference should be given to antagonists against pathogens of specific diseases by the selection of strains in the composition of therapeutic drugs.Inclusion in composition of treatment-and-preventivepreparations not only antagonists to the mixed intestinal infections, but also producers of biologically active substances will promote increase in efficiency of their action.

We have developed the probiotic of Laktovit-K on the basis of Lactobacillus brevis B-3 and of Propionibacterium shrmanii 15 differing from known by a wide spectrum of antimicrobial action against the most often found pathogenic and opportunistic microorganisms [15]. The treatment and preventive activity of preparation is established on 10-day goslings, intraperitoneally infected with E.coli serotype 04 at a dose of 1 ml of culture washes with MPA per head. Giving the drug to infected goslings at a dose of 4-6% of the daily feed rate promoted to the recovery of goslings for 6-7 days. The use of the drug as a preventive agent in a dose of 2-4% of the daily feed rate for 5 days made it possible to avoid disease and the death of goslings after infection. In the control group that did not receive the drug, death was observed on days 4–5 after infection and in the following days. At necropsy, congestive hyperemia of the liver, inflammation of the small and large intestines, pericarditis, purulent-fibrinous and myocardial dystrophy were registered.

The high treatment-and-protective efficiency of preparation against intestinal infections is proved also in farms of Almaty region on 64.5 thousand chickens, 250 calfs and 22 pigs. It is established that preparation can be used against a colibacteriosis, salmonellosis¸coccidiosisand other infections [16-18].

The ability of association from lactic acid and propionic acid bacteria also is established to suppress growth of causative agents of diseases of bees: enterobakteriosis, European and American rottenness, ascospherosis [19].

Tests of the Laktovit-K association on bees showed its high efficiency. Thus, when using the preparationin combination with carbohydrate – proteinsupplementation, a decrease in the incidence of bees by an American rottenness was noted by 90.7%, and prevalence a tick of Varroa jacobsoni - for 74.1% [20].

Our researches showed need of constant selection of strains antagonists to again allocated pathogens as because of variability of the last the strains of lactic acid bacteria which are selected earlier can be insufficiently effective.

Under the ISTC grant K-673, the Polylaktovit association was created from the lactic acid bacteria Lactobacillus plantarum 2b, Lactobacillus brevis B-3/21 and the propionic acid bacteria Propionibacteriumshrmanii -5, which is effective in the treatment of mixed enteric infection [21], as well as having a preventive effect against the virus of Newcastle disease [22].

One of the factors of efficiency of probiotics is their resistance to the reactogenic environment of a stomach and upper intestines. In this regard, adaptation of the lactic acid and propionic acid bacteria, belonging to the complex probiotic developed by us,were adapted to low pH and bile values. The variants of bacteria surpassing initial cultures in antagonistic activity, resistance to low values pH and bile are selected[23].

It is established that the studied strains of lactic acid bacteria are steady to pH 3 in the presence of the majority of the tested protective components and adsorbents. Resistance to bile at strains of microorganisms is increased by pectin, food fibersand and the starchentered into composition of nutrient medium in number of 0.5%.

It is shown that preparations from lactic acid and propionic acid bacteria possess the immunostimulating action, increase proliferation of T-lymphocytes, granulocytes, monocytes, increase synthesis of immunoglobulins of class A, increase nonspecific immunity due to increase in lizocimny activity of serum of blood and also increase concentration of hemoglobin in blood of animals [24].

It is established that the resistance to antibiotics at the studied strains of probiotic bacteria is controlled by a chromosome and it cannot be transferred to other microorganisms. The absence of translocation and colonization of the blood and internal organs of experimental rabbits by probiotic microorganisms after giving them developed probiotics has been confirmed. This indicates their safety [25, 26].

Selection of variants of the lactic acid and propionicacid bacteria surpassing initial cultures by 2-3 times in survival at sublimation drying and also on antagonistic activity is made [28, 29].

The new association on the basis of selected variants of Lactobacillus brevis B-3/75, Lactobacillus plantarum 2v/2 and 14d/A-17, Lactobacillus acidophilus 27w and Propionibacterium shermanii S-8 was compiled.It surpasses the association from initial culturesin survival during freeze-drying and antagonistic activity.

The production technology of a liquid and dry probiotic of «Polilaktovit» [30, 31] having high treatment-and-protective efficiency against the mixed intestinal infection and also pasteurellosis is developed.

The efficiency of the receiveddry and liquid preparation is tested on the basis ofLLP «Kaz. RIVS»in vivo on the rabbits infected with salmonellosis and pasteurellosis.

Rabbits of all experimental and control groups were infected with washout of daily agar cultures of reference test strains of Salmonella typhimurium 371, Pasteurella multocida 216 hypodermically to the area of a backat the doseLD_{50}. 24 hours after infection began treatment of animal experimental groups with a probiotic in comparison with an antibiotic. The working solution of a dry probiotic was prepared just before the use. For this purpose 25 g of a dry probiotic dissolved in 100 ml of the boiled and cooled up to 30 ^{0}C drinking water. The liquid probiotic was used withoutdilution.

As a result of the conducted researches the high preventive and therapeutic efficiency of the probiotic «Polilaktovit» both in combination with antibitic, and without it is established. The control rabbits infected with Salmonella typhimurium 371 or Pasteurella multocida 216 and not treated fell on 3 - the 4th day after infection. The experimental rabbits receiving for treatment only per os probiotic on 5 ml 3 times a day at salmonellosis and on 10 ml 3 *ti*mes a day in 15 minutes prior to feeding at pasteurellosis had an improvement of the general state on third day, recovery occurred on the third and fourth day.

At the experimental rabbits receiving within 10 days to infection a dry or liquid probiotic of per os and continuing to receive it on 10 ml 3 times a day in 15 minutes prior to feeding during the experiment without interruption (14 days), insignificant oppression only within the first day after infection was noted. For the 2nd day of symptoms of a disease of salmonellosis and pasteurellosis it was not noted, rabbits did not get sick.

At the rabbits receiving an antibiotic after infection, the state was normalized for the 5th day, the good appetite was noted, symptoms of a disease were not observed.

The best result is noted at treatment of salmonellosis and pasteurellosis at the rabbits receiving at the same time an antibiotic and a probiotic "Polilaktovit". After treatment by an antibiotic of the rabbits (2 injections) infected with salmonellosis and the probiotic set by per os on 5 ml 3 times a day in 15 minutes prior to feeding, animals recovered on the 2nd – the 3rd day after infection. The rabbits infected with pasteurellosis after treatment by an antibiotic (3 injections) and a liquid probiotic on 10 ml 3 times a day recovered for the fourth day.

All rabbits of experimental groups receiving a probiotic during an experiment considerably put on the weight (from 3.3-3.5 to 4.5-6.0 kg). More than others rabbits put on weight (up to 6 kg), a long time receiving a probiotic, beginning in 10 days prior to infection and until the end of experience (24 days).

On the 14th day after infection with salmonellosis and pasteurellosis, 36 rabbits of all experimental groups were slaughtered. As a result of bacteriological research, it was established that after treatment of animals with probiotic in dry and liquid form, the persistence of pathogens in the internal organs of rabbits was not observed.

Test of the dry preparation«Polilaktovit» as treatment-and-prophylactic means against dyspepsia of newborn calfs was carried out in country economy of "Habit".

For tests 30 heads at the age of five days, from them 10 heads of the patients with dyspepsia, and 20 heads for the purpose of prevention are selected. Working solution of a dry probiotic was prepared just before application. The daily dose of preparation for sick animals made 100 ml. To sick calfs received the preparation twice on 50 ml in the morning and in the evening on a hungry stomach within 2-3 days. All calfs recovered, fallen among them was not.

In order to prevent diseases, the preparation was given to calves in 50 ml once a day (in the morning on a hungry stomach),no cases of diseases among them were found.

In the same farm tested the effectiveness of the dry probiotic«Polylaktovit» as a therapeutic and prophylactic agent against dyspepsia in newborn lambs.The studies involved 20 newborn lambs, from whom 10 animals received a probiotic in dose of 15 ml per head for 15 minutes before the first feeding for 3 days.Other 10 animal probiotics did not receive and served as control. As a result of tests it is established that in group of the newborns receiving «Polilaktovit» with the preventive purpose, the incidence was not registered. In control group the number of sick animals made 30%. The probiotic was given to sick lambs 3 times a day on15 ml on the head before feedingto an absolute recovery. Recovery occurred for 2-3 days.

By results of tests the conclusion is made that the probiotic«Polylaktovit» can be used as treatment-and-prophylactic means against dyspepsia of calfs and lambs. At the same time need for application of antibiotics and other antibacterial means for treatment of sick animals disappears.

It is established that the probiotic «Polilaktovit» can be used also for prevention and treatment of mastitis at cows [32].

Research and production experiment was made in the farm «Alipov-T» on 2 groups of the lactating cows of the Alatau breed: the experimental group consisted of 24 heads, the control group included 6 heads. The animals were kept in different bases, the duration of the experience - 2 weeks.

In the experimental group a probiotic solution was applied for the irrigation of the nipples using a spray bottle after removing the milking machine.In the control group sanitary processing of an udder was carried out on the technology used in economy (processing of nipples after milking by the medicine «Dawn»).

It is established that the bactercidal effect of probiotic bacteria after processing is shown in a significant decrease in the number of potential mastitis pathogens – Staphylococcus aureus (by 78%) and Esherichia coli (by 68.9%). This is comparable with similar indicators in the experiment with the chemical agent «Dawn», which is widely used in the farms of the Republic of Kazakhstan and is one of the most effective means for treating the udder.

At the same time, an increase in the total bacterial contamination of the skin of the udder nipples in the experimental group of cows due to the tested probiotic cultures was established. The advantage of probiotic preparation is their environmental safety, low cost, positive biological effect on the skin of the nipples and udder for various damages.

In milk of skilled cows decrease in number of somatic cages which in number over 500 thousand/cm^3 are symptom of the latent mastitis is noted. There is an increase in milk of lactose, fat, casein, SOMO. The offered way of prevention of mastitis surpasses in these indicators of quality of milk opposed.

In Amiran LLP and JSC agrarian and industrial complex Adal the therapeutic efficiency of probiotic means at treatment of subclinical forms of mastitis at cows is studied. To cows of experimental group with subclinical mastitis a probiotic enteredparavaginal in a dose of 10 ml on an animal daily 1 time a day before recovery. The cows of control group treated by the traditional method accepted in farms – injection of mastisan-A to the udder in a dose of 5 ml 2 times a day after morning and evening milking before an absolute recovery.

All animals were treated before double negative reaction of tests of milk with pro-mastitis.

When using with the medical purpose of a probiotic in experimental group of cows the therapeutic efficiency was 90%, the average duration of treatment - 5.2±0.15 days. In control groupapplieding medicine mastisan-A, from 10 cows recovered 8, that made 80%. Recovery of animals happened on average in 6.0±0.82 days. Besides, at 2 cows of control group the latent mastitis in the course of treatment passed into clinical expressed.

The physical and chemical structure of milk was estimated on the content of fat, protein, SOMO and density. Sanitary and hygienic indicators of quality of milk estimated on acidity, a bacterial seedingand by quantity of somatic cages.

At cows of experimental group density and acidity of milk conformed to requirements of state standard, a mass fraction of fat and protein – basic norm. At application of a probiotic milk in the first day after recovery had negative reaction to the inhibiting substances therefore it could be realized without restrictions. When using of medicine mastisan-A containing antibiotics, the last were allocated with milk all term of treatment and four days after recovery.

Thus, the probiotic possesses more expressed therapeutic action at subclinical mastitis at cows in comparison with a widespread traditional way of treatment –introduction of medicine mastitsan-A. At the same time duration of treatment of cows of experimental group in comparison with control is reduced by 0.8 days, the therapeutic efficiency increases by 10%. The probiotic normalizes physical and chemical, sanitary and hygienic indicators of milk and increases its biological value.

Conclusions. The provided experimental data demonstrate prospects of creation of complex probiotics of a broad spectrum of activity for prevention and treatment of diseases of farm animals and birds.

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НЕГІЗІ СҮТ ЖӘНЕ ПРОПИОН ҚЫШҚЫЛЫ БАКТЕРИЯЛАРЫНАН ТҰРАТЫН ӘСЕР ЕТУ СПЕКТРІ КЕҢ ПРОБИОТИКТІ ДАЙЫНДАУ

Аннотация. Негізі сүт және пропион қышқылы бактерияларынан тұратын,ветеринарияға арналған, әсер ету спектрі кең, кешенді пробиотиктер дайындау жөнінде мәліметтер кетірілген. Пробиотиктерді даярлау кезінде, оның құрамына кіретін бактериялардың жеке бір аурудың клиникалық штамдарының қоздырғыштарына қарсы антагонизмі, сонымен қатар биологиялық белсенді заттардың – гидролитикалық ферменттердің, дәрумендердің, алмаспайтын амин қышқылдарының биосинтезіне қабылеттілігі, өт зәрі мен рН-тың төменгі мәніне, сублимациялық жолмен кептіруге төзімділігі ескерілді. Дайын препараттарда, иммунді қарқындататын, гранулоциттердің, моноциттердің, Т-лимфоциттердің пролиферациялануын арттыратын, А класына жататын иммуноглобулиндердің синтезін арттыратын, қан сары суындағы лизоцимдік белсенділікті арттыру арқылы, арнайы емес иммунитетті жоғарлатуға, сондай-ақ жануарлардың қанындағы гемоглобиннің концентрациясын арттыратын әсерлері бар екені көрсетілген.

Пробиотиктің, ауыл шаруашылығы малдары мен құстарында кездесетін аралас ішек инфекциясының қоздырғыштарына; кокцидиозға, гельминтозге, пастереллезге, ара ауруларына, сауын сиырларының маститіне қарсы тиімділігі жоғары болатыны дәлелденді. Келтірілген мәліметтер, ауыл шаруашылығы малдары мен құстарының ауруларын емдеу мен оның профилактикасы үшін әсер ету спектрі кең кешенді пробиотиктер даярлаудың болашағы зор екенін дәлелдейді.

Түйін сөздер: сүт және пропион қышқылы бактериялары, пробиотиктер, антагонизм, әсер ету спектрлі, ішек инфекциялары, пастереллез, мастит.

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СОЗДАНИЕ ПРОБИОТИКА ШИРОКОГО СПЕКТРА ДЕЙСТВИЯ НА ОСНОВЕ МОЛОЧНОКИСЛЫХ И ПРОПИОНОВОКИСЛЫХ БАКТЕРИЙ

Аннотация. Представлена информация о разработке комплексных пробиотиков на основе молочнокислых и пропионовокислых бактерий широкого спектра действия для ветеринарии. При создании пробиотиков учитывался антагонизм входящих в их состав бактерий к клиническим штаммам возбудителей конкретных заболеваний, а также способность к биосинтезу биологически активных веществ – гидролитических ферментов, витаминов, незаменимых аминокислот, устойчивость к низким значениям рН, желчи, сублимационному высушиванию. Показано, что созданные препараты обладают иммуностимулирующим действием, увеличивают пролиферацию Т-лимфоцитов, гранулоцитов, моноцитов, увеличивают синтез иммуноглобулинов класса А, повышают неспецифический иммунитет за счет увеличения лизоцимной активности сыворотки крови, а также увеличивают концентрацию гемоглобина в крови животных.

Доказана эффективность пробиотиков в отношении возбудителей смешанной кишечной инфекции, кокцидиоза, гельминтозов, пастереллеза у сельскохозяйственных животных и птиц заболеваний пчел, мастита у лактирующих коров. Представленные данные свидетельствуют о перспективности создания комплексных пробиотиков широкого спектра действия для профилактики и лечения заболеваний сельскохозяйственных животных и птиц.

Ключевые слова: молочнокислые и пропионовокислые бактерии, пробиотики, антагонизм, спектр действия, кишечные инфекции, пастереллез, мастит.

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RICE miRNAs ARE POTENTIAL REGULATORS OF HUMAN GENES EXPRESSION

Abstract. Plant miRNAs are able to enter the human cells through the gastrointestinal tract with food and to affect the processes occurring in the body like endogenous miRNAs. These exogenous miRNAs can regulate the expression of human genes, affecting various physiological processes. The interactions of miRNAs with mRNAs were calculated using the MirTarget program. Rice miRNAs were chosen, because rice, in comparison with other plants, contains the largest amount of miRNAs and is the most common nutrition of the population. It was found that 32 human genes were targets for 17 osa-miRNAs (miR408-3p, miR408-5p, miR1320-3p, miR1847.1-5p, miR1860-3p, miR2093-3p, miR2102-5p, miR2866-5p, miR2867-5p, miR2868-5p, miR2919, miR2931-5p, miR5075-3p, miR5339-5p, miR5514-5p, miR5534a-5p, miR5833-5p). Detected binding sites were located at 5'-untranslated region (5'UTR), protein-coding region (CDS) and 3'-untranslated region(3'UTR) mRNA target genes. These 32 genes were influenced by human miRNAs. Of particular interest are *RBMS2, KATNAL1* genes with binding sites for several human miRNAs. The mRNA of the *RBMS2* gene had binding sites for seven members of the miR-1273 family. The *KATNAL1* gene was target for 38 miRNAs of the large miR-548 family. Rice miRNA target genes are involved in the development of oncological, cardiovascular and neurodegenerative diseases.

Keywords: miRNA, mRNA, binding site, gene regulation, plant, human.

Introduction. MiRNAs are short RNA sequences, approximately 22 nucleotides in length that binds to targeted messenger RNAs to inhibit protein synthesis [1].On the basis of many studies, it has been shown that plant miRNAs are able to penetrate the blood and host tissues during meals. miRNAs are transferred from cell to cell with blood, being packed in exosomes [2].They are not destroyed by digestive enzymes in the gastrointestinal tract, from where they enter the blood vessels and, as a result, accumulate in the blood and internal organs [3]. Exogenous miRNA that has penetrated the host organism from food can regulate the course of physiological processes in organism by regulating the target genes of the host [4-7]. In the present work, using bioinformatics approaches, the quantitative characteristics of the interaction of rice miRNAs with mRNAs of human genes were predicted.

Materials and methods. The nucleotide sequences of the mRNAs of human genes were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The nucleotide sequences of human and rice miRNAs were taken from miRBase v.22 (http://www.mirbase.org/). The miRNA binding sites in mRNA of several genes were predicted using the MirTarget program [8]. This program defines the following features of miRNA binding to mRNA: a) the start of the initiation of miRNA binding to mRNAs from the first nucleotide of the mRNA's 5'UTR; b) the localization of miRNA binding sites in 5'UTRs, CDSs and 3'UTRs of the mRNAs; c) the free energy of interaction miRNA and the mRNA (ΔG, kJ/mole); d) the schemes of nucleotide interactions between miRNAs and mRNAs.

Results and discussion. Study of 738 osa-miRNAs binding to mRNAs of 17494 human genes revealed that only 32 genes were targets for 17osa-miRNAswith the selection criteria $\Delta G/\Delta Gm$ equal 95% and over. The interaction patterns of the nucleotide sequences of rice miRNAs with the mRNAs of human genes are presented in figure. It can be seen from the schemes that all miRNA nucleotides form hydrogen bonds. The advantage of the MirTarget program is the incorporation of hydrogen bonds in non-canonical pairs of nucleotides U-G, A-C into the free energy of miRNA interaction with mRNA [9].

The largest number of target genes was osa-miR2102-5p. miR2102-5p bound to mRNA of 11 genes (*AFAP1*, *C19orf6*, *CHSY1*, *DIRC2*, *KATNAL1*, *NR1D2*, *PDAP1*, *PPP2R5C*, *RHOBTB2*, *UHRF1BP1*, *WT1*) with energy from -115 kJ/mole to -121 kJ/mole and ΔG/ΔGm value from 95% to 100%.miR2919

<table>
<tr><th>Gene, miRNA, start of site, characteristics of binding</th><th>Gene, miRNA, start of site, characteristics of binding</th></tr>
<tr><td>*PSEN2*, miR1847.1-5p, 1785, 3'UTR, -108, 96, 21
5'-GUGCCACA**G**GCUGCAA**G**CUGCA-3'
||||||||| ||||||||||||
3'-CACGGUGU**U**-GACGUU**U**GACGU-5'</td><td>*C19orf6*, miR2102-5p, 193, CDS, -115, 95, 20
5'-G**C**GGCGGCGGCGGC**CC**GGCCC-3'
||||||||||||||||| |||
3'-C**A**CCGCCGCCGCCG**AA**C-GGG-5'</td></tr>
<tr><td>*KLHDC10*, miR1860-3p, 3134, 3'UTR, -108, 96, 22
5'-AGAGAAAACCGUAGC**C**UCCAGA**C**-3'
|||||||||| ||||||||||||
3'-UCUCUUUUGG-AUCG**A**AGGUCU**A**-5'</td><td>*ZNF442*, miR2866-5p, 1020, CDS, -98, 96, 20
5'-GAUGCUG**G**ACACAAAC**C**AGA-3'
||||||||||||||||||||
3'-CUACGAC**U**UGUGUUUG**A**UCU-5'</td></tr>
<tr><td>*OSTM1*, miR2093-3p, 848, CDS, -93, 96, 20
5'-AUGCAUU**G**AUGUGGAAGAUG**C**-3'
|||||||||| ||||||||||
3'-UACGUAA**U**UA-ACCUUCUAC**A**-5'</td><td>*ATP13A3*, miR2867-5p, 3035, CDS, -115, 95, 22
5'-U**U**GGGAUGUGUGGUGAUGGC**G**CA-3'
||||||||||||| |||||||||
3'-A**G**CCCUACACACC-CUACCG**U**GU-5'</td></tr>
<tr><td>*WT1*, miR2102-5p, 450, CDS, -121, 100, 20
5'-GUGGCGGCGGCGGCUGUGCCC-3'
||||||||||||||| |||||
3'-CACCGCCGCCGCCGA-ACGGG-5'</td><td>*HK2*, miR2868-5p, 6645, 3'UTR, -93, 96, 20
5'-UU**C**CU**G**CUACACAAAAGCCAA-3'
|||||||||||||||| ||||
3'-AA**A**GA**U**GAUGUGUUUU-GGUU-5'</td></tr>
<tr><td>*KATNAL1*, miR2102-5p, 80, 5'UTR, -117, 96, 20
5'-G**C**GGCGGCGGCGGC**C**UGCGCC-3'
|||||||||||||||||| ||
3'-C**A**CCGCCGCCGCCG**A**ACG-GG-5'</td><td>*KIAA1161*, miR2919, 3436, 3'UTR, -106, 98, 19
5'-UCUU**C**CCCCCGCCCCCCCUU-3'
|||||||||| |||||||||
3'-AGAA**A**GGGGG-GGGGGGGAA-5'</td></tr>
<tr><td>*NR1D2*, miR2102-5p, 254, 5'UTR, -117, 96, 20
5'-G**C**GGCGGCGGCGGCG**C**UGCCC-3'
|||||||||||||| ||||||
3'-C**A**CCGCCGCCGCCG-**A**ACGGG-5'</td><td>*BDNF*, miR2919, 2681, 3'UTR, -106, 98, 19
5'-UCUUUCCCCCCUCCCCCU**C**-3'
|||||||||||| |||||||
3'-AGAAAGGGGGGG-GGGGGA**A**-5'</td></tr>
<tr><td>*CHSY1*, miR2102-5p, 348, 5'UTR, -117, 96, 20
5'-G**C**GGCGGCGGCGGCG**C**UGCCC-3'
|||||||||||||| ||||||
3'-C**A**CCGCCGCCGCCG-**A**ACGGG-5'</td><td>*ADAMTS5*, miR2102-5p, 1494, CDS, -113, 93, 20
5'-GUGGCGGCGGCGGCG**CC**GC**U**C-3'
|||||||||||||| ||||||
3'-CACCGCCGCCGCCG-**AA**CG**G**G-5'</td></tr>
<tr><td>*DIRC2*, miR2102-5p, 233, CDS, -117, 96, 20
5'-G**C**GGCGGCGGCGGCG**C**UGCCC-3'
|||||||||||||| ||||||
3'-C**A**CCGCCGCCGCCG-**A**ACGGG-5'</td><td>*UFSP1*, miR2931-5p, 960, 3'UTR, -91, 96, 20
5'-UUUUGACAUCAA**U**AAUAAAAG-3'
|||||||||||||||||| ||
3'-AAAACUGUAGUU**G**UUAUU-UC-5'</td></tr>
<tr><td>*RHOBTB2*, miR2102-5p, 158, 5'UTR, -115, 95, 20
5'-GU**A**GCGGCGGCGGC**C**UCGCCC-3'
|||||||||||||||| ||||
3'-CA**C**CGCCGCCGCCG**A**A-CGGG-5'</td><td>*RPS6KA5*, miR5075-3p, 261, CDS, -121, 98, 21
5'-GCGGACGGCGGCGACGGAG**G**A-3'
|||||||||||||||||||||
3'-CGCCUGCCGCCGCUGCCUC**U**U-5'</td></tr>
<tr><td>*UHRF1BP1*, miR2102-5p, 112, 5'UTR, -115, 95, 20
5'-G**C**GGCGGCGGCGGCUGUG**U**CC-3'
||||||||||||||| |||||
3'-C**A**CCGCCGCCGCCGA-AC**G**GG-5'</td><td>*NR2F2*, miR5075-3p,350,5'UTR,-117, 95, 21
5'-GCGG**G**CGGCGGCG**G**CCGGAGA**G**-3'
||||||||||||||| ||||||
3'-CGCC**U**GCCGCCGC**U**G-CCUCU**U**-5'</td></tr>
<tr><td>*PDAP1*, miR2102-5p, 29, 5'UTR, -115, 95, 20
5'-G**C**GGCGGCGGCGGCUGGCCC-3'
||||||||||||||| ||||
3'-C**A**CCGCCGCCGCCGAACGGG-5'</td><td>*SLC35D1*, miR5339-5p, 795, CDS, -102, 96, 21
5'-UCUGAGAAG**G**UUCUC**C**AUCCUG-3'
||||||||||||||||||| ||
3'-AGACUCUUC**U**AAGAG**A**UAG-AC-5'</td></tr>
<tr><td>*PPP2R5C*, miR2102-5p, 72, 5'UTR, -115, 95, 20
5'-G**C**GGCGGCGGCGGC**CC**GCUCC-3'
|||||||||||||||||| ||
3'-C**A**CCGCCGCCGCCG**AA**CG-GG-5'</td><td>*NANOS1*, miR5534a-5p, 1967, 3'UTR, -106, 96, 21
5'-CCAUUC**C**AGCUGUUG**C**CUGUCA-3'
||||||||||||||||| ||||
3'-GGUAAG**A**UCGACAAC**A**G-CAGU-5'</td></tr>
<tr><td>*AFAP1*, miR2102-5p, 144, 5'UTR, -115, 95
5'-G**C**GGCGGCGGCGGC**CC**CGCCC-3'
|||||||||||||||| ||||
3'-C**A**CCGCCGCCGCCG**AA**-CGGG-5'</td><td>*COX20*, miR5833-5p, 396, CDS, -117, 96, 21
5'-GCCCG**G**UGAGCCCGAGGAGAG**G**-3'
||||||||||||||||||| ||
3'-CGGGC**U**ACUCGGGCUCCUC-C**U**-5'</td></tr>
<tr><td colspan="2">Note: The upper and lower nucleotide sequences of mRNA and miRNA, respectively. The bold type indicates the nucleotide of non-canonical pairs U-G, A-C.</td></tr>
</table>

Schemes of the interaction of osa-miRNA with CDS mRNA human genes had two binding sites in the 5'UTR mRNA of the *ADAMTS5*, *GPBP1L1* genes and two sites in the 3'UTR mRNA of the *BDNF*, *KIAA1161*genes with a ΔG/ΔGm value of 96% and 98%, respectively. For miR5075-3p, binding sites were found in mRNA of the *NR2F2*, *PARP2*, *RPS6KA5* genes. The rest 13 miRNAs (miR408-3p, miR408-5p, miR1320-3p, miR1847.1-5p, miR1860-3p, miR2093-3p, miR2866-5p, miR2867-5p, miR2868-5p, miR2931-5p, miR5339-5p, miR5514-5p, miR5534a-5p, miR5833-5p)could bind to mRNAs of one target gene, with a ΔG/ΔGm value of 95% to 96%. The detected miRNA binding sites were located at 5'UTR, CDS, and 3'UTR.

osa-miRNA target genes are involved in the development of various diseases, including breast cancer (*RBMS2*, *RHOBTB2*, *RPS6KA5*, *WT1*[10-13]), colorectal cancer (*ADAMTS5*, *CHSY1*[14,15]), carcinoma in various organs (*C19orf6*, *PPM1F*, *AFAP1*, *PPP2R5C*, *DIRC2*, *UHRF1BP1* [16-21]), leukemia (*HK2*, *PDAP1*[22, 23]), cardiovascular disease (*NR2F2*, *UFSP1*, *ATP13A3* [24-26]), neurodegenerative diseases (*BDNF*, *PSEN2*, *ZNF442* [27-29]).

It was important to find out whether these osa-miRNA target genes are targets for hsa-miRNA. The effect of 2565 hsa-miRNA on osa-miRNA target genes was studied. The mRNAs of almost all of these target genes could bind hsa-miRNA. The quantitative characteristics of the binding of hsa-miRNAs to the mRNAs of the target genes listed in figure are shown in table 1. Specifically, the *ADAMTS5*, *C19orf6*, *DIRC2*, *GPBP1L1*, *KIAA1161*, *KLHDC10*, *NR1D2*, *NR2F2*, *OSTM1*, *PM20D2*, *PPM1F*, *PSEN2*, *RHOBDB1*, and *RHOBTB1* geneseachhad one binding site to hsa-miRNA. Consequently, osa-miRNAs and hsa-miRNAs can bind simultaneously to enhance their suppression of translation.

Table 1 – Characteristics of hsa-miRNA binding sites in mRNA of human genes

Gene	hsa-miRNA	Start of site, nt	Region of miRNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
1	2	3	4	5	6	7
ADAMTS5	miR-511-5p	2728	CDS	-102	92	21
ATP13A3	miR-182-5p	6258	3'UTR	-110	90	24
ATP13A3	miR-183-5p	6260	3'UTR	-104	92	22
C19orf6	miR-6808-5p	2441	3'UTR	-113	90	22
CHSY1	miR-1273e	3542	3'UTR	-108	93	22
CHSY1	miR-1273g-3p	3499	3'UTR	-108	93	21
COX20	miR-3191-3p	47	5'UTR	-121	93	23
COX20	miR-5096	2121	3'UTR	-106	94	21
COX20	miR-5585-3p	2189	3'UTR	-106	91	22
COX20	miR-619-5p	2047	3'UTR	-117	96	22
DIRC2	miR-1199-5p	178	CDS	-113	93	20
GPBP1L1	miR-574-5p	3012	3'UTR	-117	96	23
HK2	miR-1273g-3p	617	5'UTR	-106	91	21
HK2	miR-1285-3p	770	5'UTR	-106	91	22
KIAA1161	miR-1273g-3p	3476	3'UTR	-108	93	21
KLHDC10	miR-4298	23	5'UTR	-113	90	22
NANOS1	miR-3960	584	CDS	-121	97	20
NANOS1	miR-5096	3285	3'UTR	-106	94	21
NANOS1	miR-551a	4459	3'UTR	-108	94	21
NANOS1	miR-619-5p	3218	3'UTR	-121	100	22
NR1D2	miR-466	3337	3'UTR	-110	95	23
NR2F2	miR-483-5p	2322	CDS	-108	91	22
OSTM1	miR-6880-3p	36	5'UTR	-110	91	21
PDAP1	miR-5096	1998	3'UTR	-106	94	21
PDAP1	miR-619-5p	1925	3'UTR	-121	100	22

Continuation of table 1

1	2	3	4	5	6	7
PM20D2	miR-1273g-3p	2662	3'UTR	-115	98	21
PPM1F	miR-6779-3p	1442	CDS	-104	91	21
PPP2R5C	miR-152-5p	435	CDS	-110	91	23
PPP2R5C	miR-6746-3p	41	5'UTR	-117	92	22
PSEN2	miR-4520a,b-5p	1988	3'UTR	-96	90	20
RHOBTB2	miR-6124	3431	3'UTR	-102	92	20
SLC35D1	miR-5695	2821	3'UTR	-102	91	22
UFSP1	miR-548az-3p	782	CDS	-93	90	21
UHRF1BP1	miR-5096	6450	3'UTR	-104	92	21
UHRF1BP1	miR-619-5p	6378	3'UTR	-119	98	22
WT1	miR-3960	573	CDS	-113	90	20
WT1	miR-466	2704-2714(6)	3'UTR	-106	91	23
WT1	miR-466	2755-2765(6)	3'UTR	-106	91	23

The *COX20*, *NANOS1*, *PDAP1*,and *UHRF1BP1* genes are targets for miR-5096, miR-5585-3p, and miR-619-5p. These miRNAs also belong to a group of unique miRNAs [30, 31]. For example, miR-619-5p targets 201 genes with fully complementary binding sites and more than three hundred that have 98% homology with its nucleotide-binding sites.

miR-5096 and miR-619-5p bound to the mRNAs of the four genes *COX20*, *NANOS1*, *PDAP1* and *UHRF1BP1*. hsa-miR-466 has polysites in two regions in the 3′UTR of *WT1* gene, forming two clusters as in the work [13].

Of particular interest are gene mRNAs with binding sites for several specific miRNAs (table 2). The mRNA of the *RBMS2* gene has binding sites for miR-1273a, miR-1273c, miR-1273e, miR-1273e, miR-1273f, miR-1273g-3p, and miR-1273h-5p. Some members of the miR-1273 family are unique in that they target several hundred genes [32]. Therefore, osa-miR408-3p can compete with the miR-1273 miRNAs to regulate *RBMS2* gene expression.

Table 2 – Characteristics of hsa-miRNA binding sites in mRNA of *RBMS2, KATNAL1*genes

Gene	hsa-miRNA	Startof site, nt	RegionofmiRNA	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
1	2	3	4	5	6	7
RBMS2	miR-1273a	4818	3'UTR	-121	92	25
RBMS2	miR-1273a	5854	3'UTR	-119	90	25
RBMS2	miR-1273c	5856	3'UTR	-110	91	22
RBMS2	miR-1273e	5919	3'UTR	-108	93	22
RBMS2	miR-1273e	4883	3'UTR	-106	91	22
RBMS2	miR-1273f	4873	3'UTR	-100	96	19
RBMS2	miR-1273f	5909	3'UTR	-98	94	19
RBMS2	miR-1273g-3p	5470	3'UTR	-113	96	21
RBMS2	miR-1273g-3p	5876	3'UTR	-113	96	21
RBMS2	miR-1273g-3p	5136	3'UTR	-110	95	21
RBMS2	miR-1273g-3p	4840	3'UTR	-108	93	21
RBMS2	miR-1273g-5p	5500	3'UTR	-108	91	22
RBMS2	miR-1273h-5p	5504	3'UTR	-115	98	21
RBMS2	miR-1285-3p	5453	3'UTR	-108	93	22
RBMS2	miR-1972	5081	3'UTR	-110	90	22
RBMS2	miR-6124	64	5'UTR	-104	94	20

Continuation of table 2

1	2	3	4	5	6	7
RBMS2	miR-6803-3p	7181	3'UTR	-110	90	22
KATNAL1	miR-1273a	3903	3'UTR	-119	90	25
KATNAL1	miR-1273g-3p	3925	3'UTR	-113	96	21
KATNAL1	miR-1972	4492	3'UTR	-113	91	22
KATNAL1	miR-548a-5p	3534	3'UTR	-98	90	22
KATNAL1	miR-548aa	3495	3'UTR	-123	100	25
KATNAL1	miR-548ab	3534	3'UTR	-98	92	22
KATNAL1	miR-548ag	3535	3'UTR	-96	90	21
KATNAL1	miR-548aj-5p	3536	3'UTR	-110	98	23
KATNAL1	miR-548ak	3535	3'UTR	-96	92	21
KATNAL1	miR-548am-5p	3534	3'UTR	-110	100	22
KATNAL1	miR-548ap-3p	3501	3'UTR	-89	100	19
KATNAL1	miR-548ap-5p	3537	3'UTR	-89	95	19
KATNAL1	miR-548aq-5p	3534	3'UTR	-102	92	22
KATNAL1	miR-548ar-5p	3535	3'UTR	-100	98	21
KATNAL1	miR-548as-5p	3534	3'UTR	-106	94	22
KATNAL1	miR-548au-5p	3535	3'UTR	-104	100	21
KATNAL1	miR-548ax	3533	3'UTR	-108	96	22
KATNAL1	miR-548ay-5p	3535	3'UTR	-100	98	21
KATNAL1	miR-548az-3p	3499	3'UTR	-93	90	21
KATNAL1	miR-548az-5p	3535	3'UTR	-104	94	22
KATNAL1	miR-548b-5p	3534	3'UTR	-102	92	22
KATNAL1	miR-548c-5p	3534	3'UTR	-110	100	22
KATNAL1	miR-548d-5p	3534	3'UTR	-106	98	22
KATNAL1	miR-548e-5p	3535	3'UTR	-106	93	22
KATNAL1	miR-548f-5p	3537	3'UTR	-98	92	22
KATNAL1	miR-548g-3p	3497	3'UTR	-93	90	22
KATNAL1	miR-548g-5p	3536	3'UTR	-110	98	23
KATNAL1	miR-548h-3p	3498	3'UTR	-104	91	23
KATNAL1	miR-548h-5p	3534	3'UTR	-104	94	22
KATNAL1	miR-548i	3534	3'UTR	-106	96	22
KATNAL1	miR-548m	3536	3'UTR	-93	90	21
KATNAL1	miR-548n	3535	3'UTR	-98	92	22
KATNAL1	miR-548o-5p	3534	3'UTR	-110	100	22
KATNAL1	miR-548q	3542	3'UTR	-113	95	22
KATNAL1	miR-548t-3p	3495	3'UTR	-123	100	25
KATNAL1	miR-548t-5p	3536	3'UTR	-96	90	21
KATNAL1	miR-548v	3495	3'UTR	-102	91	22
KATNAL1	miR-548w	3533	3'UTR	-108	93	23
KATNAL1	miR-548x-5p	3536	3'UTR	-110	98	23
KATNAL1	miR-548y	3534	3'UTR	-98	90	22
KATNAL1	miR-548z	3498	3'UTR	-104	91	23
KATNAL1	miR-574-5p	4197-4236(20)	3'UTR	-113	93	23
KATNAL1	miR-574-5p	4394-4410(9)	3'UTR	-113	93	23

The *KATNAL1* gene is the targets of miR-1273a and miR-1273g-3p. An important feature of this gene is the binding of its mRNA to members of the large miR-548 family, which consists of 38 miRNAs. miR-548aa, miR-548am-5, miR-548ap-3p, miR-548au-5p, miR-548c-5p, miR-548o-5p, and miR-548t-3p can fully bind to the 3'UTR mRNA of the *KATNAL1* gene. This gene has one another feature: its 3'UTR mRNA contains 20, and through 136 nucleotides, nine sequentially located miR-574-5p binding sites. It remains a mystery why the *KATNAL1* gene is controlled by such a large number of endogenous miRNAs. In another feature of the mRNA of the *KATNAL1* gene, the distance between the nucleotide-binding sites of miR-548h-3p and miR-548h-5p, miR-548az-3p and miR-548az-5p, and miR-548ap-3p and miR-548ap-5p is 36 nucleotides.osa-miR2102-5p binding sitewere found in mRNAs of the *KATNAL1* gene.

It is of interest to determine which human miRNAs can affect the expression of these genes. The calculation results showed that, of the 32 genes, six are not targeted by any of the 2565 hsa-miRNAs. Several genes, in addition to being targets of osa-miRNAs, have the important feature of being targets of hsa-miRNAs. The mRNA of the RBMS2 gene has binding sites for miR-1273a, miR-1273c, miR-1273e, miR-1273e, miR-1273f, miR-1273g-3p, and miR-1273h-5p. Members of the miR-1273 family are unique because some of them target several hundred genes [32]. The *KATNAL1* gene is the target of miR-1273a and miR-1273g-3p. An important feature of this gene is that its mRNA binds to members of the miR-548 family that bind to 38 miRNAs. miR-548aa, miR-548am-5, miR-548ap-3p, miR-548au-5p, miR-548c-5p, miR-548o-5p, and miR-548t-3p can bind fully to the 3'UTR mRNA of the *KATNAL1* gene. This gene has one more peculiarity: the 3'UTR mRNA contains 20, and through 136 nucleotides, nine are sequentially located miR-574-5p binding sites. It is assumed that miRNA binding to3'UTR mRNA can be significant if the gene contains repeats of site sequences as well asin coding region [33].

Conclusion. The present study provides the evidence that rice miRNAs can be transmitted with food to regulate the expression of human genes. Target genes of the osa-miRNAs are also target genes of the hsa-miRNAs.The established binding sites of osa-miRNAs and their target genes allow targeted insertion of exogenous osa-miRNAs with food to regulate their expression of these human genes.

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КҮРІШТІҢ miRNA-ЛАРЫ АДАМ ГЕНДЕРІНІҢ ЭКСПРЕССИЯСЫНЫҢ ПОТЕНЦИАЛДЫ РЕТТЕГІШТЕРІ

Аннотация. Өсімдік miRNA-лары адамның клеткаларына асқазан-ішек жолымен тағаммен бірге ене алады және эндогенді miRNAретінде организмдегі болып жатқан процестерге әсер етеді. Бұл экзогенді miRNA әртүрлі физиологиялық функцияларға әсер ететін адам гендерінің экспрессиясын реттей алады. miRNA-дың mRNA-мен өзара әрекеттесуі MirTarget бағдарламасын пайдала отырып есептелді.Басқа өсімдіктермен салыстырғанда күріштің құрамында miRNA-ның ең көп мөлшері бар және халық үшін ең көп таралған тамақтану көзі болып табылғандықтан күріш miRNA-лары таңдалды. Адам генінің 32-сі 17 osa-miRNA-ларға (miR408-3p, miR408-5p, miR1320-3p, miR1847.1-5p, miR1860-3p, miR2093-3p, miR2102-5p, miR2866-5p, miR2867-5p) 5p, miR2868-5p, miR2919, miR2931-5p, miR5075-3p, miR5339-5p, miR5514-5p, miR5534a-5p, miR5833-5p) нысана екендігі анықталды. Анықталған байланысу сайттары орындары нысана гендердің mRNA-ның 5'- трансляцияланбайтынаймақта (5'UTR), ақуызды кодтайтын аймақта (CDS) және 3'-аударылмайтын аймағында (3'UTR) орналасқан. Бұл 32 гендерге адам miRNA-лары әсер етті. Адамның бірнеше miRNA-лары мен байланысатын сайттары бар *RBMS2* және *KATNAL1* гендеріне ерекше қызығу-шылық туды. *RBMS2* генінің mRNA-дары miR-1273 тұқымдасының жеті мүшесі үшін байланысу сайттары болып табылды. *KATNAL1* гені үлкен miR-548 тұқымдасының 38 miRNA-на нысана ретінде болды. Күріш miRNA-ның нысана гендері онкологиялық, жүрек-тамыр және нейродегенеративті аурулардың дамуына қатысады.

Түйін сөздер: miRNA, mRNA, байланысу сайт, геннің реттелуі, өсімдік, адам.

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miRNA РИСА – ПОТЕНЦИАЛЬНЫЕ РЕГУЛЯТОРЫ ЭКСПРЕССИИ ГЕНОВ ЧЕЛОВЕКА

Аннотация. miRNA растений способны с пищей через желудочно-кишечный тракт попадать в клетки человека и как эндогенные miRNA влиять на процессы, происходящие в организме. Эти экзогенные miRNA могут регулировать экспрессию генов человека, влияя на различные физиологические функции. Взаимодействие miRNA с mRNA рассчитывали с помощью программы MirTarget. Были выбраны miRNA риса, потому что рис по сравнению с другими растениями содержит наибольшее количество miRNA и является самым распространенным источником питания населения. Обнаружено, что 32 гена человека были мишенями для 17 osa-miRNAs (miR408-3p, miR408-5p, miR1320-3p, miR1847.1-5p, miR1860-3p, miR2093-3p, miR2102-5p, miR2866-5p, miR2867 -5p, miR2868-5p, miR2919, miR2931-5p, miR5075-3p, miR5339-5p, miR5514-5p, miR5534a-5p, miR5833-5p). Обнаруженные сайты связывания были расположены в 5'-нетранслируемой области (5'UTR), белок-кодирующей области (CDS) и 3'-нетранслируемой области (3'UTR) mRNA генов-мишеней. На эти 32 гена влияли miRNA человека. Особый интерес представляют гены *RBMS2* и *KATNAL1* с сайтами связывания для нескольких miRNA человека. mRNA гена *RBMS2* имела сайты связывания для семи членов семейства miR-1273. Ген *KATNAL1* был мишенью для 38 miRNA большого семейства miR-548. Гены-мишени miRNA риса участвуют в развитии онкологических, сердечно-сосудистых и нейродегенеративных заболеваний.

Ключевые слова: miRNA, mRNA, сайт связывания, регуляция гена, растение, человек.

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PROSPECTS FOR THE USE OF TREE CROPS IN THE PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS AND PESTICIDES (review)

Abstract. The article presents a review of the literature on the problem of soil contamination with heavy metals and pesticides, natural and anthropogenic factors affecting their bioavailability, plant resistance mechanisms to these pollutants, and prospects for using tree crops in soil phytoremediation technology.

Heavy metals (HM) and pesticides are the most highly toxic environmental pollutants. Currently, the volumes of pollutants containing HM and pesticides are increasing annually. This situation undermines the existing ecological balance and adversely affects people's health. However, some plant species have developed tolerance or resistance to these metals naturally. Some species are able to accumulate high concentrations of HM, but tolerate them; others - reduce their flow due to their barrier functions. The most effective and cost-effective technologies for disinfecting soil and water resources are biological methods, in particular, phytoremediation. Phytoremediation is based on using the ability of plants to accumulate pollutants in aboveground and underground organs and to cause the degradation of xenobiotics in the rhizosphere zone. The primary task in the development of phytoremediation technology is to search for plants capable of accumulating environmental pollutants in the root system, and then translocate them into the aerial part. For this process, fast growing plants are ideal, creating a large biomass in a short period. The most promising species for these studies are tree cultures of the genus *Polus, Salix* and *Paulownia*. The use of these plant species in the technology of phytoremediation of HM and pesticides will reduce the level of pollution and increase the productive value of contaminated soils. Phytoremediation with the use of tree species is a modern, promising environmentally safe and cost-effective technology that can be implemented in large areas.

Key words: heavy metals, pesticides, phytoremediation, tree crops.

Introduction. Nowadays, the problem of contamination with heavy metals and pesticides has a global importance, and recently has acquired particular relevance for Kazakhstan. A preliminary study of the ecological status and rational use of the land funds of the republic showed that there is a strong pollution of the soil with various pesticides, heavy metals and an intensive decrease in its fertility. According to the land balance data, as of November 1, 2017, there are 245.4 thousand hectares of disturbed land in the republic, about 100 thousand hectares of which are subject to radical revegetation. The largest number of disturbed land is located in Karaganda, Kostanay, Mangystau, Akmola, East Kazakhstan, Aktobe, Pavlodar regions.

In all industrial regions, there are environmentally hazardous exposure zones, the total area of which is more than 60 thousand hectares [1]. In addition, in almost every region of our republic there are numerous foci of pollution with obsolete unsuitable for use pesticides - the territory of the former repositories of plant protection products and the surrounding land. More than 1.500 tons of such pesticides and their mixtures are located in warehouses and repositories of the republic, some of which are stored in unsuitable, dilapidated premises. About 10% of them belong to pesticides with POPs properties. Inventory of pesticides with POPs properties covers only 20% of the country [2].

One of the most serious aspects is that the HM, pesticides and their transformation products that have entered into the soil, are absorbed by plants and accumulate in them in concentrations that are dangerous to human and animal health. Discharges of industrial wastes that contain cadmium, lead, copper and chromium pose a potential risk to the aquatic environment, animals and people due to pollution of air, soil deposits, vegetation and water. Contamination with heavy metals causes a number of environmental problems, including a reduction in microbial activity, soil fertility and crop yields. The pollution with heavy metals usually coincides with the growth of industrialization of a given region and becomes more serious when there is neither control nor adequate environmental standards [3]. More than 55.000 different chemical compounds have been found in the environment, many of which can pose a certain hazard to various types of living organisms. Pesticides account for about 3% of this number, but they occupy one of the first places due to their harmful effects on the environment. Excessive use of these agrochemicals, as well as violation of sanitary and environmental requirements of their use, transportation and storage, leads to their sustainable accumulation in environmental objects and creates serious environmental problems [4].

Regarding to this, in order to reduce the environmental risk associated with the accumulation of polluting substances resistant to degradation in the environment, it is necessary to search for new, safe and often unconventional methods to control pollution and to develop a technology for the restoration of polluted soils. For example, phytoremediation using tree species, which is the most economically and environmentally promising, practical and successful technology. Woody species of phytoremediators with high biomass production, deep root system, high growth rate, high ability to grow in poor soils and high ability to accumulate pollutants in aboveground organs, can be an alternative for restoring soil contaminated with HM and pesticides.

The main objectives of this review are to describe the resistance of plants to HM and pesticides, the main methods of bioremediation, to demonstrate the importance of searching tree species as hyperaccumulators of these pollutants and the possibility of their potential use in the process of phytoremediation of soils with high levels of pollution.

The natural and anthropogenic factors affecting bioavailability of heavy metals and pesticides. In the structure of the chemical pollutants of the environment that able to render a significant impact on the health of the population, heavy metals and pesticides occupy a special place. This is due to their persistence in the environmental objects (soil, water, plants), pronounced by biological activity and the ability to migrate and in some cases circulate in natural biocenoses. Heavy metals are a natural component of the lithosphere whose geochemical cycles and biochemical processes have been radically altered by anthropological activity [5].

More than 40 chemical elements of the Periodic system of Mendeleev D.I., whose mass of atoms is higher than 50 atomic units, are heavy metals. The migration of the heavy metals in the agroecosystems is determined by their chemical properties, soil conditions and the biological characteristics of plants. In recent decades, the anthropogenic activity has been intensively involved in the processes of HM migration in the environment. The number of the chemical elements entering the environment as result of technogenesis, in some cases, significantly exceeds the level of their natural intake. For instant, global release of lead from the natural sources is 12 thousand tons per year, and anthropogenic emissions is 332 thousand tons [6]. By engaging in natural migration cycles, anthropogenic flows lead to the rapid spread of pollutants in the natural components of the urban landscape, where their interaction with humans is unavoidable. Volumes of pollutants containing HM annually increase and damage the natural environment, undermine the existing ecological balance and adversely reflects on the human health.

The size of the anthropogenic activity can be judged according to the data below: the contribution of the technogenic lead is 94-97% (the rest is from natural sources), cadmium – 84-89%, copper - 56-87%, nickel - 66-75 %, mercury - 58%, etc. At the same time, 26-44% of the global anthropogenic flow of these elements falls on Europe, and the share of the European territory of the former USSR is 28-42% of all emissions in Europe [7]. The level of technogenic fallout of HM from the atmosphere in different regions of the world is not the same and depends on the availability of production fields, the degree of the mining and concentrating and industrial fields, transport, urbanization of territories, etc.

The application of huge amounts of chemical fertilizers, pesticides, industrial wastes into the soil contributes to the formation of territories with altered soil composition and properties. Micronutrient pollution of the environment poses the greatest danger to industrialized countries. About industrial enterprises form technogenic regions with a high content of lead, arsenic, fluorine, mercury, cadmium, manganese, nickel and other elements in the biosphere, which represent a real danger of direct and indirect effects on the human body.

The processes and phenomena that reduce soil fertility, destroying the land resources of a country, can be divided into 4 groups: natural processes whose adverse effects on the soil surface cannot be prevented; natural processes that a person can to a certain extent prevent or reduce the negative impact on the soil, the treatment of the fields of crop rotation; natural processes, the intensive manifestation of which is due to unreasonable economic activities, such as desertification of territories, and deforestation; phenomena fully associated with human economic activity [8].

Release of heavy metals into the environment has reached a large size. This problem is particularly relevant for Kazakhstan. Metal-mining industrial complexes were built in regions with fertile soils and open water sources for irrigation. As a result, high concentrations of solid metals are found in wastewater, soil, abandoned mines, city dumps and septic tanks. A significant area of land suitable for use in agriculture is also contaminated with metals to an extreme degree and their exploitation is unsafe.

The general source of the pesticide distribution is the processed agricultural lands. In the process of pesticide application, their significant part (until 70%) gets into the soil surface that creates the prerequisites for their migration on soil-water-air and food chains, since soil is the surrounding of the basic accumulation and initial step of the movement of pesticides [9].

Mechanisms of plant resistance to HMs and pesticides. Mechanisms of plant resistance to an abundance of HM can manifest in different directions: some species able to accumulate high concentrations of HMs, but be tolerant to them; others seeks to reduce intake by maximizing their barrier functions.

For most plants, the first barrier level is the roots, where the greatest amount of HMs is retained, the next is the stems and leaves, and finally, the last is the organs and parts of plants responsible for reproductive functions.

One of the reasons for plant resistance to technogenic factors is the transport of HMs and pesticides from the root system to the aboveground part through the xylem [10]. Therefore, when developing the technology of soil phytoremediation, the paramount task is to search for plants capable of accumulating environmental pollutants in the root system, and then translocate them into the aboveground part, i.e. plants with a high coefficient of translocation. Nurzhanova A.A. and co-authors showed in their researches that in species *Artemisia annua* and *Xanthium strumarium* the coefficient of biological absorption of the underground part of plants is an order of magnitude higher than aboveground, and concluded that the root system is an active accumulator of various kind of harmful substances. Reducing the amount of pesticides in the soil also depends on the plant species. For example, some high-accumulating species (*Artemisia annua*, *Kochia scoparia* и *K. sieversiana, Rumex confertus, Erigeron canadensis*) ability to accumulate of pesticides in the rhizosphere zone after the experiment was higher than in the experiment without plants, the species *Xanthium strumarium, Solanum dulcamara* and *Aegilops cylindrical,* on the contrary, was much lower [11].

Despite substantial variability of different plants to the accumulation HMs, bioaccumulation of elements has a definite tendency, allowing them to be ordered in several groups: 1) Cd, Cs, Rb – intensive absorption elements; 2) Zn, Mo, Cu, Pb, As, Co – elements of moderate absorption; 3) Mn, Ni, Cr – weak absorption elements and 4) Se, Fe, Ba, Te – elements hard-to-reach for plants.

Another way for HMs to enter plants is non-root (foliar) absorption from airflows. It occurs when significant precipitation of metals from the atmosphere on the foliar apparatus, most often near the large industrial enterprises. The entry of elements into plants through leaves (or foliar absorption) occurs mainly by non-metabolic penetration through the cuticle. HMs absorbed by the leaves can be transferred to other organs and tissues and be included in the metabolism. Metals that are deposited with dust emissions on the leaves and stems do not pose a danger for human if the plants are thoroughly washed before eating. However, animals eating such vegetation may receive a large amount of HMs.

Heavy metals entering the soil undergo various types of transformation depending on the soil properties and the biological characteristics of the plants. The main factors affecting on the mobility of HMs in the soil, their transformation and availability for plants are the solubility of heavy metal salts, the pH of the soil environment, the content of organic matter in the soil, the particle size distribution and cation exchange capacity, the type of HMs and level of soil pollution by them, species and biological features of cultivated crops. For soils contaminated by HMs, methods that reduce their translocation into plants are based on the translation of heavy metal cations into forms that are not easily available to plants or to mobile compounds, followed by leaching. The most common methods are based on the conversion of metal cations into sedentary forms using high doses of organic fertilizers, liming, phosphate rocking and claying, as well as the use of zeolites [12, 13].

One of the most effective diagnostic indicators of soil pollution is its biological state, which can be assessed by the viability of soil microorganisms inhabiting it. It should also be borne in mind that microorganisms play a large role in the migration of HMs in the soil. In the process of life, they act as producers, consumers and transporting agents in the soil ecosystem. Many soil fungi exhibit the ability to immobilize HMs, fixing them in the mycelium and temporarily excluding from circulation. Studying the peculiarities of Pb, Cd, As and P migration in the soil-plant system under the influence of biological preparations of azotobacterin, phosphobacterin and silicon bacterin allowed to establish patterns of the influence of rhizosphere bacteria on the mobilization and immobilization of HMs and arsenic in the soil-plant system under technogenic conditions, and to identify the main factors affecting their migration, determine their ability to biosorbtion of HMs and arsenic from polluted soils [14].

This has a great practical importance for new biotechnologies in crop production and for phytoremediation of soils.

The interactions that occur in the soil-plant system are complex. The uptake of trace elements by plants varies greatly depending on the condition of the soil. At the same time, high concentrations of metals in the soil do not always indicate a correspondingly high level of these metals in plants. Toxic metal ions penetrate cells using the same processes of absorption of essential trace elements. The amount absorbed by the plant depends on the concentration and speciation of the metal in the soil solution, as well as on its successive movement from the soil to the root surface and from the root to the aboveground part [15]. The movement of metal ions to the aerial part depends on the plant species, metal, and environmental conditions [16]. Their genotoxic effects depend on the oxidation state of the metal, their concentration and the duration of exposure, and they are more pronounced at high concentrations and after a long exposure time [17].

The problem of soil pollution by pesticides is also very relevant for our republic, since in Kazakhstan almost every district has old storage facilities for chemical plant protection products. Tolerant plant species have been identified that are capable to accumulate and degrade the pesticides in the territories of former pesticide storage facilities located in the Talgar district of the Almaty region [18]. The degree of pesticide accumulation by the studied species of annual and perennial plants was different and was their specific feature. It was established that due to phytostabilization and phytoaccumulation, wild-growing species reduced the content of chlorine-containing pesticides to 28%. According to their ability to reduce or increase the concentration of pesticides in the pericheral zone, phytoaccumulators or herbal stabilizers were identified. Common phytoaccumulator plants were the common cocklebur *(Xanthium strumarium),* the sweet wormwood *(Artemisia annua)* and the common ragweed *(Ambrosia artemisifolia).* The root system of these species is able to extract pesticides from contaminated soils and accumulate them in aboveground organs. Such plants as the upland cress *(Barbarea vulgaris),* the ruderal hemp *(Cannabis ruderalis)* and amaranth *(Amaranthus retroflexus)* kept the content of pollutants in the soil at a low level and are phyto-stabilizers.

The main methods of bioremediation of pollutants. Physical, chemical and biological methods can be used for revegetation of contaminated regions. There are technologies that can eliminate and / or reduce the presence of HMs in industrial effluents, such as precipitation and coprecipitation, galvanization and electrocoagulation, membrane separation, solvent extraction, ion exchange, adsorption and biosorption [19].

However, these methods are expensive and have a large negative impact on the environment. The most effective and environmentally friendly are biological methods for cleaning contaminated areas, i.e. bioremediation, which is characterized by low cost, does not include soil removal, and also does not violate the biological and functional integrity of the soil [20].

. The advantages of bioremediation technologies are related to the capabilities of living systems, especially microorganisms, to metabolize a large number of various organic substances, to the softness of impact on the environment being cleaned, which does not lead to significant changes in the basic soil indicators. The disadvantages of soil bioremediation include the low rate of biodegradation of toxicants and the need for a thorough preliminary survey of the contaminated area to clarify the modes of biotechnological work. Bioremediation technologies are divided into different types depending on:

– whether they are carried out directly at the site of pollution or beyond;

– whether or not microorganisms are introduced into the polluted environment.

There are three main approach to bioremediation: biostimulation, bioaugmentation and phytoremediation.

Biostimulation allows the natural accumulation of microbial destructors in freshly polluted soil to be accelerated by the addition of microorganisms. Inserted cells are better adapted to the conditions of a particular habitat and the characteristics of a decomposable hydrocarbon substrate. In this case, samples of natural microbiota are extracted from the soil, which are then cultivated in fermenters to increase their remediation characteristics by adding the necessary growth factors and compounds that induce the biodegradation of the target pollutant. Then such microbiota is brought to the place of pollution.

Bioaugmentation (or bio-enhancement) is a process in which specialized microorganisms heterogenous to a given habitat that have been previously isolated from natural sources or specially genetically modified are introduced into contaminated soil. By such a method, biodegradation of hydrocarbons in the natural environment can be accomplished by stimulating the natural oil-oxidizing microflora by creating optimal conditions for its development (introduction of nitrogen-phosphorus fertilizers, aeration, etc.) or introduction of hydrocarbon-oxidizing microorganisms (introduction of active strains) along with additions of nitrogen, phosphorus, lime, etc.

Phytoremediation based on using the ability of plants to accumulate pollutants in aboveground and underground organs and cause degradation of xenobiotics in the rhizosphere zone is one of the widely used methods of bioremediation. Phytoremediation has long been recognized as a cost-effective method of disinfecting soil and water resources. From an economic point of view, phytoremediation has advantages over the "chemical" and "mechanical" methods of soil remediation, since its implementation does not involve large investments, and operating costs for the implementation of this technology are not large. Most importantly, after phytoremediation, the soil does not lose its fertility. Therefore, this technology is environmentally friendly and cost-effective. In the past few decades, studies of phytoremediation to remove various pollutants from the soil show promising prospects [21, 22].

Phytoremediation can occur by phytoextraction, phytostabilization, phyto-volatilization, phytodegradation, risodegradation, or phyto-degradation [23, 24]. This leads to the absorption of pollutants from the soil or water by the roots of plants, translocation and their accumulation in the aboveground organs. These methods are complementary. Plants can also directly degrade organic pollutants with their enzymes to inorganic compounds that accumulate in the plant. The technology of using plants for the degradation of pollutants is called phytodegradation. It is effective against organic pollutants with good mobility in the plant (herbicides, TNT, trichlorethylene).

Nowadays, about 400 plant species have been identified as accumulators of toxic metals. Some plant species have evolutionarily developed resistant forms that can survive on soils with a high concentration of HMs. Existing natural battery plants, which can accumulate large amounts of HMs, most often grow slowly and have little biomass. For the development and application of technology phytoremediation of great importance is the study of various plant species and the selection of the most promising to clean the soil from pollution.

Currently, studies in this direction have been conducted on fodder, vegetable, leguminous, herbaceous plants. For example, the cultivation of such vegetable crops as summer squash, pumpkin and spinach contributed to a decrease in the concentration of dichlorodiphenyltrichloethane (DDT) in the root zone and the rhizosphere is 10 times compared to the soil contaminated by it [25]. DDT can accumulate in large quantities in the leaves of plants and in small quantities in fruits, as well as in humus with pine needles, where it dissolves in the wax substance of pine needles [26]. The effect of the pH of the root medium on the absorption of the sulfanthrazone herbicide was studied on cotton plants. As a result of the experiments, it was found that neutral pH values contributed to an increase in the absorption of herbicide by plant roots [27]. A comparative analysis of the accumulating ability of various pesticides to cereal and herbaceous plants showed that sunflower and Indian mustard contributed to improving the degradation of pyrene and 2,4DDT in soil along with accumulation of metals. This demonstrates the potential of sunflower and Indian mustard for the simultaneous recultivation of metals, PAHs and organochlorine pesticides in mixed contaminated soils [28].

Chirakkara and Reddy [29] conducted a study on the choice of plants suitable for the simultaneous absorption of phenanthrene, lead, copper and chromium by studying the phytoremediation effectiveness of 9 species of plants. It was found that on soils where sunflower, oats, raigrass, fescue and mung were grown, a significant decrease in phenanthrene was observed, although the plants were distinguished by low survival and biomass. Huang et al. studied 23 *Ricinus communis* genotypes (palmchrist) to restore contaminated soil with cadmium and DDT and found simultaneous accumulation of metal and pesticides with some genotypes, even at higher concentrations than previously reported for other plants [30].

The use of tree crops in phytoremediation technology. The choice of plants for this technology is determined by their ability to transport the groundwater to the surface, to break down polluting compounds with the help of their enzymes and to accumulate these compounds in biomass. For plants that can be used for phytoremediation, it is required to rapidly increase their biomass with simultaneous absorption of large amounts of metals (at least 1-3% of their dry weight).

One of the main risks associated with phytoremediation is the ingress of recovered pollutants into the food chain due to the consumption of plants used for phytoremediation. Therefore, one of the promising areas in phytoremediation programs is the use of tree crops, which can be used in phytoremediation as a barrier to water, preventing leakage of contamination into the depths, and the horizontal distribution of polluted groundwater. As many researchers have noted, fast-growing plants are ideal for this process, creating a large biomass in a short period [31].

Studies to determine the mechanisms of translocation and detoxification mechanisms of herbicides in woody plants are insignificant, although many of them may be of great interest for phytoremediation. Matt A. Limmer [32], in studying the effect of perchlorate on willow *(Salix nigra Marsh),* found that perchlorate concentrations in tree sap were proportional to perchlorate concentrations in groundwater. This will allow further use of this plant species for screening of groundwater contaminated with perchlorate. Chard B.K., et al. [33] conducted a research on the accumulation of trichlorethylene and its transformation products in various organs of apple and peach trees. It was established that the greatest accumulation of these substances was observed in the leaves, then in the branches and fruits. At the end of the study, TCE was found only in the roots. Research has been conducted to better understand the potential transfer of organic compounds to edible fruits. Tyshchenko E.L. and Yakub Yu.F. [34] conducted a research of the royal paulownia as a bioindicator of the degree of soil contamination in the growing areas. It has been established that royal paulownia plants are capable to concentrate heavy and rare-earth metal ions in large quantities in wood. The accumulation of these elements in the plant depends on their content in the soil and the biological availability. The results of the research indicate that royal paulownia is a promising tree crop for inclusion in phytoremediation programs on soils containing both inorganic (HMs) and organic (for example, pesticides) pollutants.

Studies have been conducted on the use of eucalyptus trees for the accumulation of volatile organic compounds [35], studied the mechanisms of absorption and loss of trichlorethylene in growing cypress trees [36], investigated the toxicity of PAH on various types of willows [37].

It should be noted that most studies on the use of tree crops for phytoremediation are carried out on poplars. Apparently, this is due to the fact that they have an extensive dense root system, a high level of transpiration, and a high level of synthesis of degradation enzymes.

Extensive atrazine metabolism studies for phytoremediation, conducted by Burken J.G. and coauthors on hybrid poplars *(Populus deltoides x Populus nigra)*, showed that poplars are able to absorb, hydrolyze, and dealkylate atrazine to less toxic metabolites. These studies prove the possibility of vegetative detoxification of pollutants and suggests that hybrid poplars are a very promising type of plant for the phytoremediation of atrazine-contaminated soils [38]. Laboratory studies have shown that fast-growing and deep-rooted poplars also contribute to the successful restoration of groundwater [39]. Contaminated groundwater is absorbed by plants and the contaminants are then converted into organic molecules used for plant growth. This method has already successfully proven itself to restore soil contaminated with atrazine and groundwater. In a number of studies, the potential of phytoremediation using hybrid poplars of such pesticides as 1,4-dioxane, TCE, chloroacetanilide tetrachlorobiphenyl was studied [40-42].

Currently, a significant amount of research is being conducted on the interaction of microorganisms with plants in the rhizosphere and the possibility of using this to eliminate the environment polluted by pesticides. According to preliminary studies, increased degradation of atrazine, metolachlor and trifluralin was observed in polluted soils, where *Kochia sp.* species grew. Increased degradation occurred in the rhizosphere of this herbicide-resistant plant, which indicates the interaction between plants and microorganisms contribute to increased degradation in the presence of pesticides [43]. Jordahl J.L., et al. proved that poplar rhizosphere is capable of enhancing the growth of microbial populations that are involved in natural bioremediation [44]. In the root zone of *Pinus nigra* and *Salix caprea*, there were detected a polychlorinated biphenyl degrading bacteria which contribute to the decomposition of this organic pollutant [45]. All of these studies suggest that biostimulation through rizoremediation is a promising strategy to increase the degradation of various pollutants.

Plant species that are unintentionally exposed by pesticides are considered to be untargeted plants. The separation of plants into target and non-target is very important for the development of effective phytoremediation technologies, since the so-called “non-target” plants may not have a high pesticide storage capacity. At the same time, carrying out the phytoremediation technology on non-target plants will increase the efficiency of cleaning contaminated areas.

Non-target effects of plants are any direct or indirect effects that affect the survival, health or reproduction of non-target plant species. Non-targeted plants can have either one or a combination of reactions to the detoxification of a pesticide. The development of a detoxification mechanism depends on such factors as the concentration and type of pesticide present, environmental conditions and plant characteristics. Comparison of metabolic pathways, detoxification mechanisms and tolerance of various plants to pesticides will contribute to the development of a vegetative processing system for disinfecting soil and water contaminated with pesticides.

The successful application of phytoremediation technology is based on the use of various models to predict the absorption, translocation and elimination of organic contamination by plants [46]. Gopalakrishnan G. et al. [47] proposed a method for estimating the distribution of chlorinated solvents in soil and groundwater using willow and poplar tree branches. Twigs are potentially more economical and easier than mature trees. This approach is used as a quantitative means of monitoring soil and groundwater for the presence of tetrachlorethylene, trichlorethylene and carbon tetrachloride. A study of the accumulation of polychlorinated biphenyls by hybrid poplars under hydroponic conditions has shown that most PCBs are sorbed by the root system [48]. In the article of Ma X.M. and Burken J.G., the sorption and desorption separation of chlorinated solvents of trichlorethylene, 1,1,2,2-tetrachloroethane and carbon tetrachloride between the air and wood biomass of hybrid poplars was investigated. It has established that the distribution coefficients of compounds between air, water and biomass of wood cores and trunks were associated with the physico-chemical characteristics of pollutants. Tissue analysis and determination of separation factors can provide an effective way to assess the concentration of compounds in a transpiration flow in soil or groundwater in an extremely fast and cost-effective way [49].

Conclusion. Thus, the analysis of literature data showed that tree cultures are a very promising object for carrying out phytoremediation of areas contaminated with HMs and pesticides. Firstly, this is due to the fact that, unlike grass and vegetable crops, woody plants are less eaten by animals and thus reduce the risk of ingress of pesticides into animal products. Secondly, tree crops can be widely used for landscaping polluted areas. Thirdly, they are more useful in detoxifying the drains of various pesticides in order to prevent their penetration into reservoirs. In the fourth, trees prevent soil erosion, improve the appearance of contaminated sites, reduce noise and improve air quality. The most promising species for these studies are tree cultures of the genus Polus, Salix and Paulownia, which have a high phytoremediation potential of pesticides and HM. The use of these types of tree crops, on the one hand, will reduce pollution and, on the other, increase the productive value of polluted soils.

Currently, in the framework of the program-targeted financing of the Ministry of Education and Science of the Republic of Kazakhstan «Comprehensive assessment of unutilized and banned pesticides impact on genetics status and health of population of Almaty region» (BR05236379), we conduct research

on the microclonal reproduction of valuable genotypes of poplars and royal paulownia *in vitro* culture, the study of their phytoaccumulative capacity and the possibility of using for cleaning the soils polluted by HMs and pesticides of Talgar district of Almaty region.

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АУЫР МЕТАЛДАРМЕН ЖӘНЕ ПЕСТИЦИДТЕРМЕН ЛАСТАНҒАН ТОПЫРАҚ ФИТОРЕМЕДИАЦИЯСЫНДА АҒАШ ӨСІМДІКТЕРІН ҚОЛДАНУ ЖЕТІСТІКТЕРІ (шолу)

Аннотация. Мақалада топырақтың ауыр металдармен және пестицидтермен ластану проблемалары, олардың биологиялық қолайлылығына әсер ететін табиғи және антропогендік факторлар, ластаушыларға өсімдіктерге төзімділік механизмдері және топырақтың фиторемедиация технологиясындағы ағаштар дақылдарын пайдалану жетістіктері туралы әдебиеттер шолу жасау қарастырылған.

Ауыр металдар (АМ) және пестицидтер қоршаған ортаны анағұрлым улы ластаушылар болып табылады. Қазіргі уақытта, құрамында АМ және пестицидтері бар ластаушы заттардың көлемі жыл сайын артып келеді. Бұл жағдайлар қазіргі кездегі экологиялық тепе-теңдікті бұзады және халықтың денсаулығына зиянын тигізеді.

Дегенмен, кейбір өсімдіктердің түрлері табиғи жолмен осы металдарға толерантты немесе төзімді екендігі анықталды. Кейбір түрлері өзінің бойына АМ-дың жоғары концентрациясын жинайды, бірақ оларға толерантты екендігні, ал басқа түрлері - өздерінің кедергі жасай алатын қызметі есебінде азаятындығын көрсетеді. Топырақ және су ресурстарын залалсыздандырудың ең тиімді және үнемді технологиялары бірі ол биологиялық әдістер, атап айтқанда, фиторемедиация әдісі болып табылады.

Фиторемедиация өсімдіктердің жер үсті және жер асты органдарының ластауыштарын жинау және ризосфералық аймақта ксенобиотиктердің өзгеріске ұшырауын болдыратын мүмкіндіктерді пайдалануға негізделген. Фиторемедиация технологиясының дамуындағы алғышқы міндеттер - тамыр жүйесінде қоршаған ортаның ластауыштарын жинақтауға қабілетті өсімдіктерді іздестіру, сосын оларды жер үстіне ауыстыру болып табылады.

Бұл үдеріс үшін қысқа мерзім ішінде үлкен биомассаны құрайтын жылдам өсетін өсімдіктер алынады. Осы зерттеулер үшін – *Populus, Salix* және *Paulownia* орман дақылдары болып табылады. Осы аталған өсімдіктерді ТМ және пестицидтердің фиторемедиация технологиясында қолданылу ластану деңгейін төмендетеді және ластанған топырақтың өнімділік құндылығын арттырады.

Ағаш сорттарын қолданып жүргізілген фиторемедиация көптеген аймақтарға енгізілуі мүмкін қазіргі заманғы, перспективалық экологиялық қауіпсіз және экономикалық тиімді технология болып табылады.

Түйін сөздер: ауыр металлдар, пестицидтар, фиторемедиация, ағаш өсімдіктері.

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ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ ДРЕВЕСНЫХ КУЛЬТУР В ФИТОРЕМЕДИАЦИИ ПОЧВ, ЗАГРЯЗНЕННЫХ ТЯЖЕЛЫМИ МЕТАЛЛАМИ И ПЕСТИЦИДАМИ (обзор)

Аннотация. В статье представлен обзор литературы по проблеме загрязнения почв тяжелыми металлами и пестицидами, рассмотрены естественные и антропогенные факторы, влияющие на их биодоступность, механизмы устойчивости растений к данным загрязнителям и перспективы использования древесных культур в технологии фиторемедиации почв.

Тяжелые металлы (ТМ) и пестициды являются наиболее высокотоксичными загрязнителями окружающей среды. В настоящее время объемы поллютантов, содержащих ТМ и пестициды, ежегодно возрастают.

Данная ситуация подрывает существующее экологическое равновесие и негативно сказываются на здоровье людей. Тем не менее, некоторые виды растений развили толерантность или устойчивость к этим металлам естественным путем. Одни виды способны накапливать высокие концентрации ТМ, но проявлять к ним толерантность; другие – снижают их поступление за счет своих барьерных функций. Наиболее эффективными и экономически выгодными технологиями обеззараживания почвенных и водных ресурсов являются биологические методы, в частности, фиторемедиация. Фиторемедиация основана на использовании способности растений накапливать загрязняющие вещества в надземных и подземных органах и вызывать деградацию ксенобиотиков в ризосферной зоне. Первостепенной задачей при разработке технологии фиторемедиации является поиск растений способных аккумулировать загрязнители среды в корневой системе, а затем транслоцировать их в надземную часть. Для этого процесса идеальны быстрорастущие растения, создающие большую биомассу за короткий период. Наиболее перспективными видами для данных исследований являются древесные культуры рода *Populus, Salix и Paulownia*. Применение данных видов растений, в технологии фиторемедиации ТМ и пестицидов позволит снизить уровень загрязнения и повысить продуктивную ценность загрязненных почв. Фиторемедиация с использованием древесных пород является современной, перспективной экологически безопасной и экономически выгодной технологией, которая может быть внедрена на больших территориях.

Ключевые слова: тяжелые металлы, пестициды, фиторемедиация, древесные культуры.

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PROSPECTS AND THE CURRENT STATE OF THE LAKE AND COMMODITY FISHING OF THE NORTH KAZAKHSTAN REGION

Abstract. According to the authors, increasing the efficiency of fish farming in freshwater bodies is becoming increasingly important. One of the main ways of rational highly efficient use of biological resources of inland waters is the organization on their basis of commercial fish farming, including the use of a wide variety of ways and methods of artificial reproduction and rearing of fish in cages, pools and ponds. The transition to lake-based fisheries is one of the main components of sustainable economic and social development of the agricultural sector and rural areas for the Republic of Kazakhstan.

Keywords: fish farming, lakes, fisheries, prospects, ponds.

Introduction. In the Republic of Kazakhstan, for the development of the agro-industrial complex, including the commercial fish farming industry, the "Program for the development of the agro-industrial complex in the Republic of Kazakhstan for 2013-2020" AGROBUSINESS - 2020 "was adopted. As a result of its implementation, strategic goals will be achieved [1].

SWOT analysis of the agricultural industry

Strengths	Weak sides
Kazakhstan occupies the ninth place in the world in terms of the area; Kazakhstan occupies the second place in the world in terms of arable land per capita; Kazakhstan is one of the largest exporters of grain and flour; the large rural population (43% of the total population), a high share of the employed (18% of the employed population); high potential demand for food products from the markets of the CIS and Central Asia; constant growth of gross agricultural product; high potential for production and export of organic products	low share in the country's GDP (4.8%); underdevelopment of trade, including export; low level of implementation of research and development work; insufficient level of veterinary and food safety; high capital intensity; long payback period; dependence on climatic conditions; low labor productivity; low profitability of agricultural producers
Opportunities	Threats
the possibility of increasing the volume of all types of agricultural products due to the growing number and change in the nutritional structure of the population; the formation of effective government support for agricultural producers and agricultural cooperatives; expansion of the geography of deliveries and export volumes in promising sectors	adverse changes in natural climatic conditions, instability of weather conditions; spread of animal and plant diseases and environmental pollution; increased competition in international markets for certain types of products in connection with the entry into the WTO; risk of inefficient government regulation of the industry

The prospects for the development of lake-based commodity fisheries were considered by creating a mathematical model to predict its effectiveness on the example of Lake B. Tarangul, North Kazakhstan Region.

Scientific studies show that a significant increase in fish production in lakes can be achieved by performing the necessary complex of fishery activities.

The ubiquitous transition from fisheries to managed industrial fish farming should be the industry's long-term general program. The feasibility of this approach is confirmed by science and practice [7].

Scientists of NKSU named after M. Kozybaev, employees of the public association "Ecosphere" are doing a lot of work to study the current state of lakes in the North Kazakhstan region. Based on this, scientific reports and publications have been prepared which give an analysis of the hydrochemical, hydrobiological, morphometric, and other conditions of aquatic ecosystems of the North Kazakhstan region [2].

The lake content of the territory of Northern Kazakhstan is one of the highest in the Republic of Kazakhstan and averages 4.6%. In the North Kazakhstan region there are more than 2328 lakes with a water mirror area of more than 4,525 km2. Water bodies differ in their hydrological and hydrochemical parameters, overgrowth, food supply, and the composition of the ichthyofauna. Despite these differences, all of them are a favorable habitat for fish and food invertebrates. At least 1,500 water bodies have fish resources, mainly crucian carp [5].

Biological justification has been carried out for 263 reservoirs of the North Kazakhstan region today classified as fisheries [4]. According to the results of the scientific analysis, all the water bodies presented were recognized as promising for fisheries. The average productivity of reservoirs was calculated according to the methodology presented by Pismennaya OA, 2005. This technique is widely used in biological and economic calculations of the productivity of reservoirs [6].

It should be noted that a characteristic feature of the presented lakes is their periodic drying out and filling in the year and year, and the filling periods are less long, i.e. they are frost-hazardous. In particular, in 70% of undeveloped frost-hazardous water bodies, a low-value, weedy predatory species, the rotan, which is also an omnivorous ichthyophage, has divorced and increases the population. In 80% of the water bodies of the region, fishing is not carried out for several reasons, one of which is overgrowing, the other is the presence of tall fish, i.e. ubiquitous inbreeding is noted, which emphasizes the need for resettlement, in particular, crucian carp from the reservoir to the reservoir to update the population, to reduce the population of tall fish [3].

All the information presented allows us to speak not only about the possibility, but also the need for fishery reservoirs at OTRH. Moreover, the increase in fish productivity of water bodies can be carried out with small capital investments in the organization of production.

A mathematical model has been developed. Using this model, knowing the basic morphometric characteristics of the reservoir, as well as the results of hydrobiological analyzes, it is easy to determine which annual increase in ichthyomass is capable of providing this or that reservoir. Therefore, according to the state of the predicted data, it is possible to identify not only the species of fish that the reservoir can feed, but also the maximum amount necessary for stocking. Since excessive over-accumulation will lead to undermining of the natural forage base of the reservoir, which will inevitably lead to the formation of tall-growing forms of ichthyofauna, and possibly mass killing.

By the quantitative development of zooplankton, Lake B. Tarangul is medium fodder and is characterized as β-mesosaprobic. Also, according to the quantitative development of zoobenthos, the studied reservoir belongs to - mesotrophic.

According to the condition of the food supply, Bolshoi Tarangul Lake is capable of providing an annual increase in ichthyomass of “peaceful” fish up to 60 tons (or 17.8 kg / ha).

It is recommended that 60.5 tons of ichthyomass of “peaceful” fish be removed annually so as not to undermine the natural forage base. However, it must be taken into account that this value is not the total biomass of “peaceful” fish, but only an annual increase. The generally accepted optimal seizure does not exceed 30%, established by leading ichthyologists and prescribed by law.

Consequently: The total biomass of “peaceful” fish is 200 tons.

Estimation of the growth of predator biomass:

$$\Pr e = \frac{(200 - 60{,}5)}{3}$$

Hence, the annual production of predators of ichthyophages in Lake B. Tarangul may be 47.1 tons.

Total annual increase in ichthyomass in polyculture on Lake B. Tarangul may be 107.6 tons.

Speaking about efficiency, first of all, it is necessary to talk about economic efficiency. The developed model allows you to calculate and economic efficiency. Where the costs depend on the volume of withdrawal and on the area of the reservoir. Income is calculated on the basis of wholesale market prices for raw fish (in the case of processing, revenues may increase significantly).

The average annual growth rate of food production in general does not keep pace with the growth rate of consumption and incomes of the population, as a result of which the free market niche is replenished by imports and its share in domestic consumption remains very significant

Also, the program considers such indicators as profit, profitability and break-even point.

If all the necessary conditions are met in the Republic of Kazakhstan, fish productivity will increase significantly, therefore, not only Kazakhstanis will receive fish products, but it is also possible that exports to neighboring countries will increase, which will contribute to food security.

The main goal is the development of the country's fisheries, the creation of conditions for the conservation of valuable fish species and the rational use of fish stocks.

The topic under consideration is certainly important for Kazakhstan, as its expected results can make a significant contribution to ensuring the country's food security.

The increased economic potential of the country, the problem of food security, the projected population growth and increased requirements for the assortment and quality of fish products determine the need to maximize the use of all potential opportunities of freshwater fisheries.

One way to solve the problem of supplying the population with high-grade foods rich in proteins is fishery products, in particular, the development of commercial fish farming. Kazakhstan has a huge variety of ecologically clean water bodies (the total area of water bodies in Kazakhstan, excluding the Caspian Sea, is about 5 million hectares.) On which it is possible to produce environmentally friendly fish products. It should be noted that in the Republic of Kazakhstan, the export of fish products among agricultural crops ranks third after the export of grain crops (wheat and barley).

One of the main reasons for this situation is the underdeveloped system of harvesting and promotion of agricultural products from agricultural producers to sales markets, including agricultural processing enterprises. The increasing role of intermediaries in the sales network almost completely eliminated the relationship between producers and processors of raw materials. Low purchase prices for agricultural products do not stimulate an increase in their production volumes, which ultimately leads to a low share of processing of agricultural raw materials, underutilization of the capacities of processing enterprises and, ultimately, to a high share of food imports. Products of small and medium-sized domestic agricultural producers noticeably lose in quality and packaging and are not able to successfully compete with foreign suppliers.

However, the volumes of fish catch in reservoirs of fishery importance have their limits limited by the natural fish productivity of reservoirs and the ability of commercial fish species to reproduce. These limits have now been reached; an increase in the fishing load and other anthropogenic factors on water bodies does not lead to an increase in the volume of harvested fish resources. The only solution in this case is the development of commercial fish farming.

At the same time, the fish products produced must be competitive: of various assortments, of high quality, accessible to the mass consumer. Hatcheries should be able to provide fish to the population throughout the year and in the required quantities, preferably in a lively and chilled state. At the same time, the applied commercial fish farming technologies must be cost-effective, i.e., capable of ensuring the return on financial resources invested in reconstruction and technical re-equipment.

One of the factors to ensure the quality of fish products is the use of environmentally friendly technologies, which is reflected in the Concept for the transition of the Republic of Kazakhstan to the Green Economy.

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СОЛТҮСТІК ҚАЗАҚСТАН ОБЛЫСЫНЫҢ БАЛЫҚ ЖӘНЕ БАЛЫҚ АУЛАУДАҒЫ ПЕРСПЕКТИВАЛАРЫ МЕН АҒЫМДАҒЫ ЖАҒДАЙЫ

Аннотация. Авторлардың пікірінше, тұщы су қоймаларында балық өсіру тиімділігін арттыру барған сайын маңызды бола түсуде. Ішкі сулардың биологиялық ресурстарын ұтымды және тиімді пайдаланудың негізгі әдістерінің бірі балық аулаудың негізін ұйымдастыру, оның ішінде балықтарды торда, бассейнде және тоғандарда жасанды көбейту мен өсіру тәсілдері мен әдістерінің кең қолданылуы. Көлге негізделген балық аулауға көшу Қазақстан Республикасы үшін агроөнеркәсіптік кешеннің және ауылдық аймақтардың орнықты экономикалық және әлеуметтік дамуының негізгі компоненттерінің бірі болып табылады.

Түйін сөздер: балық шаруашылығы, көлдер, балық шаруашылығы, перспективалар, тоғандар.

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ПЕРСПЕКТИВЫ И СОВРЕМЕННОЕ СОСТОЯНИЕ ОЗЕРНО-ТОВАРНО РЫБНОГО ХОЗЯЙСТВА СЕВЕРО-КАЗАХСТАНСКОЙ ОБЛАСТИ

Аннотация. По мнению авторов, повышение эффективности рыбоводства в пресноводных водоемах приобретает все большую значимость. Одним из основных путей рационального высокоэффективного использования биологических ресурсов внутренних водоемов является организация на их базе товарного рыбоводства, включая в него использование самых разнообразных путей и способов искусственного воспроизводства и выращивания рыб в садках, бассейнах и прудах. Переход на озерно-товарное рыбное хозяйство является одной из основных составляющих устойчивого экономического и социального развития агропромышленного комплекса и сельских территорий для Республики Казахстан.

Ключевые слова: рыбоводство, озера, рыбное хозяйство, перспективы, водоемы.

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In vitro PROTEOLYTIC ACTIVATION OF CARCINOLYTIC PARASPORAL INCLUSIONS OF *Bacillus thuringiensis ssp. israelensis* BACTERIA

Abstract. Upon proteolytic activation, the parasporal inclusions of *Bacillus thuringiensis* bacteria break down into protein monomers (parasporins), some of which have a selective carcinolytic effect. The purpose of the study was to determine the possibility of proteolytic activation of parasporal inclusions under *in vitro* conditions of the culture medium with tumor cell lines in the presence of various concentrations of protease K. These experimental conditions were created to simulate the tumor nidus with regard to the increased concentration of proteolytic enzymes, which is characteristic of metastatic tumors and cancerous degradomes. The bacterial strain *Bacillus thuringiensis ssp. israelensis* was selected for the study as a producer of carcinolytic parasporal inclusions. The experiments were performed using monolayer tumor cell lines: Hep G2 (human hepatocarcinoma), MIA PaCa2 (human pancreatic carcinoma), RD (human rhabdomyosarcoma). Analysis of the cytotoxic effect was assessed by morphological changes in tumor cell cultures. The paper presents the results of determining optimal concentration of the protease K enzyme in tumor cell culture, at which activation of parasporal inclusions is possible without the effect of the cytotoxic action of the proteolytic enzyme itself. Current data can be used for further *in vivo* studies.

Keywords: cell lines, proteases, parasporal inclusions, bacteria.

Introduction. In 1999, Mizuki et al. first examined protein parasporal inclusions from a total pool of 1744 *B. thuringiensis* strains for cytocidal activity against human leukaemia T cells and hemolytic activity against sheep red blood cells. In general, *B. thuringiensis* strains did not have hemolytic activity (1684 strains), but 42 exhibited *in vitro* cytotoxicity against leukaemia T cells. These non-hemolytic, but toxic against tumor T cells strains, belonged to several H serovars, including *dacota, neoleonensis, shandongiensis, coreanensis* and other unidentified serogroups. The purified parasporal inclusions of the three selected strains designated as 84-HS-1-11, 89-T-26-17, and 90-F-45-14 exhibited no hemolytic activity and no insecticidal activity against dipteran and lepidopteran insects, but were extremely cytocidal against tumor T cells and other human cancer cells, demonstrating different toxicity spectra and varied levels of activity. In addition, the proteins from 84-HS-1-11 and 89-T-26-17 were able to discriminate between tumor and normal T cells, specifically killing leukaemia cells. The researchers concluded that these findings may lead to the use of *B. thuringiensis* protein inclusions for medical purposes (Mizuki E., 1999: 477).

Mizuki et al., while continuing to examine an unusual property to recognize human leukaemia cells, related to parasporal inclusions in *B. thuringiensis*, discovered a protein (named parasporin) responsible for this activity. They have subsequently cloned it (Mizuki E., 2000: 625). Parasporin, encoded by a gene 2169 bp long, was a polypeptide consisting of 723 amino acid residues with a molecular weight of 81 kDa. The parasporin sequence contained 5 conserved motifs commonly found in *B. thuringiensis* Cry proteins, but a low level of homology (<25%) was detected between parasporin and the existing classes of Cry and Cyt proteins. Parasporin exhibited cytocidal activity only when degraded by proteases into smaller molecules of 40 to 60 kDa.

The activity of parasporins as carcinolytic toxins of a protein nature has been confirmed in numerous screening studies resulting in the isolation of the most effective agents (Table 1) (Okumura S., 2013: 1889; Lee DW., 2000: 218; Okumura S., 2005: 6313; Poornima K., 2010: 348; Namba A., 2003: 29; Uemori A., 2005: 122; Yasutake K., 2005: 124; Kitada S., 2009: 121; Akiba T., 2009: 121).

Table 1 – Cytotoxic activity of various parasporins against tumor and normal human cells (Okumura S. et al.)

Cell line	Characteristics of cells	LD50 (мкг/mL)			
		Parasporin-1	Parasporin -2	Parasporin -3	Parasporin -4
MOLT-4	T-cell leukaemia	2,2	0,022	>10	0,472
Jurkat	T-cell leukaemia	>10	0,018	>10	>2
HL-60	T-cell leukaemia	0,32	0,019	1,32	0,725
T cell	Normal T cells	>10	–	>10	>2
HepG2	Hepatocellular carcinoma	3,0	0,019	2,8	1,90
HC	Normal hepatocytes	>10	1,1	>10	>2
HeLa	Cervical cancer	0,12	2,5	>10	>2
Sawano	Uterus cancer	>10	0,0017	>10	0,245
TCS	Cervical cancer	–	7,8	>10	0,719
UtSMC	Normal uterus cells	>10	2,5	>10	>2
Caco-2	Colon cancer	>10	0,013	>10	0,124

Studies on the cytotoxic effect of parasporin-2 have showed that unlike parasporin-1, it increases the permeability of the plasma membranes of tumor cells (Ohba M., 2009: 427; Petit L., 1997: 6480). Cytoplasmic lactate dehydrogenase flows out of the treated HepG2 cells, while extracellular propidium iodide enters the cytoplasm. The initial stage of the cytotoxic effect of parasporin-2 is the specific binding of the toxin to a putative receptor protein, not yet identified, which is located in a lipid raft of the plasma membrane of tumor cells susceptible to this protein. This is followed by the formation of oligomers of parasporin-2 in the plasma membranes, which leads to the pore formation and cell lysis (Petit L., 1997: 6480). Oligomerization occurs in the presence of membrane proteins, a lipid bilayer, and cholesterol. It should be noted that substantial homology exists in amino acid sequences between PS2Aa1 and *Clostridium perfringens* epsilon-toxin, whose cell action mechanism involves the toxin oligomerization in lipid rafts and pores formation in the plasma membrane (Petit L., 1997: 6480).

Abe et al. (Abe Y., 2005: 113), while examining the mechanism of action of parasporin-2, found that the toxin binds to the surface of target cells and increases the permeability of the plasma membrane. Subcellular fractionation and immunoblotting of cells treated with the toxin showed that the toxin is associated with lipid rafts and forms SDS-resistant oligomers. The binding and oligomerization of the toxin was inhibited by treating the cells with phosphatidylinositol-specific phospholipase C. The interaction of parasporin-2 with glycosylphosphatidylinositol proteins was therefore required to form an oligomeric toxin that could penetrate the plasma membrane (Abe Y., 2005: 113). Abe et al. (Abe Y., 2008: 269) examined the mechanism of action of parasporin-2 on the human HepG2 cell line (hepatocarcinoma) and showed that this Cry toxin targets lipid rafts and is assembled into oligomeric complexes in tumor cell membrane. The authors concluded that this protein is a pore-forming toxin that accumulates in lipid rafts of tumor cells. Recently, Bokori-Brown et al. (Bokori-Brown M., 2011: 4589) showed that the ε-toxin produced by *Clostridium perfringens* (the etiological agent of dysentery in newborn lambs, enteritis and enterotoxicity in goats, calves, and foals) forms heptameric pores in the membranes of the target cells in the same way as parasporin-2.

It has been found that Cyt1 protein with a molecular mass of 25 kDa isolated from parasporal inclusions of *Bacillus thuringiensis ssp. israelensis* (Bti) bacteria possesses cytotoxic activity against the L1210 murine leukaemia cell line (Yokoyama Y., 1988: 499); in later studies, this parasporin exhibited cytotoxic activity against human tumor cell lines, including MOLT-4, HeLa, and normal T-lymphocytes (Okumura S., 2004: 89; Okumura S. 2013: 1889). In *in vivo* experiments on a transplantable mouse tumor model, Cyt 1 protein turned out to be inactive on its own, but showed a strong potentiating effect in combination with the cytostatic anticancer drug Bleomycin against Ehrlich sarcoma, B16 melanoma, and Meth A fibrosarcoma (Yokoyama Y., 1992: 1079).

Previous studies for determining the acute toxicity of the Bti unactivated parasporal inclusions evaluated LD_{50} equal to 1.0 mg per adult mouse when protein is dissolved in PBS and 0.1 mg if dissolved in 50 mM Na_2CO_3 with intravenous administration (Wendy E. 1983: 181). These findings enable further studies on a transplantable mouse tumor model using non-toxic concentrations of parasporal inclusions.

The acute toxicity and genotoxicity of parasporins has been sufficiently studied. The results of toxicity study in mice of one of the parasporins, namely PS4, were recently published (Okumura S., 2014: 2115). The LD_{50} for PS4 was 160 μg/kg in ICR mice after subcutaneous introduction. Although it is less toxic than the vast majority of bacterial toxins (Gill DM., 1982: 86), it would be rated as highly toxic substance according to the toxicity rating system of Gosselin et al. (Gosselin RE, 1987), since the LD_{50} is less than 1.0 mg/kg. Moreover, the concentrations of cations, creatinine, and urea nitrogen in urine and serum indicate that PS4 impaires kidneys function in mice. A histological evaluation of kidneys in PS4-treated mice showed that protein can cause damage to the renal proximal tubule (Okumura S., 2014: 2115). It should also be mentioned that the LD_{50} value of PS2 was found to be 0.42 mg/kg after intraperitoneal injection in mice (Kyushu Institute of Technology, Japan, not published).

The tumor nidi are characterized by high vascular permeability, which is caused by the immunological process of local inflammation with the formation of pores in vessels having a diameter of up to 2 μm with the normal vascular endothelial pore width of 20 nm (Truskey GA, 2004: 427; Yuan F., 1994: 3352). This circumstance creates an opportunity for penetrating and making a local region of high concentration of crystalline parasporal inclusions having an average size of 0.4-0.7x1.2-1.5 μm (Naoya W., 2005: 988) in tumors after parenteral introduction.

At each stage, including tumor formation, growth, metastasis, and invasion into other tissues, a high concentration of proteases in these nidi is observed, the combination of which determines cancerous degradome, which is usually represented by 5 classes of proteases: serine (trypsin, protease K, etc.), cysteine, aspartic, threonine, and matrix metalloproteinases (Deu E., 2012: 10), thereby representing a natural environment for proteolytic activation of Bti parasporal inclusions.

Materials and Methods. *Bacterial strain and cultivation conditions*. The serotyped *Bacillus thuringiensis ssp. israelensis* (Bti) strain obtained from the collection at the Microbial Depository Center (Yerevan, Armenia), was used in the study. The bacterial strain was grown in MPA culture medium (pH 7) at 30 °C until sporulation was completed (approximately for 48-72 hours). To inactivate vegetative cells and stimulate spore germination, an inoculation loop full of sporulated *B. thuringiensis* bacteria was transferred to 0.5 mL of sterile deionized water, and then heated in a water bath at 75 ºC for 30 min. 0.5 ml aliquots of activated spores were placed in 250 ml of MPB culture medium (pH 7). The culture was incubated for about 24 hours at 30 °C under constant stirring at 250 rpm. The grown culture was stored in a refrigerator at 4 ºC for 4-5 days. By this time, more than 95% of the culture was presented as spores (Okumura S., 2004: 89).

Preparation of a spore-crystal mixture from a B. thuringiensis culture. Crystalline NaCl was added to a sporulated *B. thuringiensis* culture at a concentration of 1M to initiate lysis. The culture was centrifuged at 6,000 x g for 10 minutes at 4 °C, the obtained spore-crystal precipitate was then washed once with 1M NaCl and 2 times with distilled water; the resulting precipitate was further resuspended in an appropriate volume of Tris/KCl buffer (50 mM Tris/HCl, 10 mM KCl, pH 7.5). The spore-crystal mixture was aliquoted and stored at -20 °C until further use (Okumura S., 2004: 89).

Dissolution of protein parasporal inclusions. Parasporal protein inclusions were isolated from cultures by dissolving the spore-crystal mixture in 50 mM Na_2CO_3, 10 mM dithiothreitol (pH 10.5) for one hour at 37 °C under constant stirring at 1400 rpm. Insoluble spores and other artifacts were precipitated by centrifugation at 13,000 x g for 5 minutes. The resulting supernatant contained dissolved parasporal inclusions (Okumura S., 2004: 89).

Tumor cell lines and culture conditions. Tumor cell lines, including Hep G2 (hepatocarcinoma), RD (rhabdomyosarcoma), and MiaPaca-2 (pancreatic carcinoma), were grown in MEM, RPMI, and DMEM media, respectively, with the addition of 10% FBS in the presence of ampicillin (100 μg/mL), at 3 ºC in 5% CO_2. The stabilized cell lines at the 5th passage were used in the experiment (Ohba M., 2009).

Proteolytic activation of parasporal inclusions in tumor cell culture. Activation of parasporal inclusions was carried out in 96-well plates with tumor cell cultures at a concentration of 20,000 cells per well by combined introduction with protease K (Sigma). The enzyme concentration ranged from 100 to 0.75 µg/mL with a 2-fold dilution step. The solvent of parasporal inclusions was used as a negative control, Triton X-100 served as a positive control.

Results and Discussion. During experiments for the determination of the carcinolytic effect of parasporal inclusions isolated from Bti in the presence of protease K, the possibility of activation of parasporal inclusions at non-cytotoxic concentrations of the proteolytic enzyme was revealed [A. Ilin, A. Okassov "The way of cancer cells damage by Bacillus bacteria origin crystal proteins" Patent application number: 2019/0685.1 from 17.09.2019]. Data on optimal concentrations of protease K in tumor cell lines are presented in table 2.

Table 2 – *In vitro* cytotoxic concentrations of protease K in the presence and absence of Bti parasporal inclusions

Cell line	Cytotoxic concentration of protease K in the absence of parasporal inclusions, µg/mL	Cytotoxic concentration of protease K in the presence of parasporal inclusions, µg/mL
Hep G2	6	3
MiaPaca-2	6	1,5
RD	6	1,5

The control smear of the Bti sporulating culture with visible parasporal inclusions in the apical parts of the cell is shown in figure 1.

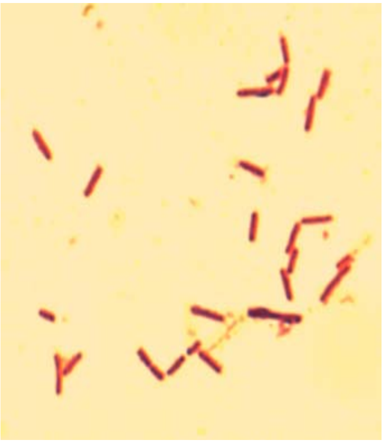

Figure 1 –
Sporulating culture
of *Bacillus thuringiensis ssp. israelensis*.
The Gram staining method (100x)

The presence of dark inclusions in the apical parts of cells indicates the initial stage of sporulation, these inclusions are crystalline Cry proteins (parasporal inclusions), the monomers of which (parasporins) have a carcinolytic effect.

The morphological picture of the cytotoxic effect of protease K and protease K in the presence of parasporal inclusions against Hep G2, MiaPaca-2 and RD cell lines is shown in figures 2, 3 and 4, respectively.

The observed pattern of changes in the morphology of tumor cells when parasporal inclusions operated in combination with protease K is characteristic of parasporins (Kim H., 2000: 16), which indicates the successful activation of parasporal inclusions by protease K. The similarity of changes in cell cultures indicates the universality of the action of activated parasporins irrespective of the cell line.

It can be seen from the above results that morphological changes in cells under the action of parasporal inclusions in the presence of a proteolytic enzyme differs sharply from the pattern of the cytotoxic effect of protease K. The rounding of cells in experimental samples is characteristic of the action of parasporins, which carry out their cytotoxic effect by changing the intracellular osmotic pressure through oligomerization in cell membranes, followed by the formation of transmembrane pores in raft domains (Yuich A. 2017: 71).

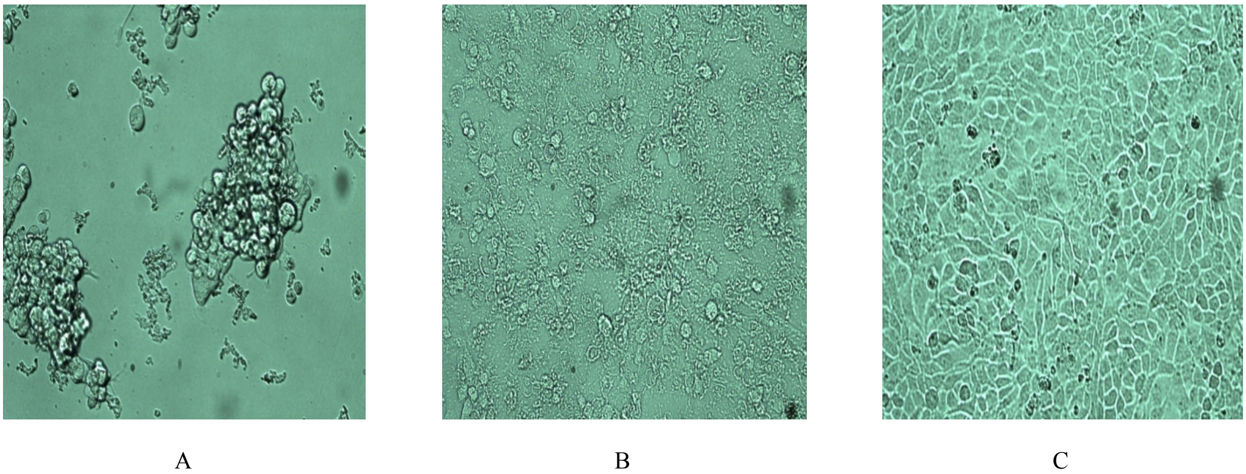

Figure 2 – Morphological picture of Hep G2 cell lines (40x):
A – cytotoxic effect of protease K (6 μg/mL) against Hep G2 cell line; B – cytotoxic effect of parasporal inclusions in the presence of protease K (3 μg/mL) against Hep G2 cell line; C – control Hep G2 cell line

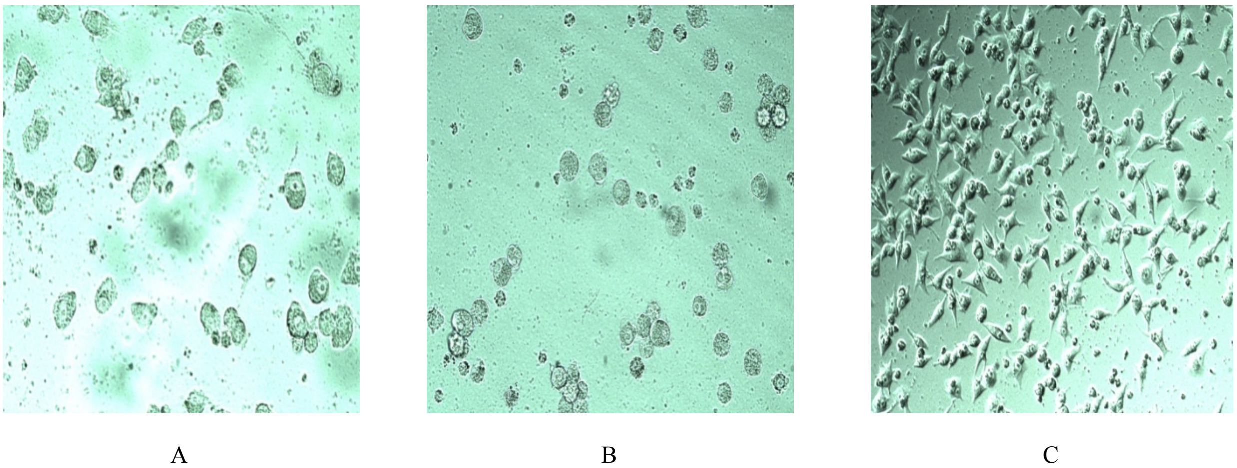

Figure 3 – Morphological picture of Mia Paca-2 cell lines (40x):
A – cytotoxic effect of protease K (6 μg/mL) against Mia Paca-2 cell line; B – cytotoxic effect of parasporal inclusions in the presence of protease K (1.5 μg/mL) against Mia Paca-2 cell line; C – normal Mia Paca-2 cell line

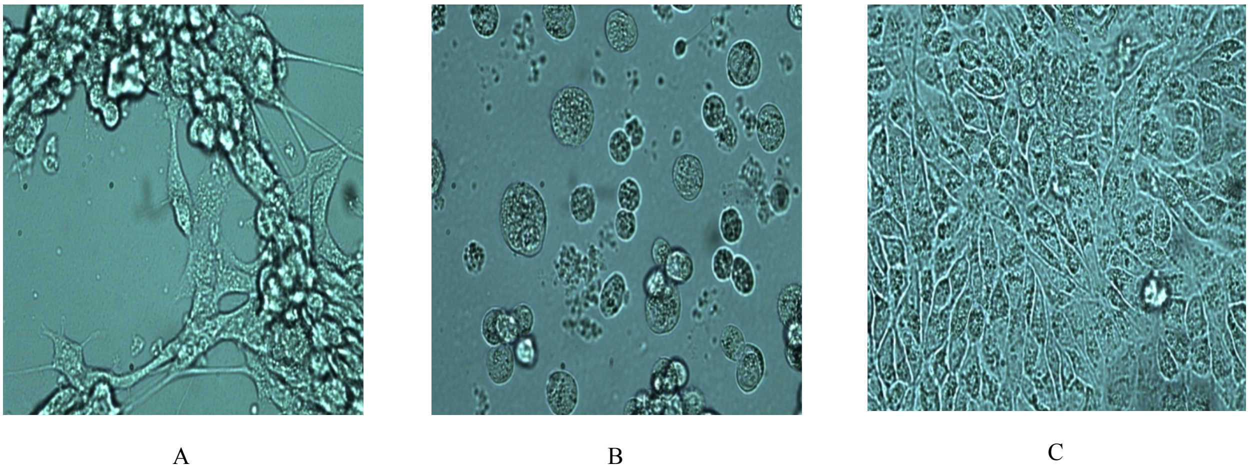

Figure 4 – Morphological picture of RD cell lines (60x): A – cytotoxic effect of protease K (6 μg/mL) against RD cell line; B – cytotoxic effect of parasporal inclusions in the presence of protease K (1.5 μg/mL) against RD cell line; C – normal RD cell line

A four-fold decrease in the cytotoxic concentration of protease K when combined with parasporal inclusions to manifest the cytotoxic effect of parasporal inclusions was observed against Mia Paca-2 and RD cell lines, and a two-fold decrease was recorded for Hep G2 cell line, as compared with the cytotoxic concentration of the proteolytic enzyme of 6 μg/mL.

Conclusion. To date we have not found published data on the precise concentrations of each of protease classes in the tumor nidus and similar studies on modeling tumor nidi. But due to one of the reasons for conditionality of the degradation of the tumor stroma and subsequent process of metastasis caused by a high concentration and cytotoxic effect of proteases (Cao H., 2016: 1099), it can be assumed that the protease concentration values are close to those necessary for activation of *in situ* parasporal inclusions.

The resulting data are the basis for further *in vivo* experiments using inactivated Bti parasporal inclusions by intravenous introduction at concentrations below toxic in order to create a local high concentration of activated parasporins in the tumor nidus without distribution of the latter among organs and tissues, thereby reducing the overall toxic effect.

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ПРОТЕОЛИТИКАЛЫҚ *Bacillus thuringiensis ssp. Israelensis* БАКТЕРИЯНЫҢ ПАРАСПОРАЛДЫ БІРІГУЛЕРІН БЕЛСЕНДІРУ *in vitro*

Аннотация. *Bacillus thuringiensis* бактерияларының параспоралды бірігуі, протеолитикалық белсендіру арқасында ақуыз монемерлерыне (параспорины) ыдырайды, жоғарыдағылардің кейбіріне селективті канцеролитикалық әрекет бар. Жұмыстың мақсаты: *in vitro* жағдайында ісік жасушаларының әр түрлі концентрациядағы протеза К қатысуымен, параспоралды бірігуідің протеолитикалық белсенділігін анықтау. Бұл тәжірибелік жағдайлар метастаздық ісіктерге және қатерлі тозу процестеріне тән протеолитикалық ферменттердің шоғырлануының жоғары бөлігінде ісік қалыптасуының фокусын модельдеу үшін жасалған. Зерттеу үшін канцеролитикалық параспоральдық қосындылардың алдын-ала расталған өндірушісі ретінде *Bacillus thuringiensis ssp israelensis,* бактерия штамдары таңдап алынды. Тәжірибе моноқабатты ісік сызығы: Hep G2 (адамның гепатокарциномасы), МАА ПаCa2 (адамның асқазан безінің қатерлі ісігі), RD (адамның рабдомиосаркомасы) ды қолдану арқылы жасалды. Мақалада протеолитикалық ферментердің цитотоксикалық әрекетсіз параспоралды бірігуі арқылы ісік жасушаларындағы протеаз К ферментерінің оңтайлы концентрациясын анықтау нәтижелері көрсетілген. Цитотоксикалық әсерді талдау ісік жасушаларының морфологиялық өзгерістерімен бағаланды. Зерттеу нәтижелері болашақ *in vivo* експерименттерде қолдану мүмкіндігі бар.

Түйін сөздер: клеткалық қыралар, протеазалар, параспоральдық қосындылар, бактериялар.

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ПРОТЕОЛИТИЧЕСКАЯ АКТИВАЦИЯ КАНЦЕРОЛИТИЧЕСКИХ ПАРАСПОРАЛЬНЫХ ВКЛЮЧЕНИЙ БАКТЕРИЙ *Bacillus thuringiensis ssp. israelensis in vitro*

Аннотация. Параспоральные включения бактерий *Bacillus thuringiensis* при протеолитической активации распадаются на белковые мономеры (параспорины), некоторые из которых имеют избирательное канцеролитическое действие. Целью работы явилось определение возможности протеолитической активации параспоральных включений в *in vitro* условиях культуральной среды с линиями опухолевых клеток в присутствии различных концентраций протеазы К. Данные условия эксперимента создавались для моделирования очага опухолевого образования в части повышенной концентрации протеолитических ферментов, характерной для метастазирующих опухолей и раковых деградомов. Для исследования был выбран продуцент канцеролитических параспоральных включений бактериальный штамм *Bacillus thuringiensis ssp.*

israelensis. Эксперименты проводились с использованием монослойных опухолевых линий: Hep G2 (гепатокарцинома человека), MIA PaCa2 (панкреатическая карцинома человека), RD (рабдомиосаркома человека). Анализ цитотоксического действия оценивался по морфологическим изменениям культур опу-холевых клеток. В статье представлены результаты исследования определения оптимальной концентрации фермента протеазы К в культуре опухолевых клеток, при которой возможна активация параспоральных включений без влияния цитотоксического действия самого протеолитического фермента. Текущие данные могут быть использованы для проведения дальнейших исследований *in vivo*.

Ключевые слова: клеточные линии, протеазы, параспоральные включения, бактерии.

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DISTRIBUTION OF TICKS OF THE GENUS DERMACENTOR KOCH, 1844 (IXODIDAE, AMBLYOMMINAE) IN THE SOUTH-EASTERN PART OF KAZAKHSTAN

Abstract. Based on the analysis of retrospective data (literature sources and collections of ixodid ticks of the RSE “Institute of Zoology” SC MES of the Republic of Kazakhstan and RSE “KSCQZD named after M. Aikimbaev” of the Ministry of Health of the Republic of Kazakhstan), as well as of own collections on the territory of Almaty, Zhambyl and Turkestan regions, the range boundaries of *Dermacentor* genus: *D. marginatus, D. niveus, D. pavlovskyi, D. reticulatus* within the south-eastern part of Kazakhstan were clarified. Significant differences in tick’s distribution between modern data and studies of the last century have been identified, which is probably due to the active human activity in this region. In the south-eastern region of Kazakhstan, *D. marginatus* is distributed mainly in foothill zones. Most of it lives in the foothills of Zailiysky and Zhetysu Alatau (Almaty region). This species is less common in the foothills of the Western Tien Shan - the Karatau ridge (Turkestan region) and the Talas ridge (Zhambyl region). The area of *D.niveus* covers desert and semi-desert territories, mainly floodplains of the Shu, Ile, Syrdariya rivers and their tributaries. *D. pavlovskyi* ticks found in the foothills of the Western Tien Shan, Karatau ridge. Their finding in 1944 by the Republican Tropical Station on small cattle in the Almaty region is probably accidental. The range of *D. reticulatus* in south-eastern Kazakhstan is limited only by the foothills of Zailiysky and Zhetysu Alatau. Finding a species in the Zhambyl and Turkestan regions is probably erroneous.

Key words: ixodid ticks, pathogens vectors, ectoparasites collections, ticks ranges, parasite fauna.

Introduction. It’s known that blood-sucking ticks are vectors of especially dangerous for humans and animals bacterial, viral, and protozoal diseases, such as tularemia, borreliosis, babesiosis, tick-borne encephalitis, Lyme disease, and horse and cattle Lyme disease [1].

In addition, in the Kyzylorda region, listeria and pasteurella were isolated from ticks of the *Hyalomma* genus. On the territory of the Volgograd region of Russia adjacent to the West Kazakhstan region, the antigen of the West Nile fever virus was detected in *Hyalomma scupense* ticks. Ticks *Rhipicephalus pumilio* are vectors of rickettsia - pathogens of Astrakhan tick-borne spotted fever.

Currently, the main problem is the lack of modern information on the status of the fauna, ecology and biology of ixodid ticks in the south and south-east of Kazakhstan, which are unsuccessful for especially dangerous zoonotic infections. Intensive studies of ixodid ticks in Kazakhstan and, in particular, in the southern region, were carried out in the middle of the last century. No such studies are currently underway. There is only information on some dangerous zoonotic infections transmitted by ixodid ticks [2]. In foreign countries, ixodid ticks are given close attention [3-5] for the above reasons. However, these studies are regional in nature, the results of which cannot always be used for the territory of South Kazakhstan. One of the urgent tasks of modern acarology is to conduct a complete inventory of the modern fauna of ixodid ticks with a clarification of the ecology, abundance, distribution and role in biocenoses as vectors of infectious zoonoses. Our studies can form the basis for the development of recommendations for sanitary-epidemiological organizations.

The study of ixodid ticks is of undoubted national and international significance. The range of some species of ticks covers both the territory of Kazakhstan and neighboring regions. In this regard, there is a likelihood of ticks spreading zoonoses of humans and animals, both to the territory of the republic and beyond.

Every year in Kazakhstan, especially in the south and south-east of the country, hundreds of cases of bites of people by blood-sucking ticks are recorded, among which there are cases with a fatal outcome from the diseases transmitted by them. Therefore, the study of ixodid ticks in Kazakhstan can be significant in solving many socio-economic problems.

Studying the issues of fauna, biology, distribution of ixodid ticks and many others can contribute to the further development of Kazakhstan's acarological science, as well as improving the sanitary-epidemiological situation in the south of the country and increasing the level of public health, as a result of which significant social and economic effects can be achieved [6].

According to the latest data, the world fauna of ticks has 896 species belonging to 3 families, while the Ixodidae family includes 702 species (14 genera) [7].

Deep and detailed research in Kazakhstan was carried out by I.G. Galuzo in the 1940-50s. According to him, the fauna of ixodid ticks of Kazakhstan counted 63 species belonging to 6 genera *(Hyalomma, Haemaphisalis, Rhipicephalus, Dermacentor, Ixodes, Boophylus*) [8]. Since then, significant changes have occurred in the systematics of ixodid ticks, and according to modern authors, who rely mainly on literature and collection materials in Kazakhstan, 42 species of ixodids belonging to 5 genera are known. Until the mid-70s of the last century, in-depth studies were carried out by G.V.Ushakova, A.V. Levit and other scientists-acarologists of Kazakhstan. On this, information about the study of ixodid ticks in the last century of Kazakhstan is exhausted.

In connection with the aggravation of the situation with tick-borne infections, the study of ixodid ticks has recently been very relevant. It is known that the territory of the south and southeast of Kazakhstan is unsuccessful for some zoonotic infections (tularemia, tick-borne encephalitis, Crimea-Congo hemorrhagic fever, etc.), which are transmitted by ixodid ticks. One of the main vectors of tularemia in the foci are ticks of the genus *Dermacentor* Koch, 1844 (*D. marginatus, D. niveus D. reticulatus*) [9-12]. In the foci of the Crimea-Congo hemorrhagic fever, along with ticks of the genus *Hyalomma, Dermacentor niveus* is one of the main vectors of the virus [13]. In this connection, the study of the distribution of ticks of the genus *Dermacentor* in the south and southeast of Kazakhstan is of no small importance.

Material and methods. The material for the work was our own collection of ixodid ticks in the territory of Almaty, Zhambyl and Turkestan regions, research materials on ticks for tularemia, reported data and collections of ticks from Taldykorgan, Zhambyl and Shymkent anti-plague stations, literary sources and collections of RSE "KSCQZD named after M. Aikimbaev"[14] and RSE "Institute of Zoology" MES RK. In our research on the collection and study of ixodid ticks, we use the technique proposed by N.A. Filippova [15]. In total, in 2018 we collected and studied 2460 individuals of *Dermacentor* genus ticks.

The geographic coordinates of tick collection sites were determined by using a GPS navigator. All information on sampling was recorded in workbooks, and then in an electronic database (EDB). Diagnostics of the specific belonging of ticks was carried out using stereoscopic microscopes. The electronic database is compiled in the Excell program. Locations of ticks are reflected on electronic maps using the ArcGIS 10 program.

Research results. The world fauna counts 34 species of ticks of the genus *Dermacentor* [7]. In his monograph, I.G. Galuzo for the fauna of Kazakhstan notes 8 species of ticks of the genus *Dermacentor*: *D. daghestanicus* Olen., 1927, *D. marginatus* Sulz., 1776, *D. nuttalli* Olen., 1927, *D. pavlovskyi* Olen., 1927, *D. pictus* Herm., 1804, *D. raskemensis* Pom., 1946, *D. silvarum* Olen., *D. variegatus kamtschadalus* Neum., 1908 [8], and the last three are not indicated for the territory of Kazakhstan. Taking into account the changes in the systematics of ixodid ticks that have occurred over the past half century, the fauna of Kazakhstan has 7 species of ticks of this genus: *D. niveus* Neum., 1897, *D. marginatus* Sulz., 1776, *D. nuttalli* Olen., 1927, *D. pavlovskyi* Olen ., 1927, *D. reticulatus* Fabr., 1794, *D. silvarum* Olen., *D. ushako-*

vae Fil., Pan., 1987 [16]. In our work, we present information on 4 species of ticks of the genus *Dermacentor* that live in the south and southeast of Kazakhstan: *D. niveus* Neum., 1897, *D. marginatus* Sulz., 1776, *D. pavlovskyi* Olen., 1927, *D. reticulatus* Fabr ., 1794.

***Dermacentor marginatus* (Sulzer, 1776).** Initially, was discovered by D.I. Blagoveshchensky [17] on dogs in the territory of South and South-East Kazakhstan, in 1931 in the city of Dzharkent, then, in 1932 on humans and cattle in the Alakul district (Uch-Aral and Ostrovka villages) of Taldy-Kurgan region and the village. Dzharkent on the dog. In subsequent years (1933-34), the species was found on horses and cattle in the Taldy-Kurgan region, Ili district, Alma-Ata region, the outskirts of the cities of Alma-Ata and Dzharkent and Chui district of the Dzhambul region. In 1937, D.I. Blagoveshchensky found this species on horse and human in the Taldy-Kurgan and October districts of the Taldy-Kurgan region and in the Kegen district of Alma-Ata region. From 1940 to 1948, ticks were found in the Alma-Ata, Aksuysky, Karatalsky, Taldy-Kurgan, Andreevsky, Sarkand, Lepsinsky, Oktyabrsky, Alakulsky districts of Taldy-Kurgan oblast and Alma-Ata, Iliysky, Chiliksky, Kugalinsky and Dzhambulsky districts of Alma-Ata oblast, Kelesky, Leng Tulkubassky, Sairamsky, Dzhuvalinsky and Karatassky regions of the South Kazakhstan region, and in the Dzhambul region.

Our studies noted the species in the Baidibek (vicinity of Almaty, Baizhansay, Zharyksay), Tolebi (floodplain of the Syrdaria River, SNNP "Sayram-Ugam" Sairam-Su gorge of Turkestan region. In the Almaty region is registered in the Sarkand (foothills of Zhetysu Alatau) and Balkash (upper reaches of the Ile river) regions (figure 1).

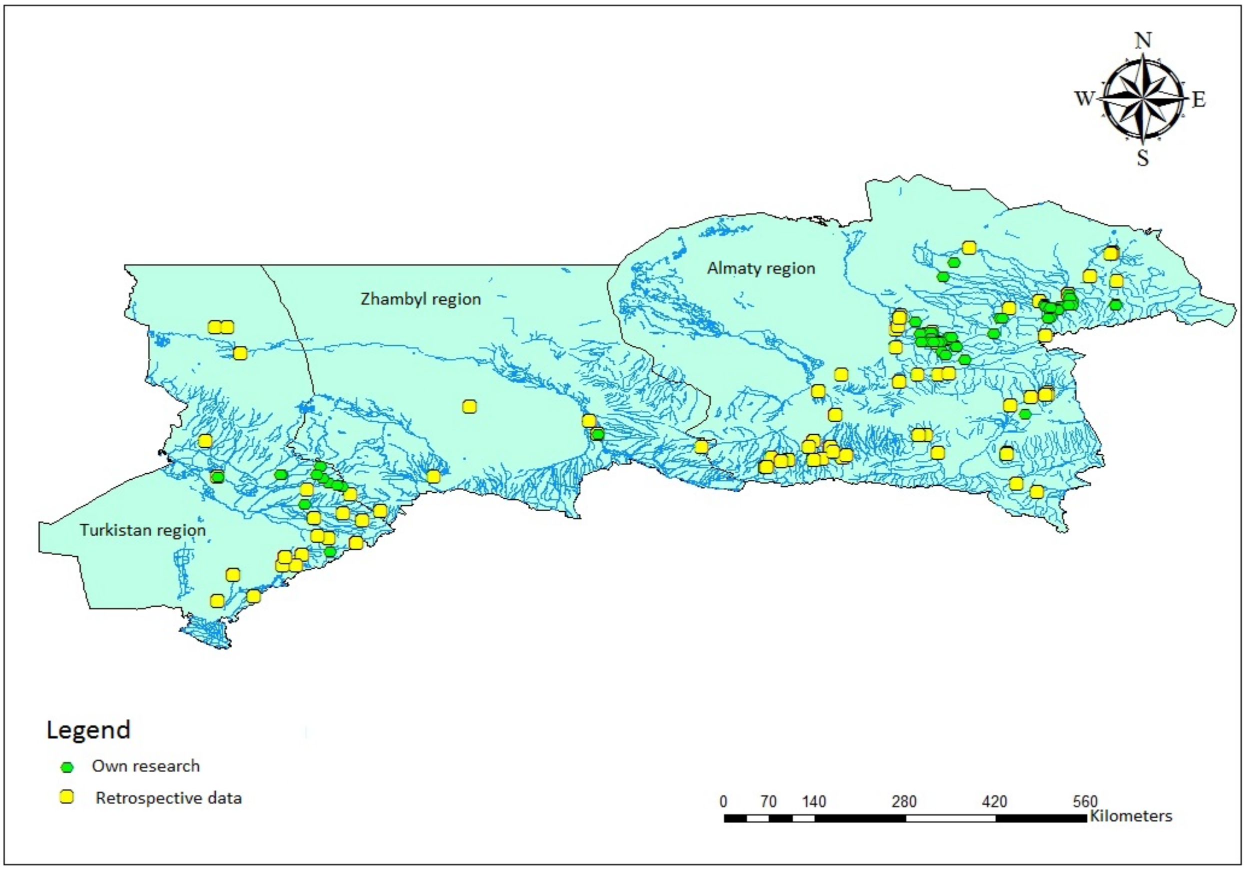

Figure 1 – Locations of finds of ticks *Dermacentor marginatus*

Material: 875♀♀, 832♂♂, 22 N, 58 L. studied. A total of 1787 individuals of *D. marginatus* own collections and from collection funds of the RSE "Institute of Zoology" SC MES of the Republic of Kazakhstan, RSE "KSCQZD named after M. Aikimbaev" Ministry of Health of the Republic of Kazakhstan, RSI "Shymkent Anti-Plague Station" of the Committee for Quality Control of Goods and

Services of the Ministry of Health of the Republic of Kazakhstan, RSE “Taldykorgan Anti-Plague Station” of the Committee for Quality Control of Goods and Services of the Ministry of Health of he Republic of Kazakhstan.

Dermacentor niveus **Neumann, 1897.** The available information about the initial finds of this species in Kazakhstan dates back to 1905 in the vicinity of the Alma-Ata city. Later, in 1929, the species was found there. In 1934, *D. niveus* was found in the Oktyabrsky, Kegen and Chui districts of the Alma-Ata region. Since the 40s of the last century, a detailed study of this species has begun. A number of researchers found the species in Enbekshi-Kazakh, Alma-Ata, Ili, Chilik, Kegen, Uigur Balkhash and Dzharkent regions of Almaty region, Kelesky, Lenger, Sairam, Turkestan, Suzak, regions of South Kazakhstan region, Aksuysky, Karatalsky, October Alakolsky, Burlyu-Tyube districts of Taldy-Kurgan region, Kurdai district of Dzhambul region. In the 50s Levit A.V. finds this species around Bilyli-Kul lake (Zhambyl region), Enbekshi-Kazakh and Balkhash regions of Almaty region, Zhambyl and Moyynkum regions of Zhambyl region. In the early 60s, Ushakova found ticks in the Balkhash district of Alma-Ata and in the Arys region of the South Kazakhstan region. In the mid-60s, studies of Karabayeva R. the ticks were collected in the Moyunkum district of the Zhambyl region, the Leninsky and Lenger regions of the South Kazakhstan region. In the late 60s, the species was found by G.Gubareva in Balkhash and by V.N. Senotrusova in the Karatalsky districts of the Alma-Ata region. The latest information we have about the findings of *D. niveus* dates back to 1970. I.G. Prygunova, K.A. Dzhanokmen, G.A. Kravets and others in the Balkhash district of Alma-Ata region (collections of the RSE “Institute of Zoology” of the National Academy of Science of the Republic of Kazakhstan).

Since 2006 of the present century, our studies of *D. niveus* have been noted in the Kerbulak and Balkash districts of the Almaty region; in the vicinity of Turkistan, Sozak, Baidibek, Otyrar, Ordabasynsky, Arys, Maktaralsky districts of the Turkistan region; Sarysu, Moyunkum districts of the Zhambyl region (figure 2).

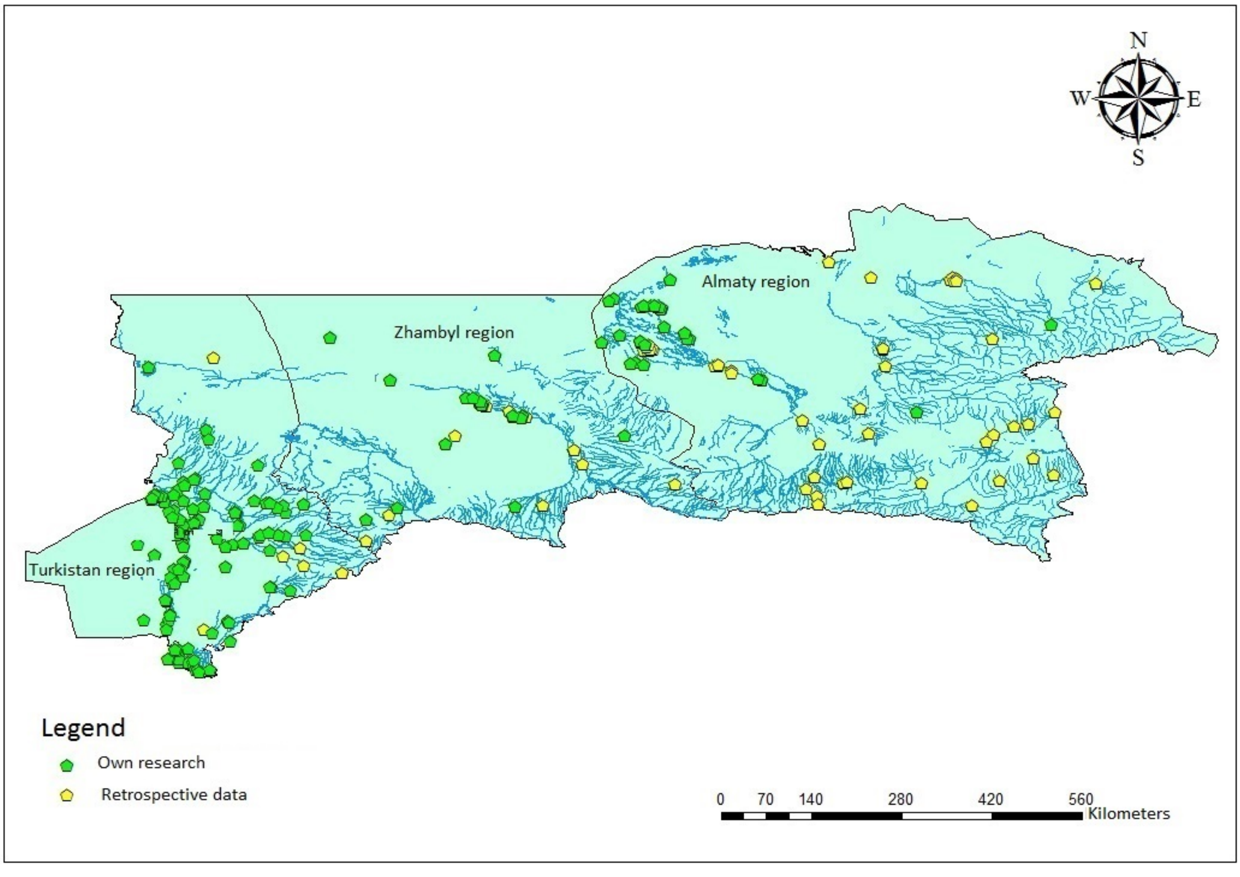

Figure 2 – Locations of finds of ticks *Dermacentor niveus*

Material: 381 ♀♀, 462 ♂♂, 19 N, 2 L. were studied. A total of 864 individuals of *D. niveus* from own collections and from collection funds of the RSE "Institute of Zoology" KN MON of the Republic of Kazakhstan, RSE "KSCQZD named after M. Aikimbaev "Ministry of Health of the Republic of Kazakhstan, RSE " Shymkent Anti-Plague Station " of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan, RSE " Taldykorgan Anti-Plague Station" of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan.

***Dermacentor pavlovskyi* Olenev, 1927.** The main habitat of *D. pavlovskyi* is the Tien Shan mountains [15]. I.G. Galuzo gives information about the first findings of the species in 1910 in the Ak-Kekil place of the Karatau ridge on the mountain sheep *Ovis polii nigrimontana*. Later, in 1928, the species was found in Turkistan. *D. pavlovskyi* was noted by the Republican Tropical Station (RTS) in 1941 on Siberian ibex and mountain sheeps in the Aksu-Dzhabagly nature reserve, in 1944 on sheep and goats in the Naydenovka village of the Dzhambul district of the Alma-Ata region. In 1953-55 the RTS finds the species again on Siberian ibex in the Aksu-Dzhabagly reserve, and in 1961 on the Menzbir marmot at the source of the river Badam (Western Tien Shan).

We collected 2 males on the flag in the Baidibek region (Karatau mountains, environs of the village of Kensai) of the Turkestan region (figure 3).

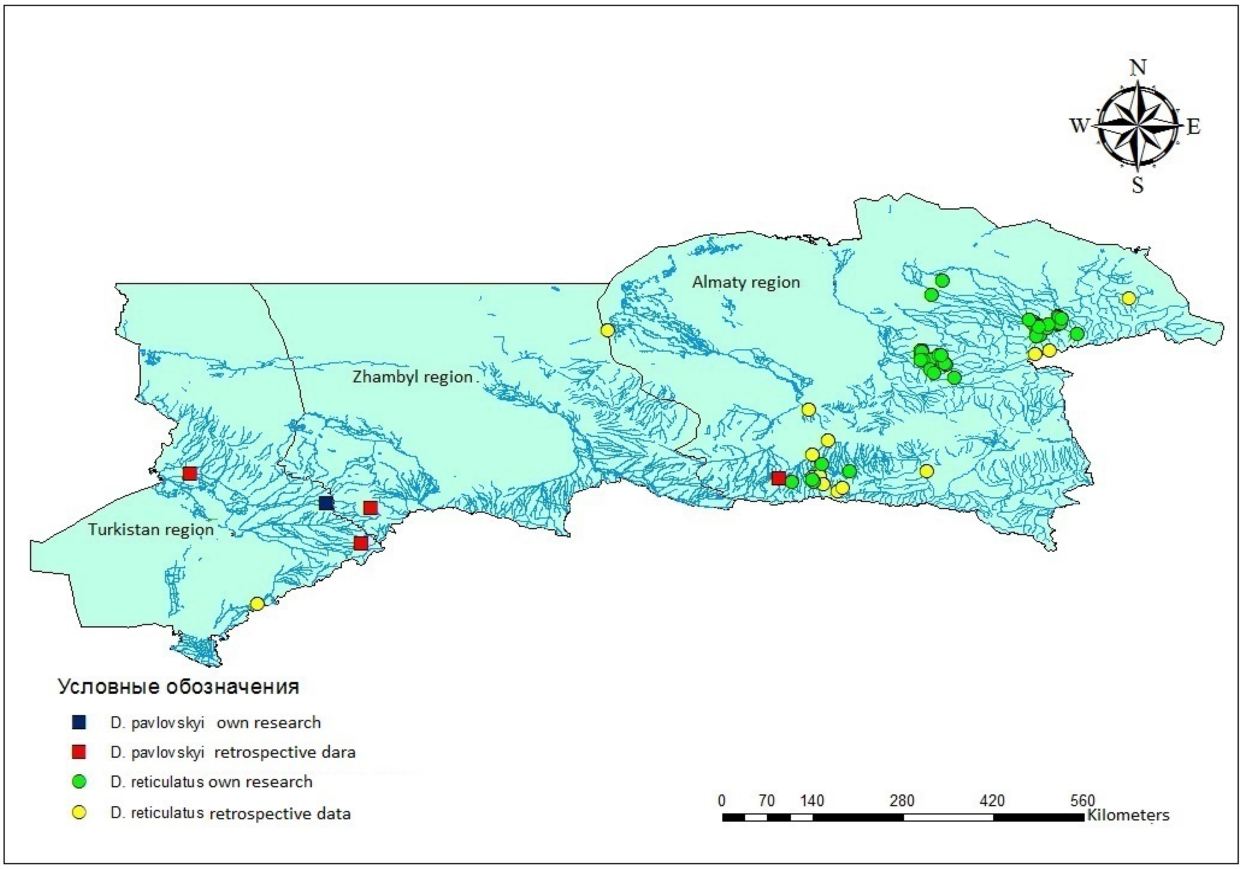

Figure 3 – Locations of ticks *Dermacentor pavlovskyi* and *D. reticulatus*

Material: 25 ♀♀, 59 ♂♂, 62 N, 4 L. of D. pavlovskyi were studied from the collection funds of the RSE "Institute of Zoology" KN MES RK, RSE "KSCQZD named after M. Aikimbaev" Ministry of Health of the Republic of Kazakhstan, RSE "Shymkent Anti-Plague Station" of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan, RSE "Taldykorgan Anti-Plague Station" of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan.

Dermacentor reticulatus **(Fabricius, 1794).** The first information about the findings of ticks of this species in 1934 on horses and cattle in the vicinity of the Alma-Ata city was given by D.I. Blagoveshchensky. Later, in 1937, he found ticks on cattle in Ili, and on hares in Alakul districts of the Almaty region [17]. In 1940-46. repeated findings of these ticks on dogs, horses, cattle, Siberian roe deer, shrews, Mongolian pikes, house mice, vegetation and humans in the vicinity of Alma-Ata (Teres-Butak, Small Almaty Gorge, upper Talgar, Broad Shchel, Medeo) and Almaty region (Bartogay, village Naydenovka, Dzhambul region, Ili, Foothills of the Dzungarian Ata-Tau) [8]. There is evidence of the presence of this species by the Republican Tropical Station in cattle in Sary-Agach (probably an accidental caught, since the species was not found in subsequent years).

Thus, information on the distribution of *D. reticulatus* in the south and southeast of Kazakhstan until the end of the 40s of the last century was limited mainly to the territory of the Almaty region [8]. Until the beginning of this century, we don’t have information about finding the species in the study area outside the Almaty region.

In 2012-16. ticks were collected from the vegetation on the flag in Almaty (Botanical Garden) and Sarkand ("Zhongar-Alatau GNPP"), Kerbulak (vicinity of Karasu), Talgar, Karasai (vicinity of Yermensay village) districts of the Almaty region. In addition, in 2017-18. ticks were collected in vicinity of Topolevka, Sarkand, Vesely, Shatyrbay, Erkin, Abay, Rudnichny, Aldabergenova, Tekeli, Budyonny, Sarkand districts of Almaty region (figure 3).

Material: 1150 ♀♀, 992 ♂♂ were studied. A total of 2142 individuals of *D. reticulatus* from the collection funds of the RSE “Institute of Zoology” KN MES RK, RSE “KSCQZD named after M. Aikimbaeva” Ministry of Health of the Republic of Kazakhstan, RSI “Shymkent Anti-Plague Station” of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan, Shymkent, Kazakhstan, RSI “Taldykorgan Anti-Plague Station” of the Committee for Quality Control of Goods and Services of the Ministry of Health of the Republic of Kazakhstan.

The discussion of the results. Since the early 2000s of this century, studies have been carried out on the current state of the fauna of ixodid ticks that live in south-eastern Kazakhstan. Based on the studies, it should be assumed that over the past half century, as a result of intensive human economic activity, the tick’s fauna underwent changes in the areas we studied. Compared with 30-60 years of the last century, significant changes have occurred in distribution of ticks of the *Dermacentor* genus [18]. Using modern research methods, we have clarified the current boundaries of the *D. marginatus, D. niveus, D. pavlovskyi, D. reticulatus* ranges within south-eastern Kazakhstan. So D. marginatus was noticed by us in the territory of Almaty, Zhambyl and Turkestan regions, mainly confined in the south-east to the foothills of Zailiysky and Zhetysu Alatau, in the south to the foothills of the Western Tien Shan. Ticks were found in mass in the foothills of Zhetysu Alatau (Almaty region), less often in the foothills of the Western Tien Shan and in single individuals in the Zhambyl region.

D. niveus is widespread in the territory of the three regions studied by us. The area of D. niveus within south-eastern Kazakhstan is confined to arid zones - these are mainly floodplains of the large rivers Shu, Ile and Syrdaria and their tributaries. *D. pavlovskyi* is registered by us only in the south of Kazakhstan in the foothills of the western Tien Shan. In Almaty and Zhambyl regions, the species was not found.

Findings. In the studies conducted in south-eastern Kazakhstan, we collected 4 species of ticks, ixodid ticks of the genus *Dermacentor*: *D. marginatus, D. niveus, D. pavlovskyi, D. reticulatus*. As a result of the analysis of retrospective data (literary sources and collections of ticks), significant differences were found in the distribution of these species of ticks in the study area compared to the 30-60 years of the last century.

Based on our studies, we can assume that not only natural and climatic conditions, but also the anthropogenic factor play a role in changing the boundaries of habitats. The earlier presence of some tick species in unusual for it territories is probably due to human economic activity - in the second half of the last century, the region was intensively developed, villages appeared and grew, irrigated agricultural enterprises have been developing.

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ҚАЗАҚСТАННЫҢ ОҢТҮСТІК-ШЫҒЫС БӨЛІГІНДЕ *DERMACENTOR* KOCH, 1844 (IXODIDAE, AMBLYOMMINAE) ТЕКТІ КЕНЕЛЕРДІҢ ТАРАЛУЫ

Аннотация. Алматы, Жамбыл және Түркістан облыстарының аумағында ретроспективті деректер мен меншікті алымдарды талдау негізінде Қазақстанның оңтүстік-шығыс бөлігі шегіндегі *Dermacentor*: *D. marginatus, D. niveus, D. pavlovskyi, D. reticulatus* текті кеңелердің таралу аймағы нақтыланды. Өткен ғасырдың қазіргі деректері мен зерттеулері арасында кенелердің таралуы бойынша елеулі айырмашылықтар анықталды, бұл адамның белсенді шаруашылық қызметімен байланысты болуы мүмкін. *D. marginatus* Қазақстанның оңтүстік-шығысында негізінен тау бөктеріндегі аймақтарда таралған. Оның негізгі бөлігі Іле және Жетісу Алатауының тау бөктерінде тұрады. Батыс Тянь-Шань – Қаратау жотасы мен Талас жотасының тау бөктерінде сирек кездеседі. *D. niveus* ареалы шөлді және жартылай шөлейтті аумақтарды қамтиды, негізінен Шу, Іле, Сырдария өзендерінің жайылмалары және олардың ағындары. *D. pavlovskyi* кенелері Батыс Тянь-Шанның тау бөктерінде, Қаратау жотасында табылған. Қазақстанның оңтүстік-шығысындағы *D. Reticulatus* ареалы тек Іле және Жетісу Алатауының тау бөктерімен шектеледі.

Түйін сөздер: иксод кеңелер, қоздырғыштардын таратушылар, эктопаразиттер коллекциясы, кеңелер таралу аймағы, празиттер фаунасы

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РАСПРОСТРАНЕНИЕ КЛЕЩЕЙ РОДА *DERMACENTOR* KOCH, 1844 (IXODIDAE, AMBLYOMMINAE) В ЮГО-ВОСТОЧНОЙ ЧАСТИ КАЗАХСТАНА

Аннотация. На основе анализа ретроспективных данных (литературных источников и коллекций иксодовых клещей РГП «Институт зоологии» КН МОН РК и РГП «КНЦКЗИ им. М. Айкимбаева» МЗ РК), а также собственных сборов на территории Алматинской, Жамбылской и Туркестанской областей уточнены границы ареалов клещей рода *Dermacentor*: *D. marginatus, D. niveus, D. pavlovskyi, D. reticulatus* в пределах юго-восточной части Казахстана. Выявлены существенные различия по распространению клещей между современными данными и исследованиями прошлого столетия, что, вероятно, связано с изменением климата и активной хозяйственной деятельностью человека в этом регионе. В юго-восточном регионе Казахстана *D. marginatus* распространен, в основном, в предгорных зонах. Основная масса его обитает в предгорьях Заилийского и Жетысу Алатау (Алматинская область). Несколько реже вид встречается в предгорьях Западного Тянь-Шаня - хребет Каратау (Туркестанская область) и Таласский хребет (Жамбылская область). Ареал *D. niveus* охватывает пустынные и полупустынные территории, в основном это поймы рек Шу, Иле, Сырдария и их притоки. Клещи *D. pavlovskyi* обнаружены в предгорьях Западного Тянь-Шаня, хребет Каратау. Нахождение их в 1944 году Республиканской тропической станцией на мелком рогатом скоте в Алматинской области вероятно случайно. Ареал *D. reticulatus* на юго-востоке Казахстана ограничен только предгорьями Заилийского и Жетысу Алатау. Нахождение вида в Жамбылской и Туркестанской областях вероятно ошибочно.

Ключевые слова: иксодовые клещи, переносчики возбудителей, коллекции эктопаразитов, ареалы клещей, фауна эктопаразитов.

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In vitro COMPARABLE ANALYSIS OF CARCINOLYTIC ACTIVITY OF MUTANT PROTEIN PARASPORIN-2 ON THE HUMAN HEPATOCARCINOMA CELL MODEL

Abstract. The paper presents the results of the study on the effect of amino acid substitution in the receptor-binding and transmembrane domain of the carcinolytic bacterial protein parasporin-2 on cytotoxic activity against the Hep G2 (human hepatocarcinoma) tumor cell line. The substitution of amino acids tyrosine for alanine and serine for cysteine was carried out by introducing a point mutation into a recombinant plasmid DNA containing the sequence of the gene encoding the paraspirin-2 protein with a polyhistidine tag. 3 new mutant parasporins 2 were prepared by substituting amino acids in two protein domains. A tenfold reduction in the cytotoxic activity of parasporin-2 was observed when the amino acid tyrosine was substituted for alanine in the receptor-binding domain of the protein; serine to cysteine substitution had no significant effect on the carcinolytic activity. The resulting data and experimental samples could be used for searching targets on the surface of tumor cells by means of affinity chromatography.

Keywords: mutations, parasporins, cell lines, cytotoxicity.

Introduction. Parasporins are genealogically heterogeneous Cry proteins synthesized by the bacterial species *Bacillus thuringiensis*. A characteristic feature of parasporins is high cytotoxic activity against human cancer cells of various origins. Proteins exhibit cytotoxic activity only after proteolytic activation (Ohba M., 2009: 427).

Mizuki et al. studied for the first time the parasporal inclusion proteins isolated from a total of 1744 *B. thuringiensis* strains. They determined the cytotoxic activity against human leukaemia T cells and hemolytic activity against sheep erythrocytes. It was concluded that the *B. thuringiensis* protein inclusions could be used for medical purposes (E. Mizuki, 1999: 477).

Further studies of the unusual properties of the *B. thuringiensis* parasporal inclusions and their ability to recognize human leukaemia cells discovered a protein named parasporin, which is responsible for carcinolytic activity. This protein was subsequently cloned (Mizuki E., 2000: 625).

Different research groups later found new strains producing carcinolytic parasporal inclusions and characterized their parasporins (Ito A., 2004: 21282; Brown K., 1992: 549; Saitoh H., 2006: 2935; Nagamatsu 2010: 494; Okumura S. 2004: 89; Okumura S., 2013: 1889).

Parasporin-2 is a carcinolytic non-hemolytic and non-insecticidal protein toxin derived from the *B. thuringiensis* parasporal inclusion. This pore-forming protein has a mass of 37 kDa, consists of 338 amino acids, the length of the coding gene is 1014 bp. To manifest cytotoxic activity, it requires the presence of GPI-anchored proteins, which indicates the possibility of pore formation in raft domains of the tumor cell membranes (Lee D., 2000: 218).

The active form of recombinant parasporin-2 was crystallized in the presence of ethylene glycol and polyethylene glycol 8000 at neutral pH (Akiba T., 2004: 2355). Hayakawa et al. reported about a new Cry protein that exhibited strong cytotoxicity against human leukaemia T cells, which was cloned from the *B. thuringiensis* Tth-E6 strain. The protein designated as parasporin-2Ab (PS2Ab) is a polypeptide composed of 304 amino acid residues with a molecular weight of 33,017. The deduced amino acid sequence of PS2Ab showed significant homology (84% identity) with parasporin-2Aa (PS2Aa) from the *B. thuringiensis* strain A1547. Upon processing of PS2Ab with proteinase K, the active form of 29 kDa was produced. The activated PS2Ab showed potent cytotoxicity against MOLT-4 and Jurkat cells, and the EC_{50} values were estimated as 0.545 and 0.745 ng/mL respectively. The cytotoxicity of PS2Ab was significantly higher than that of PS2Aa. Although both cytotoxins were structurally related, it was believed that the detected minor differences in amino acid sequence were responsible for the different degrees of cytotoxicity of PS2Ab and PS2Aa (Hayakawa T., 2007: 278). Cytological and biochemical observations on PS2Aa showed that the protein is a pore-forming toxin. To confirm this hypothesis, Akiba et al. have determined the crystalline structure of its active form with a resolution of 2.38 Å. The protein is unusually elongated and consisted mainly of long β-pleated sheets aligned along its long axis. It is similar to the aerolysin-type β-pore-forming toxins, the similarity with which confirms the pore formation hypothesis. The molecule can be divided into three domains: Domain 1, comprising small β-pleated sheets flanked by short α-helices, is probably the receptor-binding domain. The other two are both "beta-sandwich" domains, which are thought to be involved in oligomerization and pore formation. Domain 2 has a putative channel-forming β-hairpin, characteristic of the aerolysin-type toxins. The surface of the protein has an extensive track of exposed side chains of serine and threonine residues. This might orient the molecule on the cell membrane when Domain 1 binds to the target until oligomerization and pore formation are initiated. The β-hairpin has such a tight structure that it seems unlikely to be oligomerized, as postulated in a recent model of pore formation developed for aerolysin-type toxins. The spontaneous oligomerization lock model is proposed as an inactivation mechanism by the N-terminal inhibitory segment (Akiba T., 2009: 121).

Studies on the cytotoxic effect of parasporin-2 have showed that unlike parasporin-1, it increases the permeability of the plasma membranes of tumor cells (Ohba M., 2009: 427; Petit L., 1997: 6480). Cytoplasmic lactate dehydrogenase flows out of the treated HepG2 cells, while extracellular propidium iodide enters the cytoplasm. The initial stage of the cytotoxic effect of parasporin-2 is the specific binding of the toxin to a putative receptor protein, not yet identified, which is located in a lipid raft of the plasma membrane of tumor cells susceptible to this protein. This is followed by the formation of oligomers of parasporin-2 in the plasma membranes, which leads to the pore formation and cell lysis (Petit L., 1997: 6480). Oligomerization occurs in the presence of membrane proteins, a lipid bilayer, and cholesterol. It should be noted that substantial homology exists in amino acid sequences between PS2Aa1 and *Clostridium perfringens* epsilon-toxin, whose cell action mechanism involves the toxin oligomerization in lipid rafts and pores formation in the plasma membrane (Petit L., 1997: 6480).

Abe et al. (Abe Y., 2005: 113) examined the mechanism of action of parasporin-2. They found that the toxin binds to the surface of target cells and increases the permeability of the plasma membrane. Subcellular fractionation and immunoblotting of the cells treated with the toxin showed that the toxin is associated with lipid rafts and forms SDS-resistant oligomers. The binding and oligomerization of the toxin was inhibited by treating the cells with phosphatidylinositol-specific phospholipase C. The interaction of parasporin-2 with glycosylphosphatidylinositol proteins was therefore required to form an oligomeric toxin that could penetrate the plasma membrane (Abe Y., 2005: 113). Abe et al. (Abe Y., 2008: 269) examined the mechanism of action of parasporin-2 on the human HepG2 (hepatomocarcinoma) cell line and showed that this Cry toxin targets lipid rafts and is assembled into oligomeric complexes in the tumor cell membrane. The authors concluded that this protein is a pore-forming toxin that accumulates in lipid rafts of tumor cells. Recently, Bokori-Brown et al. (Bokori-Brown M., 2011: 4589) showed that the ε-toxin produced by *Clostridium perfringens* (the etiological agent of dysentery in newborn lambs, enteritis and enterotoxicity in goats, calves and foals) forms heptameric pores in the membranes of the target cells in the same way as parasporin-2.

Since little is known about the receptor molecules that bind parasporins and the mechanism of antitumor activity, Krishnan et al. (Krishnan K., 2010: 86) conducted a study with the Malaysian isolate *B. thuringiensis* 18. It produces a parasporal protein that exhibits predominant cytotoxic activity against human leukaemia T cells (CEM-SS), but is non-cytotoxic against normal T cells and other cancer cell lines, such as human cervical cancer (HeLa), human breast cancer (MCF-7) and colon cancer (HT-29), showing properties similar to those of parasporins. The study was aimed at identifying a binding protein for the *B. thuringiensis* 18 parasporin in human leukemic tissues. The protein was separated using the Mono Q ion-exchange column in HPLC system using antibodies against the purified 68-kDa parasporal protein. A receptor binding assay was used to determine the binding protein for the Bt18 paraspore protein in CEM-SS cells, the identified protein was sent for N-terminal sequencing. Double immunofluorescence staining was applied to localize *B. thuringiensis* 18 and the binding protein on the surface of CEM-SS cells. The findings of this study showed that ion-exchange separation of Bt18 parasporal proteins yielded a 68-kDa parasporal protein with cytotoxic activity. Polyclonal IgG (anti-B. thuringiensis 18) for the 68-kDa parasporal protein was successfully prepared and purified. An analysis of receptor-binding showed that the *B. thuringiensis* 18 parasporal protein is bound to a 36-kDa protein from the CEM-SS cell lysate. N-terminal amino acid sequence of the 36-kDa protein was GKVKVGVNGFGRIGG, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was found to be a binding protein. Double immunofluorescence staining showed co-localization of the *B. thuringiensis* 18 parasporin and GAPDH on the plasma membrane of the CEM-SS cells. GAPDH has been well known as a glycolytic enzyme, but it was recently discovered that GAPDH has a role in apoptosis and carcinogenesis. Pre-incubation of the anti-GAPDH antibody with CEM-SS cells decreases binding of Bt18 parasporin to the susceptible cells. Based on a qualitative analysis of the immunoblotting and immunofluorescence results, GAPDH was identified as a binding protein located on the plasma membrane of CEM-SS cells for the Bt18 parasporal protein (Krishnan K., 2010: 86).

The purpose of the study was to determine the effect of amino acid substitution in the transmembrane and receptor-binding domains in parasporin-2 on *in vitro* carcinolytic activity.

Materials and Methods. *Plasmids, bacteria and culture conditions.* The recombinant pET-23a (+) plasmid carrying the parasporin-2 gene was used (Okumura S., 2005: 6313). DH5α competent E. coli cells were used for intermediate transformation, and *E. coli* BL21-AI was used as a producer strain to prepare mutant parasporins 2. Bacteria were cultured in agar and liquid LB culture medium in the presence of 0.1% ampicillin. Plasmids were isolated using the WizardTMPlus SV Minipreps DNA Purification System adsorption kit (Promega) (Poornima K., 2010: 348).

Tumor cell line and culture conditions. Hep G2 (hepatocarcinoma) tumor cell line was grown in RPMI medium supplemented with 10% FBS in the presence of ampicillin (100 μg/mL), at 37 °C, 5% CO_2 (Kitada S., 2006: 26350).

DNA manipulations. PCR conditions: 1 cycle at 95^0C for 30 seconds, 16 cycles at 95^0C for 30 seconds, 1 minute at 55^0C, and a final incubation at 68 ^{0}C for 15 min (Okumura S., 2005: 6313).

DNA electrophoresis was performed in 6% polyacrylamide gel prepared with 0.5 X TBE buffer of the following composition: 0.089 M Tris-borate, 0.089 M boric acid, 2 mM EDTA. In order to allow gel to polymerize, 300 μl of 10% ammonium persulfate and 30 μl of TEMED (Kitada S., 2009: 80) were added to 50 ml of the solution.

DNA sequencing. The obtained PCR products were analyzed on the Beckman CEQ™ 8000 Genetic Analysis System capillary sequencer. DNA sequences were compared with the original sequence encoding parasporin-2 using the BLAST NCBI service (http://blast.ncbi.nlm.nih.gov).

Protein expression, isolation, and analysis. 2.5 ml of an overnight transformed *E. coli* BL21-AI culture was added to 250 ml of fresh LB medium comprising 0.1% ampicillin, and then cultured for 3-4 hours until an OD600nm of 0.6-0.8 was reached. The expression of target protein was induced by the addition of isopropyl-β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 0.2 μM. Protein purification was carried out on the HisTrap FF (GE) 5 ml chromatography column; the concentration was measured by the Lowry method at 750 nm. The compliance of the samples with the molecular mass of parasporin-2 was determined by polyacrylamide gel electrophoresis (Akiba T., 2009: 121).

Determination of cytotoxic activity. LD_{50} was determined by analyzing the amount of formazan (MTS test) in cell culture at a wavelength of 490 nm in 96-well plates with Cell Titer 96 Proliferation assay kit (Promega). The number of cells per well in 90 μl was 2×10^4. Triton X-100 was used as a positive control, and physiological saline served as a negative control (Brown K., 1992: 549).

Results and Discussion. Parasporin-2 was chosen as the object of study, since it is the most effective antitumor parasporin possessing low-level cytotoxicity against normal human cells (table 1) (Okumura S., 2013: 1889; Lee DW., 2000: 218; Okumura S., 2005: 6313; Poornima K., 2010: 348; Namba A., 2003: 29; Uemori A., 2005: 122; Yasutake K., 2005: 124; Kitada S., 2009: 121; Akiba T., 2009: 121).

Table 1 – Cytotoxic activity of various parasporins against tumor and normal human cells (Okumura S. et al.)

Cell line	Characteristics of cells	LD_{50} (μg/mL)			
		Parasporin-1			Parasporin-1
MOLT-4	T-cell leukaemia	2,2	0.022	>10	0.472
Jurkat	T-cell leukaemia	>10	0.018	>10	>2
HL-60	T-cell leukaemia	0,32	0.019	1,32	0.725
T cell	Normal T cells	>10	–	>10	>2
HepG2	Hepatocarcinoma	3,0	0.019	2,8	1,90
HC	Normal hepatocytes	>10	1.1	>10	>2
HeLa	Cervical cancer	0,12	2.5	>10	>2
Sawano	Uterus cancer	>10	0.0017	>10	0.245
TCS	Cervical cancer	–	7.8	>10	0.719
UtSMC	Normal uterus cells	>10	2.5	>10	>2
Caco-2	Colon cancer	>10	0.013	>10	0.124

Two main functional domains can be identified in parasporin-2: transmembrane and receptor-binding. The recognition domain is represented by beta-pleated regions and short alpha-helices, and in contrast to the transmembrane domain, has a greater number of aromatic amino acids in absolute value (Kitada S., 2009: 80).

Table 2 – Mutant parasporins obtained upon amino acid substitutions

SN	Amino acids in the original parasporin-2 subjected to substitution and their position in the sequence	Amino acids in the modified parasporins 2 and their position in the sequence	Functional Domain
1	Serine 297	Cysteine 297	Transmembrane
2	Serine 297, Cysteine 111	Cysteine 297, Serine 111	Transmembrane
3	Serine 297, Cysteine 111, Tryptophan 81	Cystein e297, Serine 111, Alanine 81	Receptor-binding

Substitution of semantic triplets was carried out using the QuikChangeTMSite-Direct Mutagenesis Kit (Stratagene) with the following primers to replace the corresponding amino acids:

S297C-forward (5’-TCATCTAGTAAACAGCGTGCTGGTTGCAC-3’),
S297C-reverse (5’-GCAACCAGCAGCGTGTTTACTAGATGAAC-3’),
C111S-forward (5’-TTTTTATAGCTATAGTATCCATATTGAGC-3’),
C111S-reverse (5’-TATGGATACTATAGCTATAAAAATGTTGG-3’),
Y81A-forward (5’-GGGATTAGTACCAGCAATAGAGGAAAATCTAG-3’),
Y81A-reverse (5’-TCCTCTATTGCTGGTACTAATCCCGATGG-3’).

The validity of the mutations was confirmed during the comparison (alignment) of DNA sequences obtained as a result of performed sequencing:

Query 43
ATGAGAGGATCGCATCACCATCACCATCACGACGTTATTCGAGAATATCTTATGTTTAAT 102
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 1
ATGAGAGGATCGCATCACCATCACCATCACGACGTTATTCGAGAATATCTTATGTTTAAT 60

Query 103
GAGTTATCAGCATTAAGTTCAAGTCCAGAAAGTGTAAGATCTAGATTTTCCTCTATTTCT 162
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct 61
GAGTTATCAGCATTAAGTTCAAGTCCAGAAAGTGTAAGATCTAGATTTTCCTCTATTTAT 120

```
    Query  163
GGTACTAATCCCGATGGTATTGCATTAAATAATGAAACGTATTTTAACGCCGTAAAACCG  222
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  121
GGTACTAATCCCGATGGTATTGCATTAAATAATGAAACGTATTTTAACGCCGTAAAACCG  180

    Query  223
CCTATTACTGCTCAATATGGATACTATAGCTATAAAAATGTTGGGACTGTTCAGTACGTA  282
           ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
    Sbjct  181
CCTATTACTGCTCAATATGGATACTATTGCTATAAAAATGTTGGGACTGTTCAGTACGTA  240

    Query  283
AATAGACCTACTGATATTAACCCAAACGTTATTCTTGCTCAAGACACATTAACAAATAAT  342
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  241
AATAGACCTACTGATATTAACCCAAACGTTATTCTTGCTCAAGACACATTAACAAATAAT  300

    Query  343
ACTAATGAACCATTTACTACAACTATCACTATAACTGGGTCTTTTACCAACACGTCTACT  402
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  301
ACTAATGAACCATTTACTACAACTATCACTATAACTGGGTCTTTTACCAACACGTCTACT  360

    Query  403
GTGACATCTAGTACAACAACAGGCTTTAAATTTACTAGTAAACTATCAATTAAAAAAGTC  462
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  361
GTGACATCTAGTACAACAACAGGCTTTAAATTTACTAGTAAACTATCAATTAAAAAAGTC  420

    Query  463
TTTGAAATTGGTGGAGAAGTTTCATTCTCTACTACAATTGGAACATCTGAAACAACTACA  522
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  421
TTTGAAATTGGTGGAGAAGTTTCATTCTCTACTACAATTGGAACATCTGAAACAACTACA  480

    Query  523
GAAACAATTACTGTATCTAAATCCGTTACGGTTACGGTTCCAGCTCAAAGTAGAAGAACT  582
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  481
GAAACAATTACTGTATCTAAATCCGTTACGGTTACGGTTCCAGCTCAAAGTAGAAGAACT  540

    Query  583
ATTCAGTTAACAGCTGAAATAGCAAAAGAATCTGCAGACTTTAGTGCTCCTATT  635
           |||||||||||||||||||||||||||||||||||||||||||||||||||||
    Sbjct  541
ATTCAGTTAACAGCTAAAATAGCAAAAGAATCTGCAGACTTTAGTGCTCCTATT  594
```

The compliance of molecular mass of the pET-23a(+) plasmid vector encoding mutant parasporins was determined by means of agarose gel electrophoresis (aigure 1).

Cytotoxic concentrations of mutant parasporins (LD_{50}) showed an almost tenfold decrease in carcinolytic activity when an aromatic amino acid tryptophan was substituted to an aliphatic amino acid alanine in the receptor-binding domain in the absence of significant changes in cytotoxic concentrations in the case of serine to cysteine substitution in transmembrane regions of parasporin-2 (figure 2).

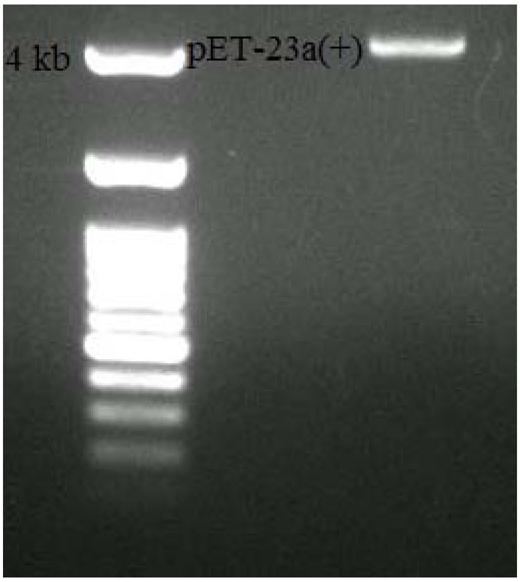

Figure 1 – Electrophoregram of pET-23a(+) plasmid

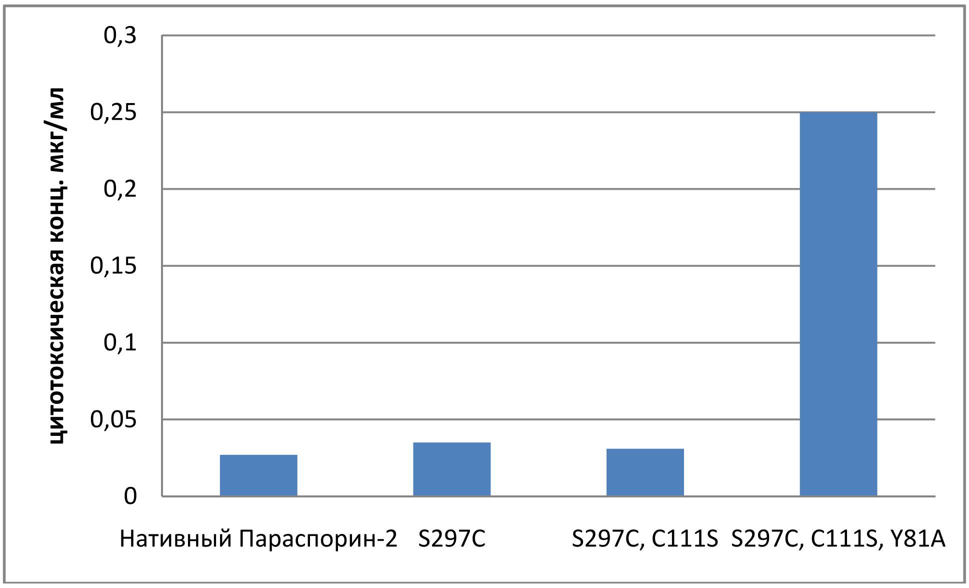

Figure 2 – Cytotoxic concentration (CTC_{50}) of mutant parasporins
and native parasporin-2 in HepG2 (human hepatocarcinoma) cell line.
Native PS-2 - 0.027 μg/mL; mutant parasporins S297C - 0.035 μg/mL;
S297C, C111S - 0.031 μg/mL; S297C, C111S, Y81A - 0.25 μg/mL.

Conclusion. The findings of this study showed the effect of amino acid (serine, alanine, and tryptophan) substitutions on the carcinolytic activity of parasporin-2. It was found that the substitution of tryptophan to alanine in the receptor-binding domain of the protein had the greatest effect, which suggests the localization of the epitope in this region, which is responsible for affinity for the potential receptor on the surface of tumor cells for this particular anticancer protein agent. We believe that the resulting mutant parasporin-2 can be used as a control upon conjugation with potential targets of tumor cells to exclude molecules that enter into non-specific interaction, for example, in Western blotting.

Serine to cysteine and cysteine to serine substitutions in the transmembrane domain of parasporin-2 did not significantly affect the cytotoxic activity. This makes it possible to use this mutant parasporin-2 for chemical crosslinking with a high molecular weight carrier (gel, chromatography column, etc.)

through the sulfhydryl group of cysteine in order to determine surface targets by the way of their conjugation with the parasporin-carrier complex.

Establishment of the mechanism of action and targets for antitumor parasporins will determine the surface antigenic determinants of cancer cells; studies on their structures make it possible to develop more effective synthetic drugs. Determination of the most significant amino acids and epitopes in the receptor-recognizing part of parasporins enables *in silico* selection of a target from a database of known tumor antigens, as well as a development of a less effective parasporin, which can be used as a negative control when conjugating with potential surface targets of tumor cells in order to exclude molecules entering into interaction in a non-specific way, such as hydrophobic interaction.

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АДАМНЫҢ ГЕПАТОКАРЦИНОМАСЫНЫҢ ЖАСУШАЛЫҚ ҮЛГІСІНДЕГІ ПАРАСПОРИН-2 МУТАНТТЫ ПРОТЕИНІНІҢ САЛЫСТЫРМАЛЫ ТАЛДАУЫ

Аннотация. Мақалада Парпринпин-2 бактериясының карцинолитикалық бактериялық ақуызының рецепторлы-байланыстырушы және трансмембраналық аймағында амин қышқылының алмастырылу әсерін зерттеп, нәтижелері бойынша Hep G2 жасушаларының (адамның гепатокарциномасы) ісік жасушакларына қарсы цитотоксикалық белсенділікке әсерін зерттеу нәтижелері келтірілген. Тирозин аминқышқылдарын аланин,серин және цистеинге алмастыру, параспорин-2 протеинін полиистидин тегімен кодтайтын геннің дәйектілігі бар рекомбинантты плазмидтік ДНҚ-ға нүктелік мутация енгізу жолымен жүзеге асырылды. Аминқышқылдарын екі ақуыз аймағына аусуымен 3 жаңа мутанты параспорина-2 алынды. Параспирин-2 цитотоксикалық белсенділігінің он есе төмендеуі аминқышқыл тирозині белоктың рецепторлы-байланыстыру аймағында аланинмен алмастырылған кезде байқалды, бұл цистеинмен серинді алмастыру карцинолитикалық белсенділігіне айтарлықтай әсер етпеді. Алынған мәліметтер мен сынама үлгілері ісік жасушаларының бетінде мақсатты хроматография арқылы іздеуге болады.

Түйін сөздер: мутациялар, параспориндер, клеткалық қыралар, цитоулылығы.

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In vitro СРАВНИТЕЛЬНЫЙ АНАЛИЗ КАНЦЕРОЛИТИЧЕСКОЙ АКТИВНОСТИ МУТАНТНОГО БЕЛКА ПАРАСПОРИНА-2 НА КЛЕТОЧНОЙ МОДЕЛИ ГЕПАТОКАРЦИНОМЫ ЧЕЛОВЕКА

Аннотация. В статье представлены результаты исследования влияния замены аминокислот в рецептор-связывающем и трансмембранном домене канцеролитического бактериального белка параспорин-2 на цитотоксическую активность против опухолевой культуры клеток Hep G2 (гепатокарцинома человека). Замена аминокислот тирозина на аланин и серина на цистеин осуществлялась посредством введения точечной мутации в рекомбинантную плазмидную ДНК, содержащую последовательность гена, кодирующую белок параспорин-2 с полигистидиновым тагом. Получено 3 новых мутантных параспорина-2 с заменой аминокислот в двух доменах белка. Было установлено десятикратное снижение цитотоксической активности параспорина-2 при замене аминокислоты тирозина на аланин в рецептор-связывающем домене белка, замена серина на цистеин не оказало значительного влияния на канцеролитическую активность. Полученные данные и опытные образцы могут быть использованы для поиска мишеней на поверхности опухолевых клеток посредством аффинной хроматографии.

Ключевые слова: мутации, параспорины, клеточные линии, цитотоксичность.

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СОДЕРЖАНИЕ

CONTENTS

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