NATIONAL ACADEMY OF SCIENCES OF UKRAINE
Palladin Institute of Biochemistry

BIOTECHNOLOGIA ACTA

Vol. 12, No 6, 2019

BIMONTHLY

Editorial Staff

Serhiy ***Komisarenko***	Editor-in-Chief; Professor, Dr. Sci., Academician; Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv
Rostislav ***Stoika***	Deputy Editor-in-Chief; Dr. Sci. in Biology, Professor, corresponding member of the National Academy of Sciences of Ukraine, Institute of Cell Biology of the National Academy of Sciences of Ukraine, Lviv
Denis ***Kolybo***	Deputy Editor-in-Chief; Dr. Sci. in Biology, Professor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Tatiana **Borsova**	Dr. Sci. in Biology, Professor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Leonid **Buchatskiy**	Dr. Sci. in Biology, Professor, Taras Shevchenko National University of Kyiv, Ukraine
Liudmila **Drobot**	Dr. Sci. in Biology, Professor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Serhiy **Dzyadevych**	Dr. Sci. in Biology, Professor, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine
Valeriy **Filonenko**	Dr. Sci. in Biology, Professor, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine
Olexander **Galkin**	Dr. Sci. in Biology, Professor, National Technical University of Ukraine “Igor Sikorsky Kyiv Polytechnic Institute”, Ukraine
Mykola **Kuchuk**	Dr. Sci. in Biology, Professor, Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine
Leonid **Levandovskiy**	Dr. of Engeneering Sci., Professor, Kyiv National University of Trade and Economics, Ukraine
Eugene **Levitsky**	Scientific Editor, Dr. Sci. in Biology, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Lyubov **Lukash**	Dr. Sci. in Biology, Professor, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine
Olga **Matyshevska**	Dr. Sci. in Biology, Professor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Olexander **Minchenko**	Dr. Sci. in Biology, Professor, corresponding member of the National Academy of Sciences of Ukraine, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine
Olexander **Obodovich**	Dr. of Engineering Sci., Institute of Technical Thermophysics of the National Academy of Sciences of Ukraine
Serhiy **Oliinichuk**	Dr. of Engineering Sci., SO “Institute of Food Resources” of the Ukrainian Academy of Agrarian Sciences, Ukraine
Yuriy **Prylutskyy**	Dr. Sci. in Phisical and Mathematical Scinces, Professor, Taras Shevchenko National University of Kyiv, Ukraine
Olexiy **Soldatkin**	Dr. Sci. in Biology, Professor, Academician of the National Academy of Sciences of Ukraine, Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine
Mykola **Spivak**	PhD, Professor, corresponding member of the National Academy of Sciences of Ukraine, Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine
Tetiana **Todosiichuk**	Dr. of Engineering Sci., National Technical University of Ukraine “Igor Sikorsky Kyiv Polytechnic Institute”, Ukraine
Alyona **Vinogradova**	Executive Editor, Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine

Editorial Council

Ahmad **Ali** (India), Yaroslav **Blume** (Ukraine), Judit **Csabai** (Hungary), Koula **Doukani** (Algeria), Mehmet **Gokhan Halici** (Turkey), Michailo **Honchar** (Ukraine), Vitaliy **Kordium** (Ukraine), Giorgi **Kvesitadze** (Georgia), Valentyn **Pidgors'kyj** (Ukraine), Jacek **Piosik** (Poland), Isaak **Rashal** (Latvia), Uwe **Ritter** (Germany), Nazım **Şekeroğlu** (Turkey), Andriy **Sibirniy** (Ukraine), Volodymyr **Sidorov** (USA), Konstantin **Scriabin** (Russian Federation), Volodymyr **Shirobokov** (Ukraine), Marina **Spinu** (Romania), Anthony **Turner** (United Kingdom), Alexei **Yegorov** (Russian Federation), Anna **Yelskaya** (Ukraine)

Editorial address:
Palladin Institute of Biochemistry of the NAS of Ukraine, 9 Leontovich Street, Kyiv, 01601, Ukraine;
tel.: +3 8 044–235–14–72. *E-mail:* biotech@biochem.kiev.ua; *Web-site:* www.biotechnology.kiev.ua

According to the resolution of the Presidium of the National Academy of Sciences of Ukraine from 27.05.2009 №1-05 / 2 as amended on 25.04.2013 number 463 Biotechnologia Acta has been included in High Attestation Certification Commission list of Ukraine for publishing dissertations on specialties “Biology” and “Technology”.

Tel.: +3 8 044–235–14–72; ***E-mail:*** biotech@biochem.kiev.ua; **Web-site:** www.biotechnology.kiev.ua

Certificate of registration of print media КВ series №19650-9450ПР on 01.30.2013

Literary editor — H. **Shevchenko**; Computer-aided makeup — **O. Melezhyk**
Authorized for printing 30.12.2019, Format — 210×297. Paper 115 g/m². Gaqrn. SchoolBookC. Print — digital. Sheets 9.1. An edition of 100 copies. Order 6.6. Make-up page is done in Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. Print — **O. Moskalenko** FOP

NATIONAL ACADEMY OF SCIENCES OF UKRAINE
Palladin Institute of Biochemistry

BIOTECHNOLOGIA ACTA

Scientific journal

Bimonthly

Vol. 12, No 6, 2019

REVIEWS

Bugyna L. M., Pallah O. V., Meleshko T. V., Bati V. V., Koval N. V., Boyko N. V.
Food products database: transnational application problems 5

Özcan Ö. Ö., Karahan M.
Cancer diagnostics, imaging and treatment by nanoscale structures targeting 12

Kirpenko N., Leontieva T.
Biotechnological perspectives of microalgae 25

EXPERIMENTAL ARTICLES

Sakovich V. V., Stohniy Ye. M., Zhernosekov D. D., Rebriev A. V., Korolova D. S., Marunych R. Yu., Chernyshenko V. O.
Metalloprotease from the cultural liquid of *Pleurotus osreatus* 35

Pirog T. P., Geichenko B. S., Zvarych A. O.
Post-harvest treatment of vegetables with exometabolites of *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 to extend their shelf life 46

Taranenko A. M., Nitovska I. O., Velykozhon L. H., MaystrovP. D., Kuchuk M. V., Morgun B. V.
Development of vectors for *Agrobacterium*-mediated genetic transformation of plants containing the synthetic *cry1Ab* gene encoding resistance to lepidopteran pests 56

Malinovska I. M.
Irection and strength of microbiological processes in layers of gray forest soil under different regimes of management 65

© Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, 2019

НАЦІОНАЛЬНА АКАДЕМІЯ НАУК УКРАЇНИ
Інститут біохімії ім. О. В. Палладіна

BIOTECHNOLOGIA ACTA

Науковий журнал

Том 12, № 6, 2019

ОГЛЯДИ

Бугіна Л. М., Паллаг О. В., Мелешко Т. В., Баті В. В, Коваль Н. В, Бойко Н. В.
Бази даних харчових продуктів: проблеми транснаціонального застосування . . . 5

Озкан О. О., Карахан М.
Діагностика, візуалізація та лікування раку з використанням нанорозмірних структур 12

Кірпенко Н.І., Леонтьєва Т.О.
Біотехнологічні перспективи мікроводоростей 25

ЕКСПЕРИМЕНТАЛЬНІ СТАТТІ

Сакович В. В., Стогній Є. М., Жерносєков Д. Д., Ребрієв А. В., Корольова Д. С., Марунич Р. Ю., Чернишенко В. О.
Металопротеїназа з культуральної рідини *Pleurotus osreatus* 35

Пирог Т. П. , Гейченко Б. С., Зварич А. О.
Післяврожайна обробка овочів екзометаболітами *Nocardia vaccinii* IMB B-7405, *Acinetobacter calcoaceticus* IMB B-7241 та *Rhodococcus erythropolis* IMB Ac-5017 для подовження терміну їх зберігання 46

Тараненко А. М., Нітовська І. О., Великожон Л. Г. , Майстров П.Д., Кучук М. В., Моргун Б. В.
Створення векторів для *Agrobacterium*-опосередкованої генетичної трансформації рослин із цільовим синтетичним геном *cry1Ab*, який кодує стійкість до шкідників ряду лускокрилих 56

Малиновська І .М.
Спрямованість та напруженість мікробіологічних процесів у горизонтах сірого лісового ґрунту за його різноцільового використання 65

UDC 338.439:339.138 https://doi.org/10.15407/biotech12.06.005

FOOD PRODUCTS DATABASE: TRANSNATIONAL APPLICATION PROBLEMS

L. M. BUGYNA, O. V. PALLAH, T. V. MELESHKO, V. V. BATI, N. V. KOVAL, N. V. BOYKO

Uzhhorod National University, Ukraine

E-mail: larina.bh@gmail.com

Received 04.10.2019
Revised 12.11.2019
Accepted 20.12.2019

The purpose of this study was to analyze and compare the existing international methods applied for classifying and identifying foods characteristics in a number of modern computerized databases such as Foodex, INFOODS/FAO, EuroFIR, and present our initial results of Regional and National Ukrainian food composition databases creation.

In this study theoretical synthesis and deductive analysis were used, literary review of foreign scientific peer-reviewed sources, characteristics of the software LanguaL, DaRiS were presented.

The demand for a language-independent thesaurus (LanguaL) and the needs for a practical, field-based food system (INFOODS) led to the attempts to link these tools and create a minimal set of standards and a consistent approach for the food products identifying and analyzing around the world. The examples of this combined approach were “systems mapping” and the “International Interface Standard for Food Databases”.

The exploitation of different tools for compiling of the first Regional (100 local products, project) and National food composition databases (53 products of 6 prioritised traditional foods within BaSeFood projects) were reported.

Key words: food composition data bases (FCDB), LanguaL, DaRis, INFOODS/FAO, EuroFIR.

The preparation of an international systematized and unified database of reliable food data requires precise nomenclature and detailed description of goods indicating their place of origin, energy and nutritional value, organoleptic properties and a number of other important hygienic and microbiological indicators [1]. Even the verified data structured according to the existing quality standards, can be a source of error, if they are derived from products, place in the single hierarchy of which has not been determined yet. To this date, there is a single international agreement of scientists on the importance of food nomenclature and food description. Preparation of reliable food data requires precise identification of the products definitions, methodology harmonization, analytical data validation, and comparison of software used.

Thus, the aim of this paper was to analyze and compare existing international methods of classification and identification of the main food characteristics in a number of most popular modern computerized databases: Foodex, INFOODS/FAO, EuroFIR and to present our initial results of Regional and National Ukrainian food composition databases creation according to the demands of Codex Alimentarius and the first results of national and regional applying of different tools namely LanguaL and DaRIS for the creation of correspondingly National and Regional Ukrainian FCDB in order to connect it with the international food indexation resources — INFOODS/FAO and EuroFIR.

The data of LanguaL, DaRiS application, theoretical synthesis and deductive analysis, and literature review of available research data were analyzed in our paper [2, 3].

The name of the food that is included in a particular database can be ambiguous because of the linguistic or geographical features of its origin. The definition, which is displayed in the web search engine of thesauruses can't be precise. Moreover, the same names for food with different scientific terms can be used in different regions. Some countries didn't manage to recognize certain terms that are used by people in other parts of the world or even in the same country as well. The situation is even more complicated by homonyms, synonyms, identical (consonant) trademarks for different products, as well as culinary or technological conditions of their production.

Since most databases use different methods of product identification, it is difficult to imagine an objective exchange of data between countries, between organizations within the same country, or even between employees in the same institution. That is why, this article laconically examines the existing identification systems used in the databases on food composition and characteristics, as the authors believe, that the international understanding of standardized food identification can enable to solve many problems arising from misunderstandings in this area.

Food classification systems. Earlier, there were two separate and seemingly opposite methods of solving food identification problems: products were classified by the "universal" general categories, or single descriptions of individual products were present in the databases. At present, the first approach has evolved and classifications have turned into complex hierarchies. Now we have many standardized classifications that have certain relevant legal documentation and are reflected in the thesaurus. Most of the national and regional databases use country-specific food classification systems which had been developed on the basis of national criteria, therefore, many specific food groups are observed.

This issue touches upon many legal aspects, highly depends on traditions of each country that, along with economic and cultural importance, actualizes this importance dramatically. For example, there is a separate group for coconut products in the databases developed in the Pachi Islands, and groups of different types of bananas, corn and corn bread in the database of Central America and Panama, a group of edible insects in the database of Thai food compositions.

National or regional classification systems are often difficult to use as an international basis, so we will not consider them in this article. Let us proceed to the known unified bases that can be applied to many modern cultures.

All these food classification systems have been developed for general information purposes. Their existing food codes are not specific and cannot replace national codes in the integrated food composition or value databases. All of the above systems refer to "classification" category. All of them were created for different purposes and reflect the peculiarities of the legislation of different groups of countries. To systematize them together does not appear to be real empirically. So demonstrating the enormous difference underlying these systems, one can see that, for example, when classifying cheeses into categories, the CIAA system differentiates them, first of all, as unripened, ripened, processed cheese. Eurocode-2 classifies cheeses, first of all, according to their consistency (soft, hard), and then, according to their fatness. PROCOME classifies all cheeses by "natural pure cheese" and "CCPR cheese" categories (with residues and contaminants). Classifications, even within a single system, can be contradictory, and their existence proves that there can be no single International classification system that can be unanimously approved and regulated. In other words, there is no single classification, which would be able to meet the needs of any food composition database compiler.

Next, we will consider systems of product identification in the special databases, which function according to the internal codes and descriptions.

The FAO/INFOODS Global Database for Pulses on Dry Matter Basis (PulsesDM1.0) provides nutrient values for pulses on a DRY MATTER BASIS — it is intended mainly for standard setting purposes [4]. Pulses are a subgroup of legumes that includes dry edible seeds with low fat. The data were recalculated to dry matter basis from the average nutrient values for 16 species published in the FAO/INFOODS Global Food Composition Database for Pulses (uPulses). In uPulses, data derived mainly from chemical analysis, complemented by data from other published sources and compiled following standards and guidelines outlined by FAO/INFOODS. PulsesDM cover minerals, vitamins, phytate, amino acids

and fatty acids fractions for foods in raw and processed forms. The data are presented in Excel format alongside with a comprehensive documentation in PDF format [5].

EuroFIR AISBL, an international, member-based, non-profit Association under Belgian law, was set up in 2009 to ensure sustained advocacy for food information in Europe. Its purpose is to develop, publish and exploit food composition information, and promote international cooperation and harmonization of standards to improve data quality, storage and access. EuroFIR AISBL draws together the best available food information globally from 26 compiler organizations in Europe, USA and Canada (FoodEXplorer) as well as validated information about bioactive compounds (eBASIS).

Food composition tables were originally produced as printed versions, and for many years this remained the only format. However, computerized databases have become increasingly important because they can hold large amounts of data and allow easy access to and manipulation of data. In more recent development, being facilitated and encouraged within Europe by EuroFIR, many national databases are now available online. A wide range of nutritional analysis software is also available [6].

In 2013, European compilers produced a food composition dataset for *EFSA* that aimed to provide an updated food composition database covering approximately 1750 foods and to expand the dataset to include harmonized information on the most common composite recipes of European countries. The dataset has been compiled to be compatible with the EFSA Guidance on Standard Sample Description for Food and Feed [7] and included additional descriptors from the EFSA FoodEx2 classification system [8].

Even using the most comprehensive and well documented food composition the databases do not guarantee robust and reliable results, as there are many errors that can arise in using food composition data. These include errors in matching foods, use of incompatible data, inappropriate strategies for dealing with missing values, and problems relating to the use of nutritional analysis software. Using food composition data to estimate nutrient intakes or the nutrient content of a recipe or menu can yield further errors owing both to the limitations of dietary assessment techniques and to errors associated with dietary assessment (e.g. conversion of reported portion descriptions to weight).

The EuroFIR FoodEXplorer facility is an innovative interface, which can be accessed online and allows its users a simultaneous search of standardized and specialized food composition databases (FCDB). Users have access to a wide range of European data, foods and nutrients through harmonized data description and associated nutrient value information.

Food classification and food description are completely different categories, since they have different purposes; however, sometimes they are "mixed" into a single whole. Classification systems have only one tendency — to group products with similar characteristics (and then, not always objectively), which means, that it is a tool of the end user of the data. Description system is a data source tool that gives a description of food as accurately as possible.

The first system that carried out sequential indexing and search of food data, carried out by means of specially built in thesauruses was the INFIC/ENFIC system for animal feed identification. There, a vocabulary control is achieved by deliberately limiting the scope of terms and through its direct reflection of hierarchical relationships. The structure allows making changes by adding new "points of view" to describe food, incorporating new information. Thus, the thesaurus is well adapted to product features that can change over time.

LanguaL thesaurus, which is used in the USA, Europe and is projected on numerical food databases, is the optimal for a person "vocabulary" existing today (Fig. 1). At first, LanguaL was called as "food factorization dictionary" (McCann et al., 1988) and was created at the end of 1970 by the Center for Food Safety and Applied Nutrition, with the help of the USA Food and Drug Administration (Hendricks, 1992). Since 1996, the European Technical Committee has taken charge of the thesaurus. In total, more than 40,000 food products have been described in different countries using this dictionary. LanguaL is a multi-modal, convenient and multilingual thesaurus organized in 14 different branches, characterizing the nutritional and/or hygienic qualities of food products, which is essential for biological and medical research.

The basic concepts of LanguaL are that:

1. Any food (or food product) can be systematically described by a combination of characteristic;

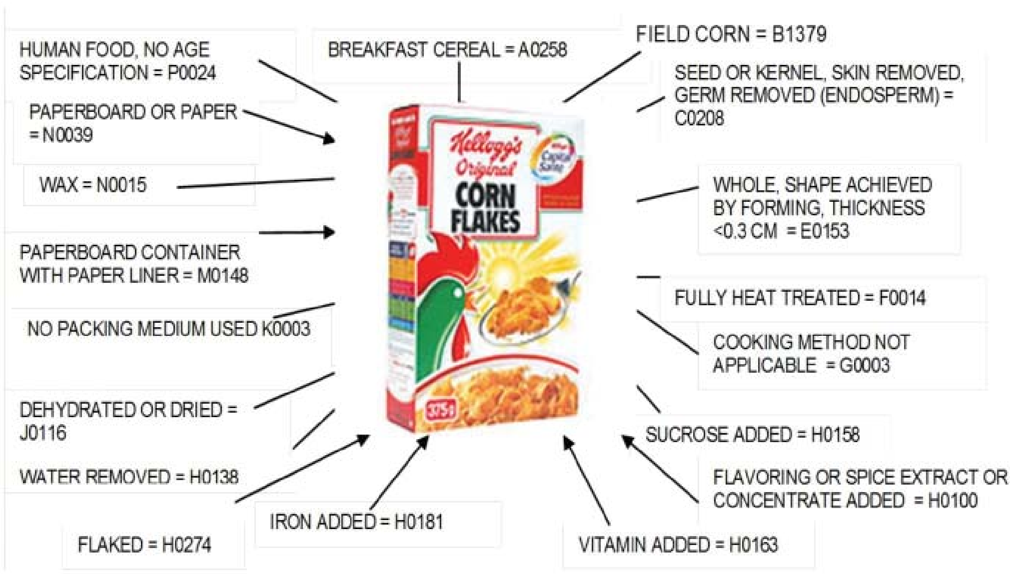

Fig. 1. **Description of food by LanguaL**

2. These characteristics can be categorized into viewpoints and coded for computer processing;

3. The resulting viewpoints/characteristic codes can be used to retrieve data about the food from external databases [9].

Each descriptor has a unique base code that points to equivalent terms in different languages, which makes the thesaurus independent of language features and peculiarities. For the past 2 years, the thesaurus has been significantly modified and now it provides open links to international food categories and coding systems. The official international version of the thesaurus was published on the LanguaL website (http://food.ethz.ch/langual), where copies of the thesaurus are available on request. The interface allows searching for products in American, Danish, French, Hungarian and a number of other databases, which maximally facilitate the exchange of information at the transnational level. However, some aspects still require further clarification, as LanguaL lacks some of the food groups that are used in the national tables. There is also a need to optimize software for searching and indexing relevant terms. The European LanguaL Technical Committee is currently working on these issues (Fig. 2).

By food description, the *INFOODS* management board prepared cognominal INFOODS system with the support of the Committee on Food Nomenclature and Terminology in 1987. The purpose of the INFOODS nomenclature system is to provide a basis for data exchange between primary sources and compilers of systems devoted to information on food composition. The system is a wide, multifaceted and open mechanism. The INFOODS management board offers criteria for determining whether the food is one-component or multi-component and provides different sets of descriptive aspects for these two large classes. However, this thesaurus is significantly inferior to LanguaL in its completeness. Also, it does not provide an indexer/retriever with a list of possible terms (synonyms) for any product of interest. A draft of the increased number of terms was repeatedly prepared, but it has not been published yet. The INFOODS system or its individual forms is used in New Zealand, the South Pacific, several African countries and 10 Latin American countries. LanguaL is common in other countries.

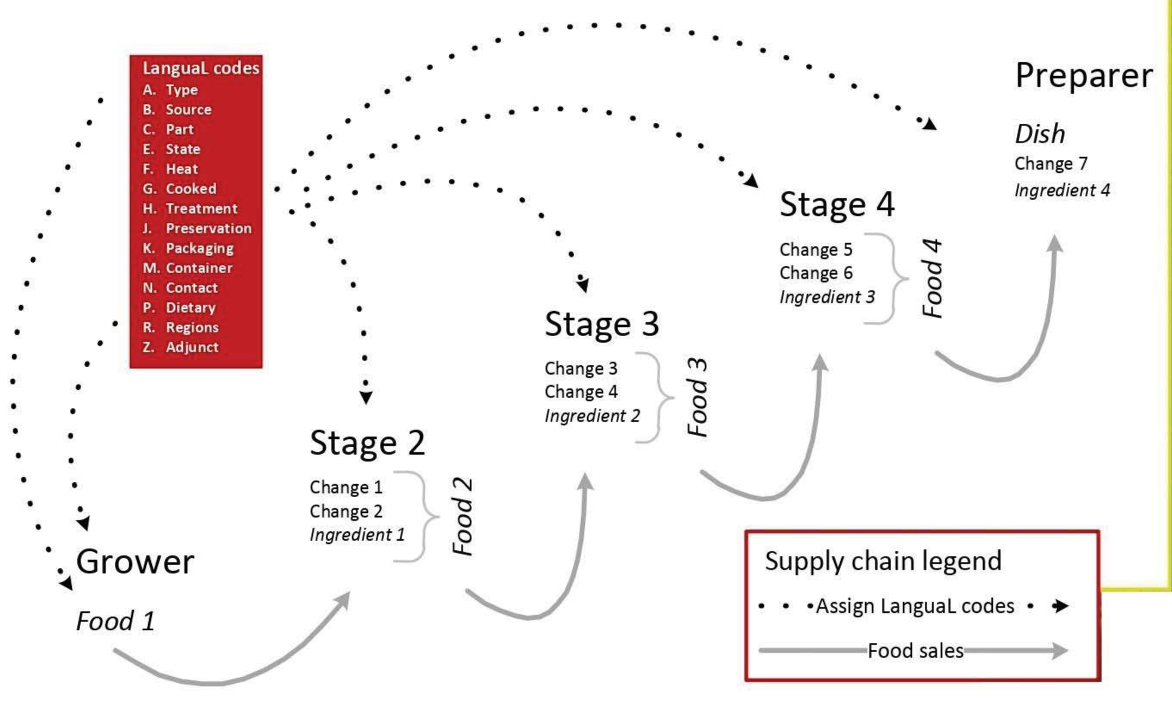

Fig. 2. **LanguaL and the food supply chain (Joanne Holden, ARS-USDA, 2010)**

Earlier, several other test food thesauruses were created to manage bibliographic information: CAB thesaurus, which is used by Nutrition Abstracts and Reviews; IFIS thesaurus, which is used in the theses of Food science and technology society; AGROVOC thesaurus (FAO, 1998), Created on FAO AGRIS and CARIS data banks. However, they were developed primarily for documentation purposes and do not have the specificity of product descriptions, which only LanguaL has.

Thus, there are two main systems that are used to describe food in food composition and characteristics databases: LanguaL thesaurus with international terms and the INFOODS system, which application depends on the national language. A comparison of these two systems was carried out by the coordinators of the regional data centers (Burlingame, 1998). LanguaL thesaurus language scored higher in relation to solving problems of the language barrier and culture, which is the reason for its unconditional adoption in Europe. However, the INFOODS system has shown some better results regarding the relationship of data compilers and local utility for ordinary users, who want to obtain data on the composition of consumable food quickly and easily. The INFOODS system is easier and faster to use and does not require searching for complex terms and codes in lists. The demand for a thesaurus that is language-independent (LanguaL), and the need for a practical, “field” system (such as the INFOODS system) for food products, have led to attempts to combine these systems and create a minimum set of standards and an agreed approach for food identification around the world. Examples of this combined approach are “System mapping” and “International Interface Standard for Food Databases”.

For the first time the Ukrainian National and Regional Food Composition Databases were created by us. In the food industry, access to food database opens up new opportunities for selection of food components for the analysis of available data, creating new recipes that will have a positive effect on production of foods. Also, it is very important for Ukrainian consumers to have access to the information about the quality and composition of foods. Especially important is the content of allergens, sugar, GMOs, information about shelf life of products, etc. To ensure consumer access

to such information scientists have to make a huge effort on creation of database of food in Ukraine [10]. One of the major problems in Ukraine is lack of reliable verified information on the composition of food products due to non-compliance by manufacturers with the relevant labeling rules. Also analytical stage where the main components of food products are analyzing have a big value in food quality control. Nowadays we do not have an unified analytical system, methods of food components analyzing and also qualified employees and compilers.

Thus, the first breakthrough in the international food identification became apparent after the scientific recognition of the benefits of using a multidimensional approach to food systematization in food databases. The second breakthrough is the recognition of the need for an alternative classification/descriptive system that would combine the advantages and exclude the disadvantages of the program products described above.

Work in the field of transnational food identification in databases is carried out with the help of the IUNS/FAO international target group and was approved at the third International Conference on Food Data. This target group will continue to review and analyze the work that will be carried out on existing food classifications and descriptions in order to harmonize the international use of the final result.

Nowadays, the Global Harmonization Initiative (GHI) is created in Europe. GHI is the international non-profit network of individual scientists and scientific organizations working together to promote harmonization of global food safety regulations and legislation and this is not possible without reliable information on the composition of food and its availability.

This work was supported by the

1. International project "BaSeFood" (FP7 — scientific cooperation) on "Bioactive components in traditional foods". Contest KBBE-2008-2-2-02: Biologically active substances in traditional foods: FP7-KBBE-2008-2B (food, agriculture and fisheries, and biotechnology).

2. INFOOD/FAO "Collection and compilation of analytical food composition data in the region of Europe". Cooperation Agreement concluded on 1.02.2016.

REFERENCES

1. *Almli V. L., Verbeke W., Vanhonacker F., Næs T., Hersleth M*. General image and attribute perceptions of traditional food in six European countries. *Food Quality and Preference*. 2011, 22(1), 129–138. https://doi.org/10.1016/j.foodqual.2010.08.008
2. *Porubská J., Giertlová A., Morochovičová M., Kováčiková E., Porubský O*. The Slovak national food composition database: New management system DaRiS. *Journal of Food Composition and Analysis*. 2014, 34(1), 26–38. https://doi.org/10.1016/j.jfca.2014.02.007
3. *Kapsokefalou M., Roe M., Turrini A., Costa H.S., Martinez-Victoria E., Marletta L., Finglas P.* Food Composition at Present: New Challenges. *Nutrients*. 2019, 11(8), 1714. https://doi.org/10.3390/nu11081714.
4. *De Victoria Muñoz, E. M*. Do we really know the composition of our foods? *Nutricion hospitalaria*. 2013, 28(5), 73–80.
5. International Network of Food Data Systems (INFOODS). Available online: http://www.fao.org/infoods/ infoods/en/ (accessed on 20 May 2019)].
6. EuroFIR. Network of Excellence and NEXUS Projects. Available online: http://www.eurofir.org/our-resources/ noe-and-nexus-projects/ (accessed on 20 May 2019). https://www.mdpi.com/2072-6643/11/8/1714/html
7. European Food Safety Authority. Standard sample description for food and feed. *EFSA J*. 2010, 8, 1457.
8. European Food Safety Authority: Food Classification. The Food Classification and Description System FoodEx2 (Revision 2). 2015. Available online: http://www.efsa.europa.eu/en/datex/datexfoodclass.htm (accessed on 20 May 2019).
9. LanguaL™ - the International Framework for Food Description Available online: https://www.langual.org/default.asp
10. *Koval A., Pauk L., Buhyna H., Costa T., Albuquerque A, Sanches-Silva J*. Development of national food composition database in Ukraine following EuroFIR and BaSeFood projects Plumb, M. Roe, P. Finglas, M. Glibetic, N. Boyko. *Traditional food international*, 4–5 October 2012, P. 89.

БАЗИ ДАНИХ ХАРЧОВИХ ПРОДУКТІВ: ПРОБЛЕМИ ТРАНСНАЦІОНАЛЬНОГО ЗАСТОСУВАННЯ

Л. М. Бугіна, О. В. Паллаг, Т. В. Мелешко, В. В. Баті, Н. В. Коваль, Н. В Бойко

Ужгородський національний університет, Україна

E-mail: larina.bh@gmail.com

Метою цього дослідження було проаналізувати і порівняти існуючі міжнародні методології, що їх застосовують для класифікації та ідентифікації характеристик харчових продуктів у сучасних комп'ютеризованих базах даних (Foodex, INFOOD/FAO, EuroFIR), а також подати наші первинні результати створення регіональних і національних українських баз даних за складом продуктів харчування.

У дослідженні використовували теоретичний синтез і дедуктивний аналіз, огляд літератури зарубіжних наукових рецензованих джерел, LanguaL, DaRiS.

Попит на незалежний від мови тезаурус (LanguaL) і потреба в практичній, польовій системі харчування (INFOODS) спонукали до спроб зв'язати цю систему та створити мінімальний набір стандартів і послідовний підхід до визначення харчових продуктів у всьому світі. Прикладами цього комбінованого підходу є «Картографування системи» та «Міжнародний стандарт інтерфейсу для баз даних стосовно продуктів харчування».

Уперше впроваджено різні інструменти для складання перших регіональних (100 місцевих продуктів) та національних баз даних про склад продуктів харчування (53 продукти із 6 пріоритетних груп традиційних страв і напоїв) у проекті BaSeFood.

Ключові слова: композиційні бази даних харчових продуктів (КБДХП), LanguaL, DaRIS, INFOODS/FAO, EuroFIR.

БАЗЫ ДАННЫХ ПРОДУКТОВ ПИТАНИЯ: ПРОБЛЕМЫ ТРАНСНАЦИОНАЛЬНОГО ПРИМЕНЕНИЯ

Л. М. Бугина, А. В. Паллаг, Т. В. Мелешко, Бати В. В., Н. В. Коваль, Н. В. Бойко

Ужгородский национальный университет, Украина

E-mail: larina.bh@gmail.com

Целью данного исследования были анализ и сравнение существующих международных методологий, применяемых для классификации и идентификации характеристик пищевых продуктов в ряде современных компьютеризированных баз данных: Foodex, INFOODS/FAO, EuroFIR, а также представление наших первоначальных результатов создания региональных и национальных украинских баз данных по составу продуктов питания.

В исследовании были использованы теоретический синтез и дедуктивный анализ, обзор литературы зарубежных научных рецензированных источников, LanguaL, DaRiS.

Спрос на тезаурус, который не зависит от языка (LanguaL), и потребность в практической, полевой системе (INFOODS) питания, привели к попыткам связать эту систему и создать минимальный набор стандартов и последовательный подход для определения пищевых продуктов во всем мире. Примерами этого комбинированного подхода являются «Картографирование системы» и «Международный стандарт интерфейса для баз данных продуктов питания».

Впервые представлены различные инструменты для составления первых региональных (100 местных продуктов) и национальных баз данных о составе продуктов питания (53 продукта из 6 приоритетных групп традиционных продуктов питания и напитков) в проекте BaSeFood.

Ключевые слова: композиционные базы данных пищевых продуктов (КБДПП), LanguaL, DaRiS, INFOODS / FAO, EuroFIR.

UDC 614.253 https://doi.org/10.15407/biotech12.06.012

CANCER DIAGNOSTICS, IMAGING AND TREATMENT BY NANOSCALE STRUCTURES TARGETING

ÖZNUR ÖZGE ÖZCAN, MESUT KARAHAN

Üsküdar University, İstanbul, Turkey

E-mail: mesut.karahan@uskudar.edu.tr

Received 10.09.2019
Revised 04.11.2019
Accepted 20.12.2019

Recent research focused on finding new strategies in cancer therapy that did not have significant side effects and was more effective than traditional modules including the surgical intervention, radiation and chemotherapeutics. In this regard the nanoscale structures provide useful approaches for cancer treatment. So, the nanoparticle systems improve the efficiency of therapeutic drugs reducing their side effects. Although many studies reported the development of novel cancer cell therapies for future, the clinical success is lacking understand the effects of nanoparticle type, size and dose with their usage areas. Thus, this review was aimed to illustrate the usage of nanoparticles in cancer diagnostic, imaging and treatment.

Key words: cancer diagnostic, imaging and treatment, nanoparticles.

Cancer continues to be one of the world's most devastating diseases with more than 10 million new cases each year [1]. The number of people diagnosed with malignancy is expected to rise to 22 million annually in the next 2 decades.

The information about various types of nanoparticles (NPs) used in cancer treatment, diagnosis and imaging was given under the title of various types of NPs used in the field of cancer treatment. After that, this review was focused on the active and passive targeting importance for the cancer apoptosis. In addition, the impact of Contemporary Advancements in active targeting nanoformulations were explained. In the last part, development and importance of different types of NPs in cancer diagnosis and imaging was discussed. Significance of this review is presentation in the field of cancer to date nanoparticles; a review with a holistic approach to their use in vaccines, drugs, diagnosis and imaging will be of great importance. Nanoparticles are very diverse and their efficacy and advantages vary according to the fields of medicine, vaccine and imaging. Which type of nanoparticles that we are going to choose for our future studies could be seen in the reviews of our previous studies. Therefore, we aimed to present a work that we think it can be as useful as possible from the active, passive targeting of cancer to the varieties used in medicine and vaccines.

There is lack of understanding concerning the effects of the nanoparticle types, working in the future without confirming its reported function. This review will be useful for better comprehension of molecular basis of nanoparticles and the advent of new diagnostic technologies can help to improve the treatment of various cancers.

Various types of nanoparticles used in the field of cancer treatment

Treatment modalities, including immunotherapy, photo thermal, photodynamic, gene and hormone therapy display promising cancer eradicating potential in preclinical studies. However, surgery, radiation and chemotherapy continue to be the first treatment option for cancers and the next major strategy for cancer treatment is highly non-specific in targeting the drugs to the cancer cells causing undesirable side-effects to the healthy tissues

[2–4]. Unfortunately, there are no alternative for cancer treatments other than surgical removal of the cancer site, radiotherapy and chemotherapy. In addition, chemotherapy and radiotherapy have thousands of side effects that kill both cancer cells and healthy cells. Therefore, we see that cellular therapies, which we believe will be more effective for cancer treatment, have started to be developed. In developing cellular therapies, nanocarriers that can be produced up to cellular dimensions have been of great importance. There are possible risks in these systems. For instance, NPs can be phagocytosed as an enemy in the body by being exposed to attacks of immune cells and may cause failure of such cancer treatment intervention. In addition, NPs can produce molecular responses such as deleterious, allergic and toxic effects in cancer cells, thereby causing greater sensitivity to cancer progression [5]. For this reason, choosing the most accurate NPs for the cancer disease will prevent patients from complications and can provide more effective therapy. During the last decades, an abundance of NPs have been developed and a real hype has been created around their potential application as diagnostic and therapeutic agents. For example, although iron oxide NPs have been suggested as potential diagnostic agents, they have not been fully preferred for clinical purposes. Therefore, pre-clinical studies on many experimental animals are ongoing [6]. This is primarily due to the ability of NPs to be biologically suitable for the body. There are some problems related to biological degradation of NPs, expulsion from the body and the possibility of toxic effects. Further studies are needed to eliminate these drawbacks of Iron oxide NPs.

Molecular imaging, when used in conjunction with said nanosystems, may make cancer diagnosis and treatment more effective. Multidisciplinary studies can be combined to provide more effective diagnosis and treatment, and these disciplines can often be made possible by collaborating with researchers in different fields such as cell biology, biochemistry, engineering, health and medicine. Recent advances in NPs technology have enabled the fabrication of NPs classes with unique sizes, shapes and materials, which in turn has facilitated major advancements in the field of nanomedicine. The promising proposition of multifunctional NPs for cancer diagnostics and therapeutics has inspired the development of the approach for improved cancer therapy. The nature of NPs is closely related with the various materials that used for their synthesis, such as metals (gold and silver), ceramics (hydroxyapatite), lipids (cholesterol and non-toxic phospholipids) and polymers [alginate, chitosan, poly(ethylene glycol (PEG)] [7].

The combination of anti-cancer drugs and different types of agents with NPs in the treatment of cancer has many advantages:

1. Enhancing the stability of hydrophobic drugs, making them suitable for application. In other words, it increases the water solubility of drugs. Especially in imaging and drug therapies developed with these nanoscales, only cancer cells can be targeted and adverse effects such as systemic chemotherapy, hair loss, immunosuppression, muscle weakness, etc. can be completely eliminated.

2. Reducing toxicity by using biocompatible nanomaterials. In both conditions it results in a therapeutic index increase, the limit between doses causing therapeutic efficacy (e.g. cancer cell apoptosis) exhibits high differential uptake efficiency in the target cells over normal cells.

3. Developing pharmacokinetics and bio distribution which increase drug efficiency on the tumor and enhance absorption of the drugs into a selected tissue (for example, solid tumor) [8].

4. One of the most important advantages of the NPs is the fact that they are able to treat cancer which is caused by cell organelle disorder. For example, effective targeting of mitochondria and nucleus has emerged as an alternative strategy in cancer chemotherapy agent which is Dual drug conjugated NP [9].

Nevertheless, before the plethora of nanodevices currently under investigation become proper for clinical usage, they have to pass the rigorous tests set forth by regulatory agencies such as Food and Drug Administration (FDA) and European Medicines Agency (EMEA) [10–12]. To date, at least 12 polymer–drug conjugates have entered Phase I and II clinical trials and we need to find more treatment model for cancer cell targeted therapy. Various biocompatible (NPs)-based drug-delivery systems such as liposomes, dendrimers, micelles, silica, quantum dots, and magnetic, gold, and carbon nanotubes have already been reported for targeted cancer treatment.

Silver Nanoparticles (AgNPs)

Therapeutic applications of silver nanoparticles (AgNPs) agents in the diagnosis and probing of many cancer diseases are noteworthy [13]. AgNPs cause apoptosis in

cancer cells as they produce reactive oxygen species (ROS) that cause oxidative stress and DNA damage that cause mitochondrial damage within the cell. Cellular penetration of AgNPs usually occurs through endocytosis [14]. In another study, it was found that AgNPs for cell morphology may affect the function of other factors. These effects include the ability to adsorb cytosolic proteins and regulate gene expression and proinflammatory cytokines, for example, microarray analysis showed that human lung epithelial cell line A549 affects cellular transcriptome analysis upon exposure to AGNPs. According to the results of the microarray study, it was found that AgNPs affect the regulation of more than 1000 genes [15]. AgNPs can induce autophagy by allowing the accumulation of autophagolysis in human ovarian cancer cells and autophagy can have a dual cell bodies. The use of autophagy inhibitors or autophagy protein 5 (ATG5) with small-mix RNAs (siRNA) in combination with AgNPs causes the death of cells in cancer cells [16]. As a result, AgNPs can induce cell death including ROS generation, leakage of lactate dehydrogenase, increasing of apoptosis and autophagy genes, cause endoplasmic reticulum stress and mitochondrial damage, activation of caspases pathways and DNA damage so that AgNPs can be used as nanoparticle that is significant to deliver drugs for cancer treatment. In addition, AgNPs has significant importance to modulate ABC transporter activity for chemotherapy in multidrug resistant cancer [17].

Gold nanoparticles

Gold nanoparticles (AuNPs) are ideal for drug targeting and also for imaging-based detection of cancer diseases at an early stage. AuNPs were first produced in 1857 by Faraday and exhibit favorable physical properties and tailored surface functionalization, providing a potential for developing cancer theranostics and they are solid balls of gold and are made by the reduction of chloroauric acid, and their diameter varies from 5 to 100 nm. They are biocompatible and less toxicity and display the relatively low rate of clearance from circulation. AuNPs was demonstrated [18] where AuNPs were conjugated with an antibody against the epidermal growth factor receptor (EGFR, it is known to overexpress on many cancers). PEGylation of AuNPs effectively downregulate this uptake by macrophages and monocytes [19]. AuNPs conjugated with carbohydrates and proteins have been utilized in novel approaches toward the development of vaccines such as Glyco-conjugated AuNPs (1–5 nm) capped with carbohydrate-based antigens that are present in cancer cells [20, 21]. Targeting mitochondria with conjugated Au- cationic NPs maltotriose-modified poly(propylene imine) (PPI) dendrimers effects on apoptosis induction in the human breast cancer cell line [22]. Doxorubicin (DOX) loaded oligonucleotides attached to (AuNPs) as a drug delivery system is useful for cancer chemotherapy [23]. Micro RNA (miR)-375 loaded AuNP exhibits high cellular uptake and preserves miR-375's activities to suppress cellular proliferation, migration/invasion, and colony formation, and to induce apoptosis in hepatocellular carcinoma cells [24].

Polymeric particles

Synthetic polymers are polylactic acid (PLA), poly(lactic-co-glycolic acid (PLGA), polycaprolactone (PCL), PEG, and poly(vinyl alcohol (PVA) are composed of commonly used natural polymers. Chemotherapeutic drugs (Trastuzumab, Pentuzumab, Paclitaxel, DOX, 5-Fluorouracil and Dexamethasone, etc.) become more effective when they are encapsulated with polymeric NPs.

NPs synthesized from PLGA, a synthetic polymer, are widely studied for anticancer drugs [26, 27]. PLGA has several advantages over cell-targeted therapies compared to other delivery systems:

1) It has been approved by the FDA for drug delivery in humans [28, 29].

2) Biodegradable.

3) Has sustained release activity, ranging from days to weeks under physiological conditions.

4) Provides long-term stability of charged bioactive molecules.

5) Shows ability to capture hydrophobic and hydrophilic drugs.

6) Has comprehensive functionalization options.

PEG is a water-soluble, biocompatible polymer commonly used for coating a wide variety of drugs to improve encapsulation efficiency. Especially in Human Epidermal Receptor 2 (HER2)-related breast cancer, mir-21 is effective in tumor immunity of Antisense Oligonucleotides (ASO) [30]. It has been demonstrated in the animal model that the antitumor effect is quite high even though MiR-21 was used previously only with targeting ligand by PLGA-PEG encapsulation of ASO [26, 31–33]. A biodegradable poly (D, L-lactide-co-glycolide) -block-poly (ethylene glycol) (PLGA-b-PEG-COOH) copolymer will be

synthesized. The most important factor in PEG expression is the prevention of immune system agents [34]. The strong buffering capacity of cationic polymers could effectively help themselves to escape from endo/lysosome as a result of "proton sponge" effect. For instance, 25 kDa polyethylenimine is well known of its excellent transfection activity *in vitro* largely due to its strong buffering capacity and these NPs is very useful for cancer immunotherapy for vaccinations [35–38].

Superparamagnetic iron oxide (SPIO) nanoparticle

Chemotherapeutic agents have been associated with SPIO-based nanocarriers through different strategies (e.g., conjugation via cleavable linker and π-π stacking with polymer layers) for delivery to tumors. Dual paclitaxel (PTX)/superparamagnetic iron oxide (SPIO)-loaded PLGA-based NPs have a potential role in tumor growth [39] in passive targeting.

"*iRGD*" peptide affects the uptake of iron oxide during labeling of panc1 cells for this reason an appropriate "*iRGD*" peptide concentration enhances the uptake of intracellular iron tumor cell proliferation in active targeting [40]. Trastuzumab is conjugated to SPIO NPs which labor as magnetic resonance imaging (MRI) contrast agents to detect HER2-positive tumors [41, 42].

Carbon nanotubes (CNTs)

Carbon NanoTubes (CNTs) are carbon allotropes with a cylindrical nanostructure which have gained intensive interest during the past 20 years because of their unique mechanical properties in addition to very interesting values in electrical and thermal conductivity and also the possibility of their surface to functionalize with a wide group or biochemical species paving the way for numerous therapeutic and drug delivery applications [43, 44]. They are able to penetrate easily through the cellular membrane and have low immunogenicity with significant uptake of delivered small interfering RNA (siRNA) and a working gene silencing effect in the tumor tissue [45]. Many of them have high general toxicity and additional drawbacks, like limited solubility and a poor non-selective biodistribution [46].

Liposomes

Liposomes are broadly used as drug delivery systems and several liposomal nanomedicines have been approved for clinical applications. Liposome-based combination chemotherapy contributes a novel avenue in

	Particle type	Composition/Structure	Properties	Applications
	Polymer	e.g., PLGA, glycerol, chitosan, DNA; monomers, copolymers, hydrogels	Some biodegradable	Drug delivery; passive release (diffusion), controlled release (triggered)
	Dendrimer	PAMAM, etc.	Low polydispersity, cargo, biocompatible	Drug delivery
	Lipid	Liposomes, micelles	Can carry hydrophobic cargo, biocompatible, typically 50–500 nm	Drug delivery
	Quantum dots	CdSe, CuInSe, CdTe, etc.	Broad excitation, no photobleaching, tunable emission, typically 5–100 nm	Optical imaging
	Gold	Spheres, rods, or shells	Biocompatibility, typically 5–100 nm	Hyperthermia therapy, drug delivery
	Silica	Spheres, shells, mesoporous	Biocompatibility	Contrast agents, drug delivery (encapsulation)
	Magnetic	Iron oxide or cobalt-based; spheres, aggregates in dextran or silica	Superparamagnetic, ferromagnetic (small remanence to minimize aggregation), superferromagnetic (~10 nm), paramagnetic	Contrast agents (MRI), hyperthermia therapy
	Carbon-based	Carbon nanotubes, buckyballs, graphene	Biocompatible	Drug delivery

Fig. 1. **NPs types and their application areas for cancer imaging and treatment [25]**

drug delivery research and has increasingly become a significant approach in clinical cancer treatment. Liposomes are grouped into two types:

1) unilamellar vesicles and
2) multilamellar vesicles.

For the treatment of multidrug resistance (MDR)/cancer immunotherapy, mixtures of siRNA/plasmid DNA and hydrophobic drug can be used with Liposomes [47].

Paclitaxel and Rapamycin (with anti-tumor and immunosuppressant properties and an inhibitor of mTOR protein kinase) are encapsulated with PEG-Liposome in breast cancer — Liposomes released in slow and sustained fashion ↑ Cell line cytotoxicity ↑ *In vivo* therapeutic effects. The system controlled the tumor growth [48].

Folate receptor, a membrane-associated folate binding protein, is overexpressed in over 90% of ovarian cancer and other epithelial types of cancer [49, 50]. Xu et al. developed a FR targeted co-delivery formulation by folate-DOX/Bmi1 siRNA liposome (FA-DOX/siRNA-L), demonstrated a great tumor targeting effect and prevented tumor growth *in vitro* and *in vivo* experiments [51, 52].

A thermosensitive magnetic liposomal delivery system is effective co-delivery of gene silencing short hairpin RNA (shRNA) vector and antitumor drug (DOX) into gastric cancer [53]. Liposomal system with an antimicrobial peptide and co-delivery of antagomir-10b could trigger cell death in the meantime besides hindering of T cells migration [54].

Polimeric Micelles

Polymeric micelles used due to their ability to load therapeutics, deliver the cargo to the site of action, improve the pharmacokinetic of the loaded drug and reduce off-target cytotoxicity. They are also developed with improved drug loading capabilities by modulating hydrophobicity and hydrophilicity of the micelle forming block co-polymers and also cancer targeted by surface modifying with tumor-homing ligands. Their classroom contains in Polymeric micelles of therapeutic applications in cancer treatment.

1. Pluronics®
2. PEG-PLA
3. PEG-PCL
4. PEG-Lipid
5. PEG-PLGA

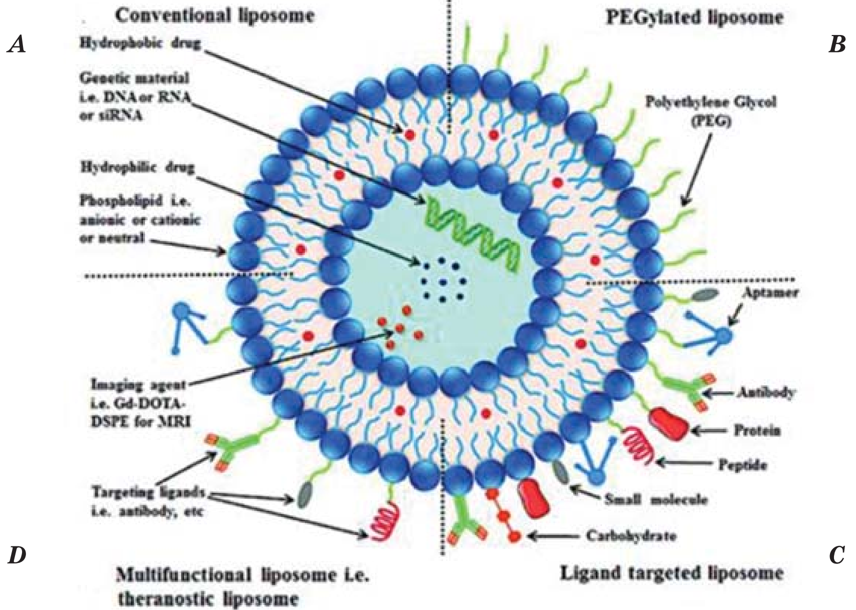

Fig. 2. **Illustration of the versatile functions of liposomes:**
A — Conventional liposomes are composed of two layers of phospholipids and when administered intravenously and remain in the circulatory system for a short time. Also they are widely used for the maintenance of hydrophilic drugs that did not pass through the cell membrane;
B — When protected with PEG, they have the ability to be protected from immune cell attacks and stay longer in circulation;
C — When liposomes are used for active targeting with small molecules such as aptamer, peptide, ligand and protein, they show very high effect on cancer cells;
D — Especially in the diagnosis and treatment of solid tumors they have teranostic effects. They are also often preferred for imaging (such as MRI) [55]

6. PEG-poly(amino acids)
7. Stimuli-sensitive polymeric micelles
8. Endogenous stimuli-sensitive polymeric micelles
9. pH-sensitive polymeric micelles
10. Reduction sensitive polymeric micelles
11. Thermo-sensitive polymeric micelles
12. Exogenous stimuli-sensitive polymeric micelles
13. Light-sensitive polymeric micelles
14. Magnetic field-sensitive polymeric micelles
15. Ultra-sound sensitive polymeric micelles
16. Margination of micro/nanoparticles: Requirement for optimum drug delivery.

Other hydrophilic block forming polymers include chitosan, poly(N-vinyl pyrrolidone) (PVP), and poly(Nisopropylacrylamide) (pNIPAAm). There are various polymer blocks used to form micellar core, including the class of polyethers such as poly(propylene oxide) (PPO), various polyesters such as PLA, PCL, PLGA, poly(β-aminoesters), polyamino acids such as poly(L-histidine) (pHis), poly(L-aspartic acid) (pAsp) and lipids such as dio leoyl(phosphatidylethanolamine) (DOPE), distearoyl (phosphatidylethanolamine) (DSPE). The assembly of block co-polymers, in which PPO attached to PEG as A-B-A triblock co-polymers (PEO-PPO-PEO) is known as Pluronics [56].

Protein Nanoparticles

Protein NPs are also used including water-soluble proteins (e.g., bovine and human serum albumin) and insoluble proteins (e.g., zein and gliadin). So far, most of proteins NPs of article are focused on the preparation and characterization of nanoparticles derived from gelatin, albumin, gliadin, legumin, Methoxy-PEG-polylactide, PEG-asparaginase and two milk proteins that have been investigated for drug delivery applications are Beta-lactoglobulin (BLG) and casein [57, 58]. To promote drug targeting ability, protein nanoparticles have been chemically modified to incorporate targeting ligands that recognize specific cells and tissues. Such modification allows targeting of albumin nanoparticles to breast cancer cells, which overexpress HER2 [59]. Gliadin NPs used as a bioactive delivery system for oral vaccines administration to aid the sustained release delivery of anticancer drugs as well as colon cancer-targeted cyclophosphamide drug therapy and effective for apoptosis of breast cancer cells [60]. Cisplatin-loaded casein is a milk protein nanoparticles demonstrated their ability to penetrate cell membranes, target tumors, and inhibit tumor growth in hepatic tumor [61].

Cancer Treatment with Active and Passive Targeting

Active Targeting Strategies

NPs are used for active targeting [71] to cancer cells including antibody and antigen, peptide, protein, aptamer and ligand fragment based targeting in figure 3 [64]. HER2 is overexpressed in approximately 25–30% of invasive breast cancer but is less expressed by normal adult tissues. Targeted treatment with humanized Trastuzumab (Monoclonal Antibody) targeting the HER2 receptor has become the mainstay of HER2 positive breast cancer. The significant effect of Trastuzumab-conjugated nanoparticles to specifically target HER2 positive cancer cells has been

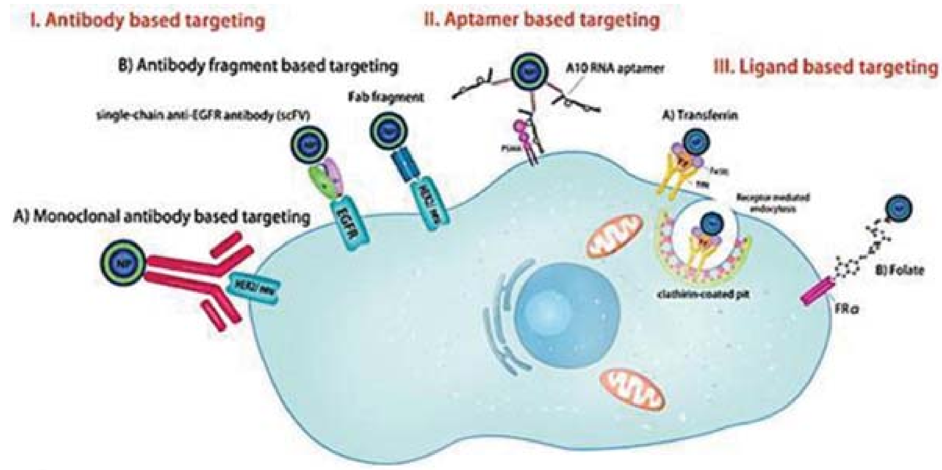

Fig. 3. **Illustration of active targeting components in cancer cells [62]**

proved *in vitro* using different cell lines and *in vivo* [62]. The ZHER2 antibody protein molecule consisting of 58 amino acids and 3 domains is designed as a high affinity linker to HER2 receptors. ZHER2 binds to the HER2 receptor, a domain point different from the point at which the therapeutic antibodies of Trastuzumab and Pertuzumab bind [63]. For this reason ZHER2 protein is highly used for diagnosis (imaging) and treatment of cancer diseases [64, 65]. Cetuximab has been targeted specifically and efficiently AuNPs in Epidermal Growth Factor Receptor (EGFR)-positive pancreatic and colorectal carcinoma cell lines [66].

Passive targeting facilitates the efficient localization of NPs in tumor interstitium but cannot further promote their uptake by cancer cells [71]. Uptake can be achieved by actively targeting NPs to receptors or other surface membrane proteins overexpressed on target cells. The addition of targeting ligands allows the delivery of drug-encapsulated NPs to uniquely identifiable cells or even subcellular sites, thereby reducing the unwanted systemic exposure of cytotoxic drug. Specific interactions between the ligands on the surface of nanocarriers and receptors expressed on the tumor cells may facilitate NPs internalization by triggering receptor-mediated endocytosis. Furthermore, active targeting of nanocarriers with small molecule therapeutic cargo has shown the potential to suppress multidrug resistance (MDR) via bypassing of P-glycoprotein-mediated drug efflux [67, 68]. As a result, although passive targeting facilitates the effective localization of NPs in the tumor interstitium, it cannot over-stimulate cellular uptake by cancer cells, such as active targeting.

NPs are functionalized with different biological molecules, peptides, antibody, and protein ligands for targeted drug delivery and also contain non-coding RNA, viral [69] and bacteria's DNA [38] or RNA for cancer immunization and cell death progress. Natural plant-based drugs that can be used in the field of pharmacology have also been found to be more effective when used with NPs such as Prosopis Cineraria. It is a leaf located in India, which can be used as NP-plant-based drug system for cancer treatment [70]. Ligands for active targeting in drug delivery approach on its great affinity to somatostatin receptors (SSTRs), which is overexpressed in several cancer cells such as core-shell type liposome co-encapsulating VEGF-targeted siRNA (siVEGF) is very effective drug system for VEGF based cancer types. SPIO NPs are accumulate in cancer through passive targeting by the EPR effect and with active targeting to help of targeting by ligands.

Passive Targetting Strategies

Although NPs refer to accumulate in the tumor cells due to the enhanced permeation and retention (EPR) effect, passive tumor targeting is dependent on the tumor vascularization and angiogenesis, and therefore lacks specificity and consistency. Both the tumor model type and conditions can seriously affect the passive targeting effectiveness [72–74]. General features of tumors include leaky blood vessels and

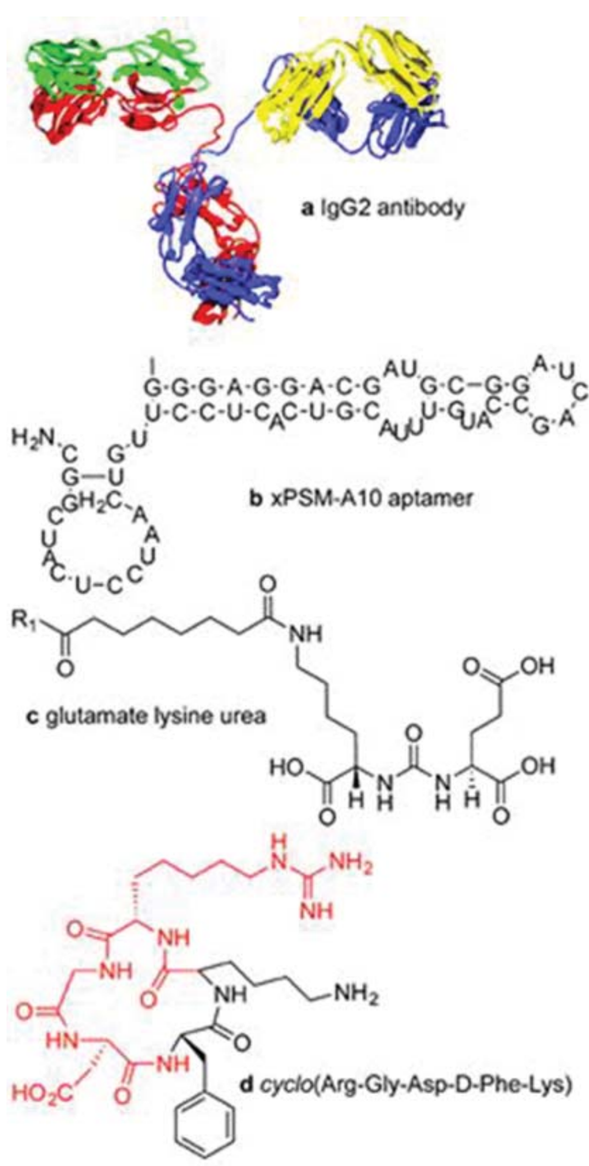

Fig. 4. **An example for Aptamer modeling to target cancer cells (generally receptors, ligands, etc.) [25]**

poor lymphatic drainage. Free drugs may be used nonspecically as a nanocarrier and can extravagate into the tumor tissues via the leaky vessels by the EPR effect. Sometimes, targeting cells within a tumor is not always feasible because some drugs cannot be used efficiently and the random nature of the approach makes it difficult to control the process which is lack of control may induce MDR.

Nanoparticle Imaging System

Imaging techniques used mostly in preclinical studies and clinical practice such as MRI, computed tomography (CT), ultrasound (US), optical imaging (OI), photoacoustic imaging (PAI), positron emission tomography (PET), and single-photon emission Computed tomography (SPECT).

Targeted NPs imaging agents provide a new technology for cancer imaging, which goes beyond anatomical characterization. It enables early detection of cancer as well as treatment monitoring at the molecular and cellular level. With the development of nanotechnology, magnetic NPs have been used in the MRI, adhering to target cells and drug release system [75, 76]. Tumor diagnosis and treatment can be obtained by the same NPs formulation and the disease can be monitored and fought by the synergistic effect of more than one therapy. The preparation and application of single multifunctional nano radiotracers based on iron oxides and enabling PET/MRI dual imaging can used for treatment and diagnosis for cancer [77]. New designed and realized a bimodal CT for SPECT and MRI with Superparamagnetic Iron Oxide NPs (SPION) privileged the magnetic properties to the CNT, while 99mTc granted the radioactive property.

Gold Nanoparticle Imaging

AuNPs are now used widely in bioimaging and phototherapy due to their tunable properties and highly sensitive optical and electronic properties of the surface plasmon resonance (SPR) [78]. AuNPs act as an active imaging probe for cancer detection facilitating whole-body scans. AuNPs can be easily functionalized with additional imaging agents by improving the AuNP-based imaging systems. That may allow the observation of tissues not only on its basic anatomic configuration but also on the molecular level for cancer diseases. For example, Au atoms using a one-step procedure for SPECT/CT imaging in an orthotropic mouse xenograft of triple-negative breast cancer (TNBC) and also PEGylation for favorable pharmacokinetics and d-Ala1-peptide T-amide (DAPTA) for targeting C–C chemokine receptor 5 (CCR5, a prognostic biomarker for breast cancer progression) [79, 80]. Multifunctional gold nanoprobe is designed for simultaneous miRNA-21 responsive fluorescence imaging and therapeutic monitoring of cancer. miRNAs provides a simple but powerful protocol with great potential in cancer imaging, therapy, and therapeutic monitoring [81].

Superparamagnetic iron oxide (SPIO) Imaging

Superparamagnetic iron oxide (SPIO) NPs were studied for the development of contrast agents in MRI for cancer diagnosis. First-generation of SPIO NPs had diagnostic capabilities only, whereas a new model of SPIO NPs has multifunctional characteristics for combined therapeutic and diagnostic applications for cancer. The magnetite (Fe_3O_4) and maghemite (Fe_2O_3) cores of SPIO NPs can be readily detected with MRI, thereby enabling real-time *in vivo* drug tracking. To provide colloidal stability of the magnetic core and better biocompatibility, SPIO NPs have been stabilized with polysaccharides (e.g., dextran and chitosan), PEG, polypyrrole (PPy), PLA, PLGA and their copolymers. Compared with other coating materials such as silica, polymer advantages with great biocompatibility and biodegradability to facilitate MRI-guided drug delivery, gene delivery, photo thermal therapy (PTT), photodynamic therapy 5 (PDT) or magnetic hyperthermia [82] growth through the EPR effect and available real-time *in vivo* drug tracking with MRI [83].

Discussion

Current cancer treatments include surgical intervention, radiation and chemotherapeutic agents. Side effects are an integral part of these treatment modules. So, recent studies focused on finding new strategies without any major side effects and were more effective instead of these modules. However, better comprehension of molecular basis of tumor and the advent of new diagnostic technologies (such as active and passive targeting models) and treatments can be decreased mortality rate in cancer patients. So the researchers continue to find strategies to improve the chances of survival and quality of cancerous patient's lives. NPs are used in the search for this new treatment method and can be preferred in imaging methods as well [84].

Nanoparticles and related biotechnologies provide needed augmented presentation for development of vaccine, treatments, diagnosis, imaging active and passive strategies for cancer and undoubtedly nanoparticle engineering. For this purpose an exciting ongoing and future studies and an increasing focus of clinical trials cancer treatments will remain.

Consequently, this review provides a brief manual for anyone in the field of nanotechnology for the diagnoses, treatment and vaccination of the cancer disease.

This work was supported by the Üsküdar University.

REFERENCES

1. *Stewart B. W., Kleihues P.* World Cancer Report. *World Health Organization Press.* Avaible at https://publications.iarc.fr/Non-Series-Publications/World-Cancer-Reports/World-Cancer-Report-2003 (accessed, Geneva, 2003).
2. *Jemal A., Siegel R., Xu J., Ward E.* Cancer statistics. *Cancer J. Clin.* 2010, V. 60, P. 277–300.
3. *Peer D., Karp J. M., Hong S., Farokhzad O. C., Margalit R., Langer R.* Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* 2007, V. 2, P. 751–60.
4. *Kumari P., Ghosh B., Biswas S.* Nanocarriers for cancer-targeted drug delivery. *J. Drug Targeting*. 2015, 24 (3), 179–191. https://doi/org/10.1038/nnano.2007.387
5. *Kononenko V., Narat M., Drobne D.* Nanoparticle interaction with the immune system *Arch. Industr. Hygiene Toxicol.* 2015, 66 (2), 97–108. https://doi/org/10.1515/aiht-2015-66-2582
6. *Baetke S. C., Lammers T., Kiessling F.* Applications of nanoparticles for diagnosis and therapy of cancer. *Brit. J. Radiol.* 2015, 88 (1054), 20150207. https://doi/org/10.1259/bjr.20150207
7. *Yih T, Al-Fandi M*. Engineered nanoparticles as precise drug delivery systems. *J. Cel. Biochem.* 2006, V. 97, P. 1184–1190. https://doi/org/10.1002/jcb.20796
8. *Jabir N. R., Tabrez S., Ashraf G. M., Shakil S., Damanhouri G. A., Kamal M. A.* Nanotechnology-based approaches in anticancer research. *Int. J. Nanomedicine.* 2012, V. 7, P. 4391. https://doi/org/10.2147/IJN.S33838
9. *Mallick A., More P., Ghosh S., Chippalkatti R., Chopade B. A., Lahiri M., Basu* S. Dual Drug Conjugated Nanoparticle for Simultaneous Targeting of Mitochondria and Nucleus in Cancer Cells. *ACS Applied Materials & Interfaces*. 2015, 7 (14), 7584–7598. https://doi/org/10.1021/am5090226
10. *Stammati A. P., Silano V., Zucco F.* Toxicology investigations with cell culture systems. *Toxicology.* 1981, V. 20, P. 91–153.
11. *Borm P., Klaessig F. C., Landry T. D., Moudgil B., Pauluhn J.* Research strategies for safety evaluation of nanomaterials, part V: role of dissolution in biological fate and effects of nanoscale particles. *Toxicol. Sci.* 2006, V. 90, P. 23–32. https://doi/org/10.1093/toxsci/kfj084
12. *Costa E. C., Gaspar V. M., Marques J. G., Coutinho P., Correia I. J.* Evaluation of Nanoparticle Uptake in Co-culture Cancer Models. *PLoS ONE.* 2013, 8 (7), e70072. https://doi/org/10.1371/journal.pone.0070072
13. *Zhang X.-F., Liu Z.-G., Shen W., Gurunathan S.* Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. *Inter. J. Mol. Sci.* 2016, 17 (9), 1534. https://doi/org/10.3390/ijms17091534
14. *AshaRani P. V., Mun G. L. K., Hande M. P., Valiyaveettil S.* Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano.* 2009, V. 3, P. 279–290. https://doi/org/10.1021/nn800596w.
15. *Foldbjerg R., Irving E. S., Hayashi Y., Sutherland D. S., Thorsen K., Autrup H., Beer C.* Global gene expression profiling of human lung epithelial cells after exposure to nanosilver. *Toxicol. Sci.* 2012, V. 130, P. 145–157. https://doi.org/10.1093/toxsci/kfs225
16. *Lin J., Huang Z., Wu H., Zhou W., Jin P., Wei P., Zhang Y., Zheng F., Zhang J., Xu J.* Inhibition of autophagy enhances the anticancer activity of silver nanoparticles. *Autophagy.* 2014, V. 10, P. 2006–2020. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6454023
17. *Kovács D., Szőke K., Igaz N., Spengler G., Molnár J., Tóth T., Kiricsi M.* Silver nanoparticles modulate ABC transporter activity and enhance chemotherapy in multidrug resistant cancer. *Nanomed. Nanotechnol., Biol. Med.* 2016, 12 (3), 601–610. https://doi.org/10.1016/j.envpol.2019.113880
18. *Sokolov K., Follen M., Aaron J.,* Pavlova I. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res.* 2003, 63 (9), 1999–2004. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2773158
19. *Kah J. C., Wong K. Y., Neoh K. G., Song J. H., Fu J. W., Mhaisalkar S.* Critical parameters in the pegylation of gold nanoshells for

biomedical applications: An in vitro macrophage study. *J. Drug Target*. 2009, V. 17, P. 181–193. https://doi.org/10.1080/10611860802582442
20. *Svarovsky S. A., Szekely Z., Barchi J. J.* Synthesis of gold nanoparticles bearing the thomsen–friedenreich disaccharide: A new multivalent presentation of an important tumor antigen. *Tetrahedron Asymmetry*. 2005, V. 16, P. 587–598. https://doi.org/10.1016/j.tetasy.2004.12.003
21. *Ojeda R., de Paz J. L., Barrientos A. G., Martin-Lomas M., Penades S.* Preparation of multifunctional glyconanoparticles as a platform for potential carbohydrate-based anticancer vaccines. *Carbohydrate Res*. 2007, V. 342, P. 448–459. https://doi.org/10.1016/j.carres.2006.11.018
22. *Mkandawire M. M., Lakatos M., Springer A., Clemens A., Appelhans D., Krause-Buchholz U., Mkandawire M.* Induction of apoptosis in human cancer cells by targeting mitochondria with gold nanoparticles. *Nanoscale*. 2015, 7 (24), 10634–10640. https://doi.org/10.1039/C5NR01483B
23. *Lee C. S., Kim H., Yu J., Yu S. H., Ban S., Oh S., Jeong D., Im J., M. J., Kim T. H.* Doxorubicin-loaded oligonucleotide conjugated gold nanoparticles: A promising in vivo drug delivery system for colorectal cancer therapy. *Europ. J. Med. Chem*. 2017, V. 142, P. 416–423. https://doi.org/10.1016/j.ejmech.2017.08.063
24. *Chang Y., Yan W., He X., Zhang L., Li C., Huang H., Nace G., Geller D. A., Lin J., Tsung A.* miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. *Gastroenterol.* 2012, V. 143, P. 177–187 e8. https://doi.org/10.1053/j.gastro.2012.04.009
25. *Dawidczyk C. M., Russell L. M., Searson P. C.* Nanomedicines for cancer therapy: state-of-the-art and limitations to pre-clinical studies that hinder future developments. *Frontiers in Chemistry*. 2014, V. 2. https://doi.org/10.3389/fchem.2014.00069
26. *Devulapally R., Sekar N. M., Sekar T. V., Foygel K., Massoud T. F., Willmann J. K., Paulmurugan R.* Polymer Nanoparticles Mediated Codelivery of AntimiR-10b and AntimiR-21 for Achieving Triple Negative Breast Cancer Therapy. *ACS Nano*. 2015, 9 (3), 2290–2302. https://doi.org/10.1021/nn507465d
27. *Fernandez-Fernandez A., Manchanda R., McGoron A. J.* Theranostic Applications of Nanomaterials in Cancer: Drug Delivery, Image-Guided Therapy, and Multifunctional Platforms. *Appl. Biochem. Biotechnol.* 2011, V. 165, P. 1628–1651. https://doi.org/10.1007/s12010-011-9383-z
28. *Lu J. M., Wang X., Marin-Muller C., Wang H., Lin P. H., Yao Q., Chen C.* Current Advances in Research and Clinical Applications of PLGA-Based Nanotechnology. *Expert Rev. Mol. Diagn.* 2009, V. 9, P. 325–341. https://doi.org/10.1586/erm.09.15
29. *Mundargi R. C., Babu V. R., Rangaswamy V., Patel P., Aminabhavi T. M.* Nano/Micro Technologies for Delivering Macromolecular Therapeutics Using Poly(D,LLactide-Co-Glycolide) and Its Derivatives. *J. Control. Release.* 2008, V. 125, P. 193–209.
30. *Mattos-Arruda L., Giulia Bottai, Paolo G. Nuciforo, Luca Di Tommaso, Elisa Giovannetti, Vicente Peg, Agnese Losurdo, José Pérez-Garcia, Giovanna Masci, Fabio Corsi, Javier Cortés, Joan Seoane, George A. Calin, Libero Santarpia.* MicroRNA-21 links epithelial-to-mesenchymal transition and inflammatory signals to confer resistance to neoadjuvant trastuzumab and chemotherapy in HER2-positive breast cancer patients. *Oncotarget*. 2015, V. 6, P. 37269–37280. https://doi.org/10.18632/oncotarget.5495
31. *Malhotra M., Thillai Veerapazham Sekar, Jeyarama S. Ananta, Rammohan Devulapally, Rayhaneh Afjei, Husam A. Babikir, Ramasamy Paulmurugan, Tarik F. Massoud.* Targeted nanoparticle delivery of therapeutic antisense microRNAs presensitizes glioblastoma cells to lower effective doses of temozolomide in vitro and in a mouse model. *Oncotarget.* 2018, V. 9, P. 21478–21494. https://doi.org/10.18632/oncotarget.25135
32. *Mohammadian F., Pilehvar-Soltanahmadi Y., Mofarrah M., Dastani-Habashi M., Zarghami N.* Down regulation of miR-18a, miR-21 and miR-221 genes in gastric cancer cell line by chrysin-loaded PLGA-PEG nanoparticles. *Artif. Cel., Nanomed., Biotechnol.* 2016, 44 (8), 1972–1978. https://doi.org/10.3109/21691401.2015.1129615
33. *M ller V., Gade S., Steinbach B., Loibl S., von Minckwitz G., Untch M., Schwarzenbach H.* Changes in serum levels of miR-21, miR-210, and miR-373 in HER2-positive breast cancer patients undergoing neoadjuvant therapy: a translational research project within the Geparquinto trial. *Breast Cancer Research and Treatment*. 2014, 147 (1), 61–68. https://doi.org/10.1007/s10549-014-3079-3
34. *Harris J. M., Chess R. B.* Effect of Pegylation on Pharmaceuticals. *Nat. Rev. Drug Discov.* 2003, V. 2, P. 214–221. https://doi.org/10.1038/nrd1033
35. *Mintzer M. A. Simanek E. E.* Non Viral Vectors for Gene Delivery. *Chem. Rev.* 2009, V. 109, P. 259–302. https://doi.org/10.4103/2277-9175.98152
36. *Yin H., Kanasty R. L., Eltoukhy A. A., Vegas A. J., Dorkin J. R., Anderson D. G.* Non

Viral Vectors for Gene-based Therapy *Nat. Rev. Genet*. 2014, V. 15, P. 541–555.

37. *Pietersz G. A., Tang C. K., Apostolopoulos V.* Mini Rev. *Med. Chem.* 2006, V. 6, P. 1285–1298.
38. *Hu Q., Wu M., Fang C., Cheng C., Zhao M., Fang W., Tang G.* Engineering Nanoparticle-Coated Bacteria as Oral DNA Vaccines for Cancer Immunotherapy. *Nano Letters*. 2015, 15 (4), 2732–2739. https://doi.org/10.1021/acs.nanolett.5b00570
39. *Schleich N., Sibret P., Danhier P., Ucakar B., Laurent S., Muller R. N., Danhier* F. Dual anticancer drug/superparamagnetic iron oxide-loaded PLGA-based nanoparticles for cancer therapyand magnetic resonance imaging. *Inter. J. Pharmac*. 2013, 447 (1–2), 94–101.
40. *Zuo H. D., Yao W. W., Chen T. W., Zhu J., Zhang J. J., Pu Y., Zhang X. M.* The Effect of Superparamagnetic Iron Oxide with iRGD Peptide on the Labeling of Pancreatic Cancer CellsIn Vitro: A Preliminary Study. *BioMed Res. Inter*. 2014, P. 1–8. https://doi.org/10.1155/2014/852352
41. *Huh Y. M., Jun Y. W., Song H. T., Kim S., Choi J. S., Lee J. H., Yoon S., Kim K. S., Shin J. S., Suh J. S., Cheon J. In vivo* magnetic resonance detection of cancer by using multifunctional magnetic nanocrystals. *J. Am. Chem. Soc.* 2005, 127 (35), https://doi.org/10.1021/ja052337c
42. *Oghabian M. A., Jeddi-Tehrani M., Zolfaghari A., Shamsipour F., Khoei S., Amanpour S.* Detectability of Her2 positive tumors using monoclonal antibody conjugated iron oxide nanoparticles in MRI. *J. Nanosci. Nanotechnol.* 2011, 11 (6), 5340–5344. https://doi.org/ 10.1166/jnn.2011.3775
43. *Marchesan S., Kostarelos K., Bianco A., Prato M.* The winding road for carbon nanotubes in nanomedicine. *Mater. Today.* 2015, V. 18, P. 12–19. https://doi.org/10.1016/j.mattod.2014.07.009
44. *Lacerda L., Bianco A., Prato M., Kostarelos K.* Carbon nanotubes as nanomedicines: From toxicology to pharmacology. *Adv. Drug Deliv. Rev.* 2006, V. 58, P. 1460–1470. https://doi.org/10.1016/j.addr.2006.09.015
45. *Siu K. S., Chen D., Zheng X., Zhang X., Johnston N., Liu Y., Yuan K., Koropatnick J., Gillies E. R., Min W. P.* Non-covalently functionalized single-walled carbon nanotube for topical siRNA delivery into melanoma. *Biomaterials*. 2014, V. 35, P. 3435–3442. https://doi.org/10.1016/j.biomaterials.2013.12.079
46. *Sanginario A., Miccoli B., Demarchi D.* Carbon Nanotubes as an Effective Opportunity for Cancer Diagnosis and Treatment. *Biosensors.* 2017, 7 (4), 9. https://doi.org/ 10.3390/bios7010009.
47. *Zununi Vahed S., Salehi R., Davaran S., Sharifi S.* Liposome-based drug co-delivery systems in cancer cells. *Mater. Sc. Engin: C*. 2017, V. 71, P. 1327–1341. https://doi.org/10.1016/j.msec.2016.11.073
48. *Eloy J. O., Petrilli R., Topan J. F., Antonio H. M., Barcellos J. P., Chesca D. L., Serafini L. N., Tiezzi D. G., Lee R. J., Marchetti J. M.* Co-loaded paclitaxel/rapamycin liposomes: development, characterization and in vitro and in vivo evaluation for breast cancer therapy, Colloids Surf. *B. Biointerfaces*. 2016, V. 141, P. 74–82. https://doi.org/10.1016/j.colsurfb.2016.01.032
49. *Elnakat H., Ratnam M.* Distribution, functionality and gene regulation of folate receptor isoforms: implications in targeted therapy. *Adv. Drug Deliv. Rev.* 2004, V. 56, P. 1067–1084. https://doi.org/ 10.1016/j.addr.2004.01.001
50. *Chaudhury A., Das S.* Folate receptor targeted liposomes encapsulating anti-cancer drugs, Curr. Pharm. *Biotechnol.* 2015, V. 16, P. 333–343 https://doi.org/ 10.2174/1389201016666150118135107
51. *Wu D., Zheng Y., Hu X., Fan Z., Jing X.* Anti-tumor activity of folate targeted biodegradable polymer-paclitaxel conjugate micelles on EMT-6 breast cancer model. *Mater. Sci. Eng. C.* 2015, V. 53, P. 68–75. https://doi.org/10.1016/j.msec.2015.04.012
52. *Yang T., Li B., Qi S., Liu Y., Gai Y., Ye P., Yang G., Zhang W., Zhang P., He X., Li W., Zhang Z., Xiang G., Xu C.* Co-delivery of doxorubicin and Bmi1 siRNA by folate receptor targeted liposomes exhibits enhanced anti-tumor effects *in vitro* and *in vivo*. *Theranostics.* 2014, V. 4, P. 1096–1111. https://doi.org/ 10.7150/thno.9423
53. *Peng Z., Wang C., Fang E., Lu X., Wang G., Tong Q.* Co-delivery of doxorubicin and SATB1 shRNA by thermosensitive magnetic cationic liposomes for gastric cancer therapy. *PLoS One*. 2014, V. 9, P. e92924. https://doi.org/ 10.1371/journal.pone.0092924
54. *Connelly C. M., Uprety R., Hemphill J., Deiters A.* Spatiotemporal control of microRNA function using light-activated antagomirs, *Mol. BioSyst*. 2012, V. 8, P. 2987–2993. https://doi.org/10.1039/c2mb25175b
55. *Riaz M., Riaz M., Zhang X., Lin C., Wong K., Chen X. Yang Z.* Surface Functionalization and Targeting Strategies of Liposomes in Solid Tumor Therapy: A Review. *Inter. J. Mol. Sci.* 2018, 19 (1), 195. https://doi.org/10.3390/ijms19010195
56. *Biswas S., Kumari P., Lakhani P. M., Ghosh B.* Recent advances in polymeric micelles for anti-cancer drug delivery. *Europ. J. Pharmac. Sc*. 2016, V. 83, P. 184–202. https://doi.org/10.1016/j.ejps.2015.12.031

57. *Lohcharoenkal W., Wang L., Chen Y. C., Rojanasakul Y.* Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy. *BioMed Res. Inter.* 2014, P. 1–12. https://doi.org/10.1155/2014/180549
58. *Weber C., Coester C., Kreuter J., Langer K.* Desolvation process and surface characterisation of protein nanoparticles. *Inter. J. Pharmac.* 2000, 194 (1), 91–102. https://doi.org/
59. *Teng Z., Luo Y., Wang T., Zhang B., Wang Q.* Development and application of nanoparticles synthesized with folic acid conjugated soy protein. *J. Agricult. Food Chemy.* 2013, V. 61, P. 2556–2564. https://doi.org/10.1021/jf4001567
60. *Gulfam M., Kim J., Lee J. M., Ku B., Chung B. H., Chung B. G.* Anticancer drug-loaded gliadin nanoparticles induced apoptosis in breast cancer cells. *Langmuir.* 2012, V. 28, P. 8216– 8223. https://doi.org/10.1021/la300691n
61. *Elzoghby A. O., Saad N. I., Helmy M. W., Samy W. M., Elgindy N. A.* Ionically-crosslinked milk protein nanoparticles as flutamide carriers for effective anticancer activity in prostate cancer-bearing rats. *Europ. J. Pharmac. Biopharmac.* 2013, 85 (3), part A, 444–451. https://doi.org/10.1016/j.ejpb.2013.07.003
62. *Bazak R., Houri M., El Achy S., Kamel S., Refaat T.* Cancer active targeting by nanoparticles: a comprehensive review of literature. *J. Cancer Res. Clin. Oncol.* 2014, 141 (5), 769–784. https://doi.org/ 10.1007/s00432-014-1767-3
63. *Eigenbrot C., Ultsch M., Dubnovitsky A., Abrahmsen L., Hard T.* Structural basis for high-affinity HER2 receptor binding by an engineered protein. *Proc. Nat. Acad, Sci. USA.* 2010, 107 (34), 15039–15044. https://doi.org/10.1073/pnas.1005025107
64. *Zhang J. M., Zhao X. M., Wang S. J., Ren X. C., Wang N., Han J.-Y., Jia L. Z.* Evaluation of 99mTc peptide ZHER2: 342Affibody®molecule forin vivomolecular imaging. *The Brit. J. Radiol.* 2014, 87 (1033), 20130484.
65. *Ghanemi M., Pourshohod A., Ghaffari M. A., Kheirollah A., Amin M., Zeinali* M., *Jamalan M.* Specific Targeting of HER2-Positive Head and Neck Squamous Cell Carcinoma Line HN5 by Idarubicin ZHER2 Affibody Conjugate. *Curr. Cancer Drug Targets.* 2019, 19 (1), 65–73. https://doi.org/ 10.2174/1568009617666170427105417
66. *Glazer E. S., Massey K. L., Zhu C., Curley S. A.* Pancreatic carcinoma cells are susceptible to noninvasive radio frequency fields after treatment with targeted gold nanoparticles. *Surgery.* 2010, 148 (2), 319–324. https://doi.org/https://doi.org/10.1016/j.surg.2010.04.025
67. *Talekar M., Kendall J., Denny W., Garg S.* Targeting of nano- particles in cancer: drug delivery and diagnostics. *Anticancer Drugs.* 2011, 22 (10), 949–962. https://doi.org/10.1097/CAD.0b013e32834a4554
68. *Wang Z., Gu F., Zhang L., Chan J. M., Radovic-Moreno A., Shaikh M. R.* Biofunctionalized targeted nanoparticles for thera- peutic applications. *Expert Opin. Biol. Ther.* 2008, 8 (8), 1063–1070. https://doi.org/10.1517/14712598.8.8.1063
69. *Lebel M.-È., Chartrand K., Tarrab E., Savard P., Leclerc D., Lamarre A.* Potentiating Cancer Immunotherapy Using Papaya Mosaic Virus-Derived Nanoparticles. *Nano Letters.* 2016, 16 (3), 1826–1832. https://doi.org/10.1021/acs.nanolett.5b04877.
70. *Jinu U., Gomathi M., Saiqa I., Geetha N., Benelli G., Venkatachalam P.* Green engineered biomolecule-capped silver and copper nanohybrids using Prosopis cineraria leaf extract: Enhanced antibacterial activity against microbial pathogens of public health relevance and cytotoxicity on human breast cancer cells (MCF-7). *Microbial Pathogenesis.* 2017, V. 105, P. 86–95. https://doi.org/10.1016/j.micpath.2017.02.019
71. *Joshi M. D., Patravale V., Prabhu R.* Polymeric nanoparticles for targeted treatment in oncology: current insights. *Inter. J. Nanomed.* 2015, P. 1001. https://doi.org/ 10.2147/IJN.S56932
72. *Torchilin V.* Tumor delivery of macromolecular drugs based on EPR effect. *Adv. Drug Delivery Rev.* 2011, V. 63, P. 131–135. https://doi.org/10.1016/j.addr.2010.03.011
73. *Greish K.* In Cancer Nanotechnology: Methods and Protocols. Ed. R. S. Grobmyer and M. B. Moudgil. *Humana Press, Totowa, NJ.* 2010, 25–37.
74. *Maeda H. Nakamura, Fang J.* The EPR effect for macromolecular delivery to solid tumors: improvement of tumor uptake lowering of systemic toxicity and distinct tumor imaging *in vivo*. *Adv. Drug Deliv. Rev.* 2013, V. 65, P. 71–79. https://doi.org/10.1016/j.addr.2012.10.002
75. *Sun C., Lee J. S., Zhang M.* Magnetic nanoparticles in MR imaging and drug delivery. *Adv. Drug Deliv. Rev.* 2008, V. 60, P. 1252–1265. https://doi.org/ 10.1016/j.addr.2008.03.018
76. *Aires A., Ocampo S. M., Simoes B. M., Rodríguez M. J., Cadenas J. F., Couleaud P., Spence K., Latorre A., Miranda R., Somoza A., Clarke R. B., Carrascosa J. L., Cortajarena A. L.* Multifunctionalized iron oxide nanoparticles for selective drug delivery to CD44-positive cancer cells. *Nanotechnol.* 2016, V. 27, P. 065103.
77. *Marciello M., Pellico J., Fernandez-Barahona I., Herranz F., Ruiz-Cabello J., Filice M.* Recent advances in the preparation and application of multifunctional iron oxide and liposome-

based nanosystems for multimodal diagnosis and therapy. *Interface Focus*. 2016, 6 (6), 20160055. https://doi.org/10.1098/rsfs.2016.0055
78. *Guo J., Rahme K., He Y., Li L.-L., Holmes J., O'Driscoll C.* Gold nanoparticles enlighten the future of cancer theranostics. *Inter. J. Nanomed.* 2017, V. 12, P. 6131–6152. https://doi.org/10.2147/IJN.S140772
79. *Jin Y.* Multifunctional compact hybrid Au nanoshells: a new generation of nanoplasmonic probes for biosensing, imaging, and controlled release. *Acc. Chem. Res.* 2014, 47 (1), 138–148. https://doi.org/10.1021/ar400086e
80. *Zhao Y., Pang B., Luehmann H.* Gold nanoparticles doped with (199) Au atoms and their use for targeted cancer imaging by SPECT. *Adv. Healthc. Mater.* 2016, 5 (8), 928–935. https://doi.org/10.1002/adhm.201500992
81. *Liu J., Zhang L., Lei J., Ju H.* MicroRNA-Responsive Cancer Cell Imaging and Therapy with Functionalized Gold Nanoprobe. *ACS Appl. Mater. Interfaces*. 2015, 7 (34), 19016–19023. https://doi.org/10.1021/acsami.5b06206
82. *Li K., Nejadnik H., Daldrup-Link H. E.* Next-generation superparamagnetic iron oxide nanoparticles for cancer theranostics. *Drug Discov. Today*. 2017, 22 (9), 1421–1429. https://doi.org/ 10.1016/j.drudis.2017.04.008
83. *Daldrup-Link H. E. Mohanty S., Ansari C., Lenkov O., ShawF., Ito K., Hong S. H., Hoffmann M., Pisani L., Boudreau N., Gambhir S. S., Coussens L. M.* Alk5 inhibition increases delivery of macromolecular and protein-bound contrast agents to tumors. *JCI Insight*. 2016, V. 1, P. e85608. https://doi.org/ 10.1172/jci.insight.85608
84. *Zaimy M. A., Saffarzadeh N., Mohammadi A., Pourghadamyari H., Izadi P., Sarli A., Tavakkoly-Bazzaz J.* New methods in the diagnosis of cancer and gene therapy of cancer based on nanoparticles. *Cancer Gene Ther.* 2017, 24 (6), 233–243.

ДІАГНОСТИКА, ВІЗУАЛІЗАЦІЯ ТА ЛІКУВАННЯ РАКУ З ВИКОРИСТАННЯМ НАНОРОЗМІРНИХ СТРУКТУР

О. О. Озкан, М. Карахан

Університет Ускюдар, Стамбул, Туреччина

E-mail: karahan@uskudar.edu.tr

Сучасні дослідження спрямовані на пошук нових стратегій лікування раку, які не мають значних побічних ефектів і більш ефективні порівняно з традиційними методами, включаючи хірургічне втручання, променеву терапію і хіміотерапію.Застосування нанорозмірних структур уможливлює використання новітніх підходів для лікування раку. Системи наночастинок підвищують ефективність терапевтичних препаратів, знижуючи їхні побічні ефекти. Хоча в багатьох дослідженнях повідомляється про розробку нових методів лікування ракових клітин в майбутньому, клінічний успіх дає змоги зрозуміти вплив типу, розміру та дози ночастинок на зони їх застосування. Таким чином, в цьому огляді проілюстровано можливість використання наночастинок в діагностиці, візуалізації та лікуванні раку.

Ключові слова: діагностика раку, візуалізація та лікування, наночастинки.

ДИАГНОСТИКА, ВИЗУАЛИЗАЦИЯ И ЛЕЧЕНИЕ РАКА С ИСПОЛЬЗОВАНИЕМ НАНОРАЗМЕРНЫХ СТРУКТУР

О. О. Озкан, М. Карахан

Университет Ускюдар, Стамбул, Турция

E-mail: karahan@uskudar.edu.tr

Современные исследования направлены на поиск новых стратегий лечения рака, не имеющих значительных побочных эффектов и более эффективных по сравнению с традиционными методами, включая хирургическое вмешательство, лучевую терапию и химиотерапию. Применение наноразмерных структур дает возможность использовать полезные подходы для лечения рака.Системы наночастиц повышают эффективность терапевтических препаратов, снижая их побочные эффекты. Хотя во многих исследованиях сообщается о разработке новых методов лечения раковых клеток в будущем, клинический успех не позволяет понять влияние типа, размера и дозы наночастиц на области их применения. Таким образом, в настоящем обзоре проиллюстрирована возможность использования наночастиц в диагностике, визуализации и лечении рака.

Ключевые слова: диагностика рака, визуализация и лечение, наночастицы.

UDC 582.26: 574.62: 574.55 https://doi.org/10.15407/biotech12.06.025

BIOTECHNOLOGICAL PROSPECTS OF MICROALGAE

N. KIRPENKO, T. LEONTIEVA

Institute of Hydrobiology of the National Academy of Sciences of Ukraine, Kyiv

E-mail: nativnativ@ukr.net

Received 25.10.2019
Revised 04.12.2019
Accepted 20.12.2019

The current state and perspectives of biotechnological use of microscopic algae were analyzed. The main directions of algobiotechnology, due to the physiological and biochemical features of these organisms, the volume of algae production in the world, the types of microalgae that had already been used or had practical prospects, ways of biomass obtaining and productivity increasing of industrial algae cultivation were given. The state of this problem, expediency of algobiotechnology development and prospects of microalgae cultivation in Ukraine were discussed.

Key words: algobiotechnology, microalgae, industrial cultivation, aquaculture.

Microscopic algae are considered as a new agricultural crop due to their valuable biochemical composition, high rate of reproduction and lability of metabolism. The industrial production of microalgae biomass — algobiotechnology — is a prospect for obtaining the renewable raw materials of various purposes, including replenishment of food and feed resources [1–7]. A significant advantage of this technology is that it does not increase the load on natural ecosystems, most of which are currently significantly depleted. The United Nations Organization [8] emphasizes the importance of enhancing the food security for the introduction of new technologies without sacrificing the environment, including the adjustment of production and use of food microalgae. At the same time, these organisms can find application not only as a food resource but also in many other areas.

Areas of use and perspective species of microalgae

In world practice, algae are used quite widely due to their physiological and biochemical characteristics. The most famous and investigated are *Chlorella vulgaris* Beijer., *Spirulina platensis* (Gomont) Geitler (*Arthrospira platensis* Gomont), *Dunaliella salina* Teod. Meanwhile, many other algae species are also suitable for practical application.

High content of proteins, carbohydrates, lipids, pigments, vitamins, polyunsaturated fatty acids, including essential, provides nutritional value of these organisms. Proteins content in the cells of algae is up to 45–65%, however they are well balanced by the content of essential amino acids and can be used for the enrichment of the amino acid composition of food.

Some of the microalgae have therapeutic and preventative effect in violation of the activity of the immune, endocrine, digestive, cardiovascular and nervous systems of animals and humans, having antitumor, antidiabetic, radioprotective and immunomodulatory activity. They are used in the medical and pharmacological fields, in diet nutrition, therapeutic cosmetology, in the production of biologically active supplements.

In a number of microalgae, regenerative properties were identified, so they are used to treat wounds and burns. In particular, in the Institute of Hydrobiology of the National Academy of Sciences of Ukraine, a method of chlorophyll-carotene paste obtaining from the algae as the basics of the medicinal preparation “Algofin”, an ointment with regenerative and anti-inflammatory properties, was developed [9].

In general, microalgae are a promising raw material for the production of antioxidants,

vitamins, biomarkers, β-carotene, phycocyanin and others. These organisms are a rich source of natural food dyes that are used, in particular, in baby foods. Algae ability to direct biosynthesis of certain biologically active substances such as iodine-containing compounds of hormonal nature, alkaloids, steroids, etc. contributes to increasing an interest of their use.

Microscopic algae have prospects in the field of creating natural balanced feed for livestock, poultry and fish farming, including the cultivation of fish and invertebrates in aquaculture. Inland aquatic ecosystems undergo often significant anthropogenic changes. Loss and degradation of hydrobionts habitat, water pollution, overexploitation and intake, the introduction of alien species endanger the sustainability, biodiversity of hydro-ecosystems and formation of biological resources, which necessitates their artificial reproduction [10]. The use of green algae in fish farming increases the productivity of fishponds and the forage base for other aquatic organisms, as well as prevents their "flowering" and improves the hydrochemical status [11].

The advantages of microalgae use in livestock are animals productivity increase by improving immunity and cost reduction of veterinary products, feed consumption increased efficiency, the possibility of year-round feed enrichment with vitamins and natural biologically active compounds. The latter is of particular importance, as a large number of products are currently manufactured using food substitutes and synthetic preparations, which is a significant danger to living organisms consuming them.

Chlorella vulgaris and *Arthrospira platensis* are the most widely used for the needs of livestock [2, 3, 6, 7, 12–15]. More effective is introduction into the feed of a native suspension of algae, which contains a significant amount of valuable extracellular substances — the antibiotic chlorelin, arachidonic acid, amino acids, vitamins, enzymes, especially in the initial stages of culture growth [16].

In livestock and poultry farming, positive results were obtained when using other algae — *Chlorococcum, Spirogyra, Scenedesmus, Navicula, Nitzschia* and others [17]. It should be added that the biomass of the algae can be enriched with iodine, selenium or other essential elements [18, 19].

Microalgae in the form of dry powder, paste or suspension can be used in crop production to increase soil fertility and microbial activity, increase crop yields and accelerate their vegetation, reduce application standards of fertilizers, pesticides and growth regulators [20]. Algae have the most positive effect on crop yield in temperate zones and non-irrigated agriculture.

For soils recultivation, especially irrigated soils, it is advisable to use a suspension of cells of green algae (*Chlorella vulgaris*, representatives of the genus *Scenedesmus*) or nitrogen-fixing cyanobacteria (*Tolypothrix tenuis, Nostoc punctiforme, Anabaena cylindrica*). At one time, the effectiveness of seston using as a valuable organic fertilizer during "flowering" of the Dnieper reservoirs was proven [21]. Seston can also become a basis for the production of eco-friendly pure glue "Fitton", developed with the participation of specialists from the Institute of Hydrobiology of the National Academy of Sciences of Ukraine [22, 23], which is promising for agricultural plant seed pelleting. It should be added that some cyanobacteria (*Lyngbya majuscula* Harvey ex Gomont.) produce toxins that are active against phytopathogenic fungi [24], which may also find a use in plant production.

A considerable amount of research is related to the possibility of algae biomass usage to create alternative fuel types — biodiesel, bioethanol, hydrogen, methane [25–27]. It is commonly known that microalgae contain neutral and polar lipids. Polar lipids are mainly synthesized under favorable conditions, are characterized by high biological activity and are commonly used as food and dietary supplements. Neutral lipids are accumulated more in unfavorable conditions or under stress, are the main reserve substances of cells and are promising for the production of biofuels, biopolymers, etc. [28, 29]. Such biofuel is CO_2-neutral and its use will reduce the amount of gaseous emissions contributing to global climate change.

In order to improve biofuel production technologies, the search for promising species and strains of microalgae, ways of optimizing their cultivation and increasing the amount of lipid fraction, the methods of algae mass processing, in particular, methods for destruction of a cell membrane and extraction of lipid substances, development of photobioreactors structure, etc. are still under way [30–34]. Improving the mode of thermal treatment of algae biomass allows to reduce its duration and to convert from 50 to 65% of the raw material to so-called Biocrude, "artificial oil". This technology does not require pre-

dehydration of biomass, whereas usually high moisture content impedes complete phase separation and reduces the efficiency of lipid extraction. Additional catalytic treatment of microalgae biomass allows including proteins and carbohydrates in the biofuel production process, the destruction of which increases the yield of the product [35].

A number of microalgae species characterized by high lipid content were proposed as feedstock for alternative bioenergy: *Chlorella* sp., *Neochloris oleoabundans, Nannochloropsis* sp., *Botryococcus braunii, Dunaliella tertiolecta, Scenedesmus* TR -84 [35, 36]. In addition, autotrophic cyanobacteria and green algae are considered as promising objects capable of producing hydrogen for hydrogen energy [37], and many of the carbohydrate compounds can be used as a substrate for bioethanol production [38].

Work in the field of "green energy" is carried out in Ukraine. At the National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", the scientific and technological bases for the conversion of algae biomass into biofuel are developed [39]. At the Kremenchuk Mykhailo Ostrohradskyi National University, it is offered to receive biogas from seston during "flowering" of Dnieper reservoirs. The developers are convinced that the invention can help to clean up the Dnieper and solve energy problems, in particular for the heat supply of small settlements [40].

Microalgae are one of the most important components in the system of biological treatment of domestic and industrial wastewater. As it is known, in Ukraine, most wastewater treatment plants use traditional biotechnologies that do not provide effective removal of phosphates and nitrates. At the same time, microalgae are able to use for growth only biogenic compounds, in addition, they saturate water with oxygen, which accelerates the oxidative processes and mineralization of organic impurities in wastewater [41, 42]. The possibility of using green algae for bioremediation of the aquatic environment contaminated with petroleum products and waste from pulp and paper enterprises was demonstrated [43, 44].

The cultivation of green algae on the runoff of livestock complexes enables to remove the excess of organic matter, to normalize odor and color, with a considerable part of Nitrogen returned to algae biomass and again to animals feed [45].

Algae cultivation allows the use such by-products of technological processes as heat and carbon dioxide excess, reducing their flow into the atmosphere. In intensive conditions of cultivation, algae are capable of 70% of CO_2 removing within eight hours [46]. In particular, the possibility of cultivation of some green chlorococcal algae using CO_2 concentrations in gas-air mixture from 0.2 to 16% was shown [47]. Our own research shown that green microalgae, in particular, representatives of the genus *Chlorella* and genus *Desmodesmus* had significant carbon dioxide assimilation potential, significantly increasing the growth rate [48–49].

It is known that in the formation of 1 kg of phytomass, microalgae absorb more than 1.8 kg of CO_2 from the surrounding air space, in addition, they are able to assimilate nitrogen oxides with partial conversion into gaseous nitrogen, as well as other mineral compounds, which include biogenic elements Sulfur, Potassium, Magnesium, Calcium, etc. [50]. In this regard, it is important to look for algae strains with increased ability to assimilate CO_2 and resistant to Sulfur and Nitrogen oxides [36].

Detailed studies of the tolerance limits and adaptive capacity of algae have considerable practical promise. For example, it is known that for many species the influence of temperatures above 35–40 °C is critical and is usually accompanied by loss of cell physiological activity. At the same time, at the incubation of the strain *Acutodesmus obliquus* (Turpin) Hegewald & Hanagata Syko-A Ch-055-12 IPPAS isolated from the activated sludge of the pulp and paper enterprise aerotanks, at 40–45 °C, some cells survived and continued vegetation, which made it possible to recommend a strain for sewage treatment in the temperature range from +15 to + 41 °C [51].

Nostoc muscorum Elenkin, *Scenedesmus acutus* Meyer, *Chlorella vulgaris* Beijer. species of *Neospongiococcum* genus can be used to treat sewage of the forestry complex, *Acutodesmus obliquus* — for urban wastewater treatment. These algae reduce the biochemical consumption of Oxygen in the effluents; accumulate ferrum, capable of decomposing phenolic compounds, etc. [52, 53].

Diatomic algae capable of synthesizing fats and fat-like substances as a basis for biodiesel production, mucopolysaccharides, and some unusual pigments (e.g. marennin) have considerable biotechnological potential [54]. Thus, *Cylindrotheca closterium* (Ehremberg) Lewin & Reimann is characterized by high content of polyunsaturated fatty acids and carotenoids, in particular fucoxanthin,

which has antioxidant, antimutagenic and anticancerogenic properties and is used as a feed supplement for bivalve molluscs [55].

It was shown that representatives of genus *Euglena* produce protein, *Chlamidomonas* genus — carbohydrates, *Ankistrodesmus* genus — lipids, *Dunaliella* genus — carotene and tocopherol [56]. To obtain the ketocarotinoid astaxanthin, an extremely valuable preparation for aquaculture, the content of which reaches 4% in the dry matter of cells, in Japan (Fuji Chemical Industry) and the USA (Cyanotech), cultivation of *Haematococcus pluvialis* Flotow., thoroughly researched at one time by the specialists of the lapsed Institute of South Sea Biology of the National Academy of Sciences of Ukraine, has been mastered [57, 58].

The filamentous green algae *Cladophora* and *Rhizoclonium* are noteworthy, which develop abundantly in low-flowing water reservoirs. The cell envelope of these algae contains a significant amount of fiber that can be used in the production of various grades of paper and building materials.

Thus, microalgae have considerable potential for practical use. In this regard, algobiotechnology requires increased attention and expansion of biotechnological work directions, which involves the search for new strains and a detailed study of their biochemical characteristics and physiological properties.

Depending on the ultimate goal of algobiotechnologies, algae must first of all, grow and produce significant biomass in well-defined conditions — at the required temperature, light, pH value, medium composition, etc. Secondly, they must differ in some matters of metabolic features. Thus, to obtain feed materials, species with the optimal ratio of proteins, carbohydrates, lipids, biologically active substances (vitamins, carotenoids, coenzymes, etc.), with high nutritional quality and digestibility are required. For energy raw materials, it is necessary to select species with a high content of energy components, first of all, lipids. If it is necessary to treat sewage, algae should be tolerant of high concentrations of organic or mineral biogenic substances or contaminants (phenols, metal oxides, carbon dioxide, etc.). The selected species should also be characterized by stability of the main used characteristics, while having a labile metabolism and the ability to programmatically respond to external influences.

Ways to obtain biomass and increase the efficiency of industrial microalgae culture

In the southern latitudes, the cultivation systems for microalgae cropping can be placed in open areas, and in more moderate conditions — indoors. For the needs of animal husbandry, crop production, wastewater treatment, energy production, open systems (ponds, trays, and pools) can be used. For food or medical and pharmaceutical needs, where there are high requirements for algological and microbiological purity and product composition, closed photobioreactors can be used. In the first case, the process of algae growing is relatively uncomplicated and the cost of the biomass produced is low. Yet there is greater need for areas and high quality of production is not guaranteed. In the second case, product value increases significantly due to using the special equipment and more sophisticated technology. In such case the size of the occupied space decreases, and the complete control of the cultivation conditions ensures stable predicted quality of the product.

Despite considerable advances in the field of optimization and intensification of algae cultivation [59–64], the work is ongoing on improving the structures of closed photobioreactors and finding new materials [49, 65]. Thus, the use of polyethylene film has become widespread, it has been proposed to grow algae in tris-acetate-phosphate-pluronic, which is capable of being transformed from liquid into gel and vice versa when the temperature changes [16]. This improves lighting conditions, facilitates harvesting, and reduces energy consumption and duration of the cultivation process.

The use of genetic engineering methods to create highly productive algae strains capable of actively synthesizing certain compounds is becoming widespread. Thus, with the help of point genetic engineering, the new strains of microalgae were obtained on the basis of the genome of the freshwater chlorococcal *Acutodesmus dimorphus*, which should combine the best features of several planktonic species [66].

Despite the long history of biotechnology research, the potential of this field is not yet fully exploited. This applies both to the range of "new crops" and to the ways in which they are used and how to increase the content of valuable components. For example, according to the known patterns, the amount of lipids and carbohydrates increases at the stationary stage of algae growth or under stressful conditions. In this regard, in order to enhance the yield of these compounds, it is recommended to use

two-stage technology: first to create optimal conditions for high crop yields, in the second stage algae should be stressed [29]. It was observed that lipid accumulation was facilitated by a decrease in the concentration of available nitrogen compounds, enhanced carbohydrate synthesis — by phosphate deficiency. However, such techniques significantly complicate the technology. At the same time, our studies shown that some algae had not only a high content of lipids, but also maintained it throughout the life cycle under normal cultivation conditions [67, 68]. Thus, active algobiotechnological studies would help to expand the range of promising algae species, optimize and reduce the cost of algae technologies.

Volumes of microalgae biomass production in the world

Analysis of scientific literature, press and internet publications shows that the production of microalgae biomass is gradually becoming traditional in many countries of the world. Symposia of the European Society of Microalgae Biotechnologies regularly take place in Hungary, analyzing new developments and current industry challenges [69]. In the United States, the first microalgae growing plants in artificial ponds were established in 1977, and industrial production of microalgae is gradually increasing [8, 26]. The largest capacities are concentrated in the USA, China, India, Japan, Thailand, Germany, Australia, and Israel. Well-known microalgae biomass producers are Royal, DutchShell (Hawaii), AlgaeBioFuels and Solazyme (USA), Aquaflow Bionomic Corporation (New Zealand), Mitsubishi (Japan) companies. In Europe Ingrepro B.V. (Netherlands) company offers the technological schemes for lipid-enriched biomass obtaining of microalgae.

In Europe and America, a variety of chlorella products are known, “Japan Chlorella” company produces its biomass for food purposes; about 1.5 thousand tonnes of dry biomass are produced annually in Taiwan; Malaysia and Philippines consume for food needs over 500 tonnes of algae. In Africa and Mexico, a significant amount of protein concentrates are produced from spirulina, using alkaline lakes to grow them. Italy develops spirulina cultivation technology in seawater and in closed-type cultivators.

There are some small enterprises in Russia (OOO, limited liability companies under the laws of Russian Federation, such as “Ecofactor”, “Legion Center”, “Solixant”) that produce chlorella as a pure suspension or with lactic and bifidobacteria for livestock, and spirulina, as well as Omega-3 polyunsaturated fatty acids and carotenoids from freshwater and marine algae. “Energotehnoprom” company (Kazan) produces bioreactors of different capacity for growing chlorella [70]. It is traditional to grow microalgae for agriculture in Central Asia — for animal husbandry, crop production, fur farming and silk production [56, 64]. There is growing interest in this problem in Belarus, where a large complex of biotechnological works and patenting of development is being performed [63].

Prospects for microalgae cultivation in Ukraine

In Ukraine, active cultivation of microalgae and their use in animal husbandry began in the 1970s [71]. Significant achievements in this area were obtained by scientists of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine, headed by prof. L.Ya. Sirenko. At the Institute, up to now there is a collection of living microalgae cultures created by Lydia Yakimivna (Fig. 1). The staff of the Institute performed a large complex of biotechnological works to find new directions for algae biomass using, ways of seston utilization of Dnieper reservoirs, development of microalgae cultivation technology in tubular photobioreactors (Fig. 2) and introduction of it into the department of microalgae industrial cultivation on the basis of Ladyzhinskaya Thermal Power Plant for the needs of livestock and fish farming, etc. [21, 61, 62, 72–76].

At present, unfortunately, in Ukraine the market for this product is almost not filled, the needs are met mainly due to foreign supplies (partly from Europe and mostly from China) and only a few companies supply biomass of domestic algae. In particular, the limited liability company under the laws of Ukraine, “Mercury-II”, in the framework of a joint scientific project with of the lapsed Institute of South Sea Biology of the National Academy of Sciences of Ukraine, started growing *Spirulina platensis* (the trademarks “Living Spirulina AlgaeLife” and “SpirulinaLive”) since 2007 in the Kharkiv region to implement it in Ukraine and abroad, continuing to further develop and improve the technology. Within this company, the scientific-production firm “Prombiotechnika” (Odesa) offers bioreactors for the cultivation of microalgae. In Vinnitsa region Bar branch of the company “Tsukorpromvodonaladka” grows *Chlorella* and *Scenedesmus* for the treatment of sewage of food industry enterprises. “Chlorella Ukraine” (Bila Tserkva) private enterprise offers chlorella

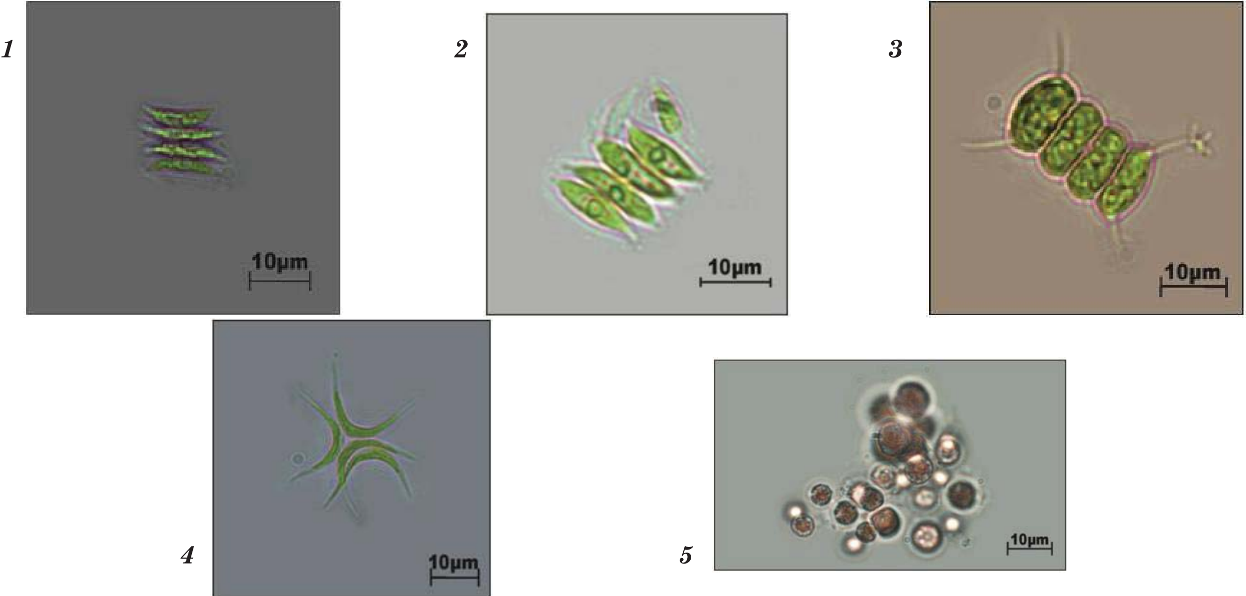

Fig. 1. **Microscopic algae from the collection of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine HPDP**: *1 — Tetradesmus dimorphus* (Turpin) M.J. Wynne HPDP–108 (=*Acutodesmus dimorphus* (Turpin) P. Tsarenko); *2 — Tetradesmus obliquus* (Turpin) M.J. Wynne HPDP–104 (=*Acutodesmus obliquus* (Turpin) E. Hegew. et Hanagata); *3 — Desmodesmus communis* (E. Hegew.) E. Hegew. HPDP–109; *4 — Messastrum gracile* (Reinsch) T.S. Garcia HPDP–115 (=*Selenastrum gracile* Reinsch); *5 — Porphyridium purpureum* (Bory) K.M. Drew et R. Ross HPDP–141 (=*Porphyridium cruentum* (Gray) Nägeli)

for various needs in the form of suspension, concentrate, paste or dry powder [78].

Recently, Ukrainian entrepreneurs are focusing on the development of aqua farming. For this purpose, they are given the opportunity to rent parts of reservoirs for fish breeding in seas, reservoirs, garden farms, create favorable conditions for investment and credit. The state is trying to promote aquaculture and mariculture development in accordance with world standards, with the aim of producing organic aquaculture products, in particular, the cultivation of a number of freshwater and marine fish and shellfish species. In this regard, it is also promising to grow microalgae as a component of fish and invertebrates feed.

A significant impediment to the implementation of algobiotechnology, especially in Ukraine, is the unwillingness of domestic entrepreneurs to make long-term investments and the high cost of algae production. Intensification of research activities aimed at increasing the productivity of algae and increasing the yield of target products, improving the methods of cultivation and integrated processing of biomass, as well as the use of local resources — waste heat from Thermal Power Plants, food production waste, CO_2 from flue gases of industrial enterprises, etc. can contribute to its reduction [29, 79].

The cultivation of microalgae is promising for the renewable raw materials obtaining of various purposes. The development of algobiotechnology research will promote the replenishment and improvement of the food base, the creation of medical products and new technical resources, and safety issue resolution of the environmental as well.

Algae production volumes are subject to UN Organization structures accounting and expansion, in particular through aquaculture. Meanwhile, the amount of biomass of freshwater microalgae produced is incomparably lower than that of marine algae. In particular, world production of *Spirulina* spp., concentrated in Australia, Israel, India, Malaysia, Myanmar, Japan according to available statistics from the Food and Agriculture Organization of United Nations (FAO), does not exceed 100 thousand tones, compared to hundreds and million thousands of tons for marine species [80].

Analysis of the achievements of algobiotechnology indicates the feasibility of creating a new and complete agriculture in Ukraine to solve various technological problems related to wastewater treatment, utilization of excess potential biogenic resources (biogenic compounds, organic substances, carbon dioxide), obtaining natural balanced feeds for animals, fish and

Fig. 2. **Experimental tubular photobioreactor of biotechnological complex of the Institute of Hydrobiology of the National Academy of Sciences of Ukraine**

poultry, as well as raw materials for further processing (production of biologically valuable compounds, biogas, biodiesel). For this purpose, given the considerable scientific and practical achievements of specialists of many organizations (academic institutes, universities, state and private enterprises), it is advisable to create a register (in the future — a bank) of algae cultures with different ranges of ecological valence and useful physiological properties, to study adaptive potential of different types of algae with respect to fluctuations of abiotic and biotic factors, to determine ways of purposeful regulation of productivity, biochemical composition and accumulation of certain valuable compounds in biomass.

REFERENCES

1. Microalgae — a promising agricultural culture. Available at: http://infoindustria.com.ua/2015/09/18/. (accessed 18 september 2018).
2. *Abdelnour S. A., Abd El-Hack M. E., Arif M., Khafaga A. F., Taha A. E.* The application of the microalgae Chlorella spp. as a supplement in broiler feed. World's Poult. *Sci. J.* 2019, 75, 305–318. http://dx.doi.org/10.1017/S0043933919000047
3. *Abdelnoiur Sameh., Almeer Rafa, Alkahtani Saad, Alarifi Saud, Abdel-Daim Mohamed, Albashed Gadah.* Impacts of Enriching Growing Rabbit Diets with Chlorella vulgaris Microalgae on Growth, Blood Variables, Carcass Traits, Immunological and Antioxidant Indices. *Animals*. 2019, 9, 788p.
4. *Becker E. W.* Micro-algae as a source of protein. *Biotechnol. Advances*. 2007, 25, 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002
5. *Vonshak A.* Scaling up microalgal cultures to commercial scale. *European Journal of Phycology*. 2017, 52(4), 407–418, https://doi.org/110.1080/09670262.2017.1365177
6. *Gadiev Rinat, Khaziev Danis, Galina Chulpan, Farrakhov Albert, Farhutdinov Kamil, Dolmatova Irina, Kazanina Marina, Latypova Gulnara.* The use of chlorella in goose breeding. *AIMS Agriculture and Food*. 2019, 4, 349–361. https://doi.org/10.3934/agrfood.2019.2.349
7. *Karunkyi O., Reznik T., Kulidzhanov Ye. Chlorella* suspension and its usage in finishing pigs' rations. *Grain Products and Mixed Fodder's*. 2019, 19, 46-49. https://doi.org/49.10.15673/gpmf.v19i1.1321
8. The use of food microalgae in the fight against acute malnutrition in order to overcome the consequences of humanitarian disasters and achieve sustainable development. United Nations A/C.2/60/L.14. General Assembly. Sixtieth Session. 31 October 2005.
9. *Sirenko L. A., Sakevych O. J., Kirpenko Yu. O.* Method for obtaining chlorophyll-carotene paste with reparative-regenerative properties. *Ukraine. Patent 13945,* April 24, 1997.
10. FAO Fisheries and Aquaculture Technical Paper. *Rome: FAO*. 2013, 583, 67 p.
11. *Lebedeva L. P., Dzhokebayeva S. A.* Optimization of growth processes of chlorella and spirulina and the use of pure extracts as biologically active additives to fish feed. Available at: https://bulletin-ecology.kaznu.kz/index.php/1-eco/article/view/303 (accessed 05 December 2019).
12. *Melnykov S., Manankyna E.* Use of chlorella in the feeding of farm animals. *Nauka i innovacii*. 2010, 5(50), 40–43. (In Russian).
13. *Plutakhin G., Machneva N., Koshchayev A.* Chlorella and its use in poultry farming. Available at: http://webpticeprom.ru/ru/articles-birdseed.html?pageID= 1309028642. (accessed 05 december 2019).
14. *Salnikova M. Ya.* Chlorella — a new kind of feed. *Moskva: KOLOS*, 1977, 96 p. (In Russian).
15. *Shatskikh Ye., Gafarov S. H., Boyarintseva G., Safronov S.* Use of chlorella in feeding piglets. *Kormleniye selskokhozyaystvennykh zhivotnykh i kormoproizvodstvo*. 2006, 7, 53–58. (In Russian).
16. *Ponomarev A. V.* Development and scientific support of the process of mass transfer during the cultivation of microalgae in a film photobioreactor. The dissertation author's abstract on scientific degree editions. Voronezh, 2011. (In Russian).
17. *Muzafarov A. M., Taubaev T. T.* Cultivation and use of microalgae. *Tashkent: Fan*, 1984, 185 p. (In Russian).

18. *Frolova M. V.* The effectiveness of the use of chlorella suspension, enriched with iodine and selenium, when growing young pigs (Ph.D. dissertation). Volgograd, 2012, 115 p. (In Russian).
19. *Bodnar O. I.* Effect of trace elements on lipid metabolism in Chlorella vulgaris Beijer. *Visnyk ONU. Biologiya*. 2018, 2(43), 11-22. https://doi.org/10.18524/2077-1746.2018.2(43).146950
20. *Lukjanov V. A., Styfeev A. Y.* Applied aspects of the use of microalgae in agrocenosis. *Kursk: Kursk State Agricultural Academy*, 2014, 181 p. (In Russian).
21. *Brantsevich L. T., Vorobyeva N. M., Karasik V. M.* Guidelines for the use of seston as fertilizer for. agricultural plants. *Kyiv: Naukova dumka*, 1980, 37 p.
22. *Kirpenko N. I.* Ways of using biopolymers of microalgae. *Naukovi zapysky Ternopilskoho natsionalnoho peduniversytetu. Ser.biol*. 2004, 3–4(24), 48–52. (In Ukrainian).
23. *Sirenko L., Panferov V., Sabluk V.* A method of obtaining a glue base of a composition for pre-sowing seed treatment. *Ukraine. Patent 13946,* April 25, 1997.
24. *Osborne N. J, Webb P. M, Shaw G. R.* The toxins of *Lyngbya majuscula* and their human and ecological health effects. *Environ Int*. 2001, 27(5), 381–392. https://doi.org/10.1016/s0160-4120(01)00098-8
25. *Amin-Ul' Mannan M., Dipannita Khazra, Arun Karnval, Diban Chakravarti Kannan.* Algae as a source of raw materials for the production of biofuels. *Algologiya*. 2017, 27(3), 337–356. (In Russian).
26. *Zolotareva O. K., Shnyukova Ye. I., Syvash O. O., Mykhaylenko N. F.* Prospects for the use of microalgae in biotechnology. *Kyiv: Alterpres.* 2008, 234 p. (In Ukrainian).
27. *Chernova N. I., Kiseleva S. V., Popel O. S.* Efficiency of biodiesel production from microalgae. *Teploenerhetyka*. 2014, 6, P. 14–21. (In Russian). https://doi.org/10.1134/S0040363614060010
28. *Meshcheryakova Yu. V., Nagornov S. A.* Production of raw materials for biodiesel based on microalgae oil Chlorella. *Vestnyk VIESH*. 2013, 4(13), 33–35. (In Russian).
29. *Dvoretskiy D. S., Dvoretskiy S. I., Temnov M. S.* Technology for producing lipids from microalgae. *Tambov: FGBOU VPO "TGTU"*. 2015, 103 p. (In Russian).
30. *Nagornov S. A., Meshcheryakova Ju. V.* Production of raw materials for biodiesel based on microalgae oil Chlorella. *Innovatsii v selskom xozyaystve*. 2013, 3(5), 39–41. (In Russian).
31. *Tsarenko P. M., Borisova Ye. V.* The IBASU-A microalgae crop collection is a potential bio-resource for biodiesel production. *Algologiya*. 2014, 24(3), 409–412. (In Russian).
32. *Chokshi K., Pancha I., Trivedi K., George B., Maurya R. Ghosh A., Mishra S.* Biofuel potential of the newly isolated microalgae *Acutodesmus dimorphus* under temperature induced oxidative stress conditions. *Biores. Technol.* 2015, 180, 162–171. https://doi.org/10.1016/j.biortech.2014.12.102
33. *Xin L., Hu H. Y., Ke G., Sun Y. X.* Effects of different nitrogen and phosphorus concentration on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus sp. Biores. Technol.*, 2010, 101, P. 5494–5500. https://doi.org/10.1016/j.biortech.2010.02.016
34. *Sarsekeyeva F. K., Usserbaeva A. A., Zayadan B. K. Mironov K. S., Sidorov R. A., Kozlova A. Yu, Kupriyanova E. V., Sinetova M. A., Los D. 1.* Isolation and Characterization of a New Cyanobacterial Strain with a Unique Fatty Acid Composition. *Advances in Microbiology*. 2014, 4(15), 1033–1043. http://creativecommons.org/licenses/by/4.0/
35. *Sorokina K. N., Yakovlev V. A., Piligayev A. V.* The potential use of microalgae as a feedstock for bioenergy. *Kataliz v promyshlennosti*. 2012, 2, 63–72. (In Russian).
36. *Tsarenko P., Borysova O., Blume Ya.* High biomass producers and promising candidates for biodiesel production from microalgae collection IBASU-A (Ukraine). *Oceanological and Hydrobiological Studies.* 2016, 45(1), 79–85. https://doi.org/10.1515/ohs-2016-0008
37. *Zolotareva Ye. K., Shnyukova Ye. I., Podorvanov V. V.* Microalgae as producers of water conduit. *Algologiya*. 2010, 20(2), 225–249. (In Russian).
38. *Markov V. A., Devyanin S. N., Nagornov S. A., Akimov V. S.* Biodiesel fuels from various raw materials. *Transport na alternativnom toplive*. 2011, 3, 25–31. (In Russian).
39. *Golub N. B.* Scientific and technological bases of conversion of renewable raw materials into biomass, biomethane and biodiesel. The dissertation author's abstract on scientific degree editions. Kyiv, 2015. (In Ukrainian).
40. *Synelnikov O. D., Nykyforov V. V., Malovanyy M. S., Kharlamova O. V.* Method of producing biogas from blue-green algae. *Ukraine. Patent 105896,* April 11, 2016.
41. *Denisov A. A., Zhuykov V. Yu., Sirenko B. I.* Wastewater treatment in open water from organic and mineral contamination with. Moskva: *Dostizheniya nauki i tekhniki APK*, 2007, 54–56. (In Russian).
42. *Leonova L. I., Stupina V. V.* Algae in the post-treatment of wastewater. *Kyiv: Naukova dumka*, 1990, 184 p.
43. *Gogonin A. V., Shchemelinina T. N., Volodin V. V.* The use of microalgae in the process of

wastewater treatment at a pulp and paper mill. *Biodiagnostics of the state of natural and natural-man-made systems: Materials of the XVth All-Russian Scientific-Practical Conference with International Participation. Vyatskiy gos. in-t biologii Komi nauchnogo tsentra Uralskogo otdeleniya RAN*, 2017. (In Russian).
44. *Sharapova I. E., Shubakov A. A., Mikhaylova Ye. A., Volodin V. V.* Investigation and use of microalgae for bioremediation of aqueous media. *Innovatsii v nauke*. 2012, 1(13), 38–45. (In Russian).
45. *Zhuykov V. Yu.* Post-treatment and disinfection of wastewater with algae-bacterial microflora of biological ponds. The dissertation author's abstract on scientific degree editions. Schelkovo, 2009. (In Russian).
46. *Markov S. A.* Using algae to produce biofuels and remove excess carbon dioxide from the atmosphere. *Alternative energy and ecology: Proceedings of the International Conference*. 2009, 2 (70), 83–90.
47. *Kulabukhov V., Karyakin D., Maltsevskaya N.* Prospects for the use of microalgae for the absorption of CO2 from the flue gases of industrial enterprises. *Ekologiya i promyshlennost Rossii*. 2016, 20(9), 4–8. (In Russian). https://doi.org/10.18412/1816-0395-2016-9-4-8
48. *Romanenko V. D., Kirpenko N. I., Konovets I. M., Krot Yu. G.* Species-specific peculiarities of the green algae growth at additional carbon nutrition. Report 1. Growth rate of the green algae at maximal saturation of medium by CO_2 in open cultivation system. *Hydrob. J.* 2010, 3 (46), 60–72. https://doi.org/10.1615/HydrobJ.v46.i3.50
49. *Romanenko V. D., Kirpenko N. I., Konovets I. M., Krot Yu. G.* Species-specific peculiarities of the green algae growth at additional carbonic nutrition. Report 2. Growth of *Chlorella vulgaris* Beijer. strain LARG-3 at CO_2 utilization in close cultivation system. *Hydrob. J.* 2010, 5 (46), 44–50. https://doi.org/10.1615/HydrobJ.v46.i5.50
50. *Sister V. H., Ivannikova Je. M., Cirkov V. H., Kozevnikov Ju. A.* The use of microalgae in energy-generating cycles that consume liquid hydrocarbon fuel of fossil origin. *Alternativnaya energetika i ekologiya*. 2013, 9(131), 36-41. (In Russian).
51. *Shchemelinina T. N., Anchugova Ye. M., Tarabukin D. V.* The strain of the green microalga Acutodesmus obliquus, intended for the purification of wastewater from pollutants in the utilities and pulp and paper industry. *Russia. Patent 2556131*, July 10, 2015.
52. *Shchemelinina T. N., Markarova M. Yu., Zlobina N. V.* Biological product "Universal" and microalgae in the conditions of hydrocarbon pollution. *Vestn. biotekhnol. i fiz.-him. biol.* 2014, 10(2), 18–22. (In Russian).
53. *Doria E., Longoni P., Scibila L., Iazzi N., Cella R., Nielsen E.* Isolation and characterization of a *Scenedesmus acutus* strain to be used for bioremediation of urban wastwater. *J. Appl. Phycol.* 2012, 24, 375–383.
54. *Davidovich N. A., Davidovich O. I., Podunay Yu. A.* Reproductive characteristics of diatoms: importance for cultivation and biotechnology. *Fiziologiya rasteniy*. 2015, 62 (2), 167–175. (In Russian).
55. *Zheleznova S. N., Ryabushko V. I., Gevorgiz R. G.* The method of producing biomass of the diatom cylindrotheca closterium. *Russia. Patent* RU 2582182. 20 April, 2016.
56. *Ismailkhodzhayev B. SH.* Physiological and biochemical features of green and euglean microalgae and the prospects for their use. *The dissertation author's abstract on scientific degree editions.* Tashkent, 1994. (In Russian).
57. *Minyuk G. S., Yerokhin V. Ye., Gordiyenko A. P.* Physiological, biochemical and biophysical characteristics of unicellular algae *Haematococcus pluvialis* — promising astaxanthin. *Yu. N. Tokareva, Z. Z. Fynenko, N. V. Shadryna. Sevastopol*: *EKOSY-Hidrofizika*. 2008, 353–382. (In Russian).
58. *Chubchikova I. M., Drobetskaya I. V., Minyuk G. S.* Screening of green microalgae as potential sources of natural ketocarotenoids 2. Features of growth and secondary carotenogenesis in representatives of the genus Bracteacoccus (Chlorophyseae). *Morskyy ekolohichnyy zhurnal*. 2011, 91–97. (In Russian).
59. *Lisovskiy G. M.* Managed cultivation of microalgae. Moskva: *Nauka*. 1964, 153 p.
60. *Onishchenko Ye. M.* On the question of ways to improve the efficiency of ground-based open systems for the cultivation of microalgae. Available at: *http://www.jbks.ru/archive/issue-14/article-11/*
61. *Romanenko V. D., Krot Yu. G., Sirenko L. A., Solomatina V. D.* Biotechnology cultivation of hydrobionts. *Kyiv: Instytut hidrobiolohii NAN Ukrainy*. 1999, 264 p. (In Ukrainian).
62. *Kirpenko N. I.* Dynamics of the content of nitrogen forms in the medium during the intensive cultivation of chlorella. *J. Hydrobiological.* 2000, 36 (6), 74–81. (In Russian).
63. Technology and equipment for the cultivation of photoautotrophs and tissue culture. *Sbornik nauchnykh trudov. Moskva*, 1984, 145 p.
64. *Zhumadilova Zh. Sh., Sapargaliyeva G. M., Izimbet A. P.* Cultivation of microalgae to obtain biomass in the laboratory. *Mezhdunarodnyi zhurnal prikladnykh i fundamentalnykh issledovaniy.* 2015, 10(5), 838–839. (In Russian).
65. *Koller Martin*. Design of Closed Photobioreactors for Algal Cultivation. *Algal Biorfineries, 2015*, 133–186. https://doi.org/10.1007/978-3-319-20200-6_4

66. *Szyjka Sh. J., Mandal Sh., Schoepp N.* Evaluation of phenotype stability and ecological risk of a genetically engineered alga in open pond production. *Algal Research.* 2017, 24, 378–386. https://doi.org/10.1016/j.algal.2017.04.006
67. *Kirpenko N. I, Tsarenko P. M., Usenko O. M., Musiy T. O.* Strain of green microalgae Monoraphidium griffithii (Berk.) Komark.-Legner. HPDP-105 is a high-lipid biomass producer. *Ukraine. Patent 09629*, September 25, 2018.
68. European Society of Microalgal Biotechnology. Available at: https://uia.org/s/or/en/1100056397 (accessed 05 december 2019).
69. *Kirpenko N. I., Usenko O. M., Musiy T. O.* Comparative analysis of the content of proteins, carbohydrates, and lipids in the cells of green microalgae. *J. Hydrobiological.* 2018, 54 (2), 81–91.
70. Bioreactor for growing chlorella. Available at: www.biovet-service.ru/uslugi/chlorellarost.html (accessed 05 December 2019).
71. *Bakay S. M., Shelest V. P., Volokh V. N*. The use of chlorella in the diet of pigs. Pig breeding. *Kyiv: Urozhai.* 1966, 2, 63–67. (In Russian).
72. *Myslovich V. O., Karpenko V. I., Sirenko L. A., Kirpenko N. I.* The use of microalgae for the purification of liquid waste poultry farms *Kyiv: Dep. v VINITI*, 1987, 11 p.
73. *Sirenko L. A., Kozitskaya V. N., Konyshev B. I., Krot Yu. G., Kirpenko N. I., Komarenko Ye. I.* Growing algae on the combustion gases of oil producing and refining enterprises. *Thesis report of the All-Russian Conference "Industrial cultivation of microalgae", Uzbekistan: Andizhan*,1990.
74. *Sirenko L. A., Kirpenko N. Y., Komarenko O. I.* Resources of biomass of macro- and microalgae in Ukraine — a source for the preparation of biologically active substances. *Abstracts of the Fourth International Conference on Medical Botany.* Kyiv, 1997, 51–53.
75. *Bilous O. P., Nezbrytska I. M., Klochenko P. D., Kirpenko N. I.* Collection of microalgae cultures HPDP. Kyiv, 2018, 36 P.
76. *Sirenko L. A., Rybak N. V., Parshykova T. V., Pakhomova M. N.* Collection of living cultures of microscopic algae (acronym collection — HPDP. *Kyiv: Fitosotsiotsentr*, 2005, 53 P. (In Ukrainian).
77. Live spirulina is grown on an aqua farm in Ukraine. Available for address http://spirulinalive.com.ua
78. Official site of PE "Chlorella Ukraine". Available at: https://hlorella.jimdo.com/
79. *Trifonov V. Yu.* The use of flue gases generated in the process of thermal processing of municipal solid waste for the cultivation of microalgae Spirulina platensis. *Ecological Bulletin of Russia*. 2009, 11, 28–32.
80. The state of world fisheries and aquaculture. Contribution to universal food security and nutrition. World production of aquatic plants in aquaculture. *Rome: FAO*, 2016, 216 p.

БІОТЕХНОЛОГІЧНІ ПЕРСПЕКТИВИ МІКРОВОДОРОСТЕЙ

Кірпенко Н. І., Леонтьєва Т. О.

Інститут гідробіології НАНУ, Київ

E-mail: nativnativ@ukr.net

Проаналізовано сучасний стан та перспективи біотехнологічного використання мікроскопічних водоростей. Наведено основні напрями альгобіотехнології, зумовлені фізіолого-біохімічними особливостями цих організмів, обсяги одержуваної в світі водоростевої продукції, види мікроводоростей, що вже набули застосування чи мають практичні можливості, шляхи одержання біомаси та підвищення продуктивності промислового культивування водоростей. Розглянуто стан цієї проблеми, доцільність розвитку альгобіотехнології та умови вирощування мікроводоростей в Україні.

Ключові слова: альгобіотехнологія, мікроводорості, промислове культивування, аквакультура.

БИОТЕХНОЛОГИЧЕСКИЕ ПЕРСПЕКТИВЫ МИКРОВОДОРОСЛЕЙ

Кирпенко Н. И., Леонтьева Т. А.

Институт гидробиологии НАНУ, Киев

E-mail: nativnativ@ukr.net

Проанализировано современное состояние и перспективы биотехнологического использования микроскопических водорослей. Приведены основные направления альгобиотехнологии, обусловленные физиолого-биохимическими особенностями этих организмов, объемы производимой в мире водорослевой продукции, виды микроводорослей, уже нашедшие применение или имеющие практические возможности, пути получения биомассы и повышения продуктивности промышленного культивирования водорослей. Рассмотрены состояние этой проблемы, целесообразность развития альгобиотехнологии и условия выращивания микроводорослей в Украине.

Ключевые слова: альгобиотехнология, микроводоросли, промышленное культивирование, аквакультура.

EXPERIMENTAL ARTICLES

UDC 577.151.6

https://doi.org/10.15407/biotech12.06.035

METALLOPROTEASE FROM THE CULTURAL LIQUID OF *Pleurotus ostreatus*

V. V. Sakovich[1]
Ye. M. Stohnii[2]
D. D. Zhernosekov[1]
A. V. Rebriev[2]
D. S. Korolova[2]
R. Yu. Marunych[2]
V. O. Chernyshenko[2]

[1]Polesskii State University, Pinsk, Republic of Belarus

[2]Palladin Institute of Biochemistry
of the National Academy of Sciences of Ukraine, Kyiv

E-mail: mrs.valeryia@mail.ru

Received 22.09.2019
Revised 14.11.2019
Accepted 20.12.2019

The aim of this work was to identify and to study physical and chemical properties of the enzyme preparation which was obtained from the cultural liquid of *Pleurotus ostreatus*.

The protease containing fraction was obtained from the cultural liquid by sodium chloride precipitation followed by dialysis and concentration procedures. Gelatinase and milk-clotting activity were defined by standard methods. The content of the protein component of the fraction was analysed by HPLC, Laemmli electrophoresis and MALDI-TOF analysis. Protease activity was proved by enzyme-electrophoresis. To identify the protease, mass-spectrometry was carried out using the MatrixScience database. To study the specificity of protease action, the series of chromogenic substrates were used: S2238; S236; S2251; S2765; Leu-pNa; Ala-pNa and S2302. The inhibitory analysis was carried out using EDTA, benzamidine, PMSF, PCMB.

The obtained fraction possessed maximal protease activity at 45 °C. Meanwhile maximal milk-clotting activity was observed at 35 °C. The highest milk-clotting activity was shown at pH 5.0 and less than 3.0. The highest protease activity was shown at pH 6.0. Using HPLC method, it was found the main protein component and some minor proteins. According to the electrophoresis results, the main protein component of the fraction had molecular mass 45 kDa. Enzyme electrophoresis demonstrated that protease activity of the fraction was present in the zone corresponding to 45 kDa. When identifying trypsinolysis products, no homology was found with other known proteinases. It was shown that the protease hydrolyzed peptide bonds which were formed by carboxyl group of amino acids with hydrophobic side chains. The enzyme was inhibited by EDTA (IC50 = 2.5 mM). The maximal enzyme activity towards gelatin and Leu-pNa was shown in the presence of 5 mM calcium chloride.

The new calcium-dependent metalloprotease with molecular weight 45 kDa was found in the cultural liquid of *P. ostreatus*. The enzyme had no homology with other known proteases and hydrolyzes peptide bonds formed by carboxyl groups of amino acids with hydrophobic side chains.

Key words: basidiomycetes, proteolytic enzymes, milk-clotting activity, physical and chemical properties.

Proteases of animal origin became common use in dairy industry in particular in cheese production. Nowadays, the main sources of these proteases are pancreas and gastric mucosa of cattle and pigs. This resource is limited, so substitution of the expensive rennet enzyme by mushroom proteases is cost effecting and promising. It was shown that the level of milk-clotting activity of basidiomycetes was comparable to that of the traditionally used commercial rennet enzymes [1].

The requirements for rennet substitutes are strict. Their enzymatic properties should be as close as possible to natural renin. This means that, along with high milk-clotting activity, substitute enzymes should have a slight total proteolytic activity [1, 2]. Due to the concomitant proteolytic activity,

the obtained clots often have a bitter taste, which negatively affects the quality of cheese products [1, 3]. An analysis of literary sources shows that the search for substitute enzymes in the macromycete group is quite successful [4, 5]. It is known that *Pleurotus ostreatus* contains proteases with milk-clotting activity (MCA) [3, 6]. There are data that the extract of fruit bodies of *P. ostreatus* is similar to preparations used in the dairy industry and after purification can be used in cheese making.

In our previous investigations we selected nutrient media and optimal conditions for the deep cultivation of *P. ostreatus* [7]. We showed that the enzyme preparation from the cultural liquid of *P. ostreatus* possessed both: milk-clotting and proteolytic activity [8, 9].

Materials and Methods

Obtaining of the protease containing fraction. The experiments were performed on a "wild" strain of *P. ostreatus*, which was isolated from fruiting bodies growing on a cultivated poplar (*Populus* sp.). In all experiments, a potato-sucrose medium was used, as described in [7]. The mycelium was planted under a laminar box to minimize the risk of contamination. The inoculum was introduced in the form of fragments of a carpet of stock culture of mycelium with an area of 1 cm^2. Cultivation was carried out for 14 days in dark at a temperature of 27 °C on a shaker of WiseShake SHO model at 70 rpm. At the end of the incubation, the culture liquid was collected and frozen. As the initial stage of purification of the enzyme preparation from the culture liquid, salting out with sodium chloride (100% saturation) was used.

The salt was removed by dialysis. For long-term storage of the preparation, the method of freeze drying was used with a combination of a temperature of −51 °C and a pressure of 1.370 mBar. Protein concentration was determined spectrophotometrically [10].

Gelatinase activity. Proteolytic activity (PA) was determined according to the method described by Leighton et al. [11]. A mixture containing 0.15 ml of the enzyme preparation and 0.25 ml of the substrate (1% gelatin in 0.2 M acetate buffer, pH 5.0) was incubated for 60 min in the absence of light (gelatin final concentration $6.85{\times}10^{-6}$ M). The reaction was stopped by the addition of 10% trichloroacetic acid. The mixture was centrifuged (8.000 rpm) for 15 min at 4 °C. Then 1.4 ml of 1 M NaOH was added to the obtained supernatant (0.8 ml). For one unit of PA, an enzyme amount was taken that promotes an increase in absorbance of 0.01 in one hour at 440 nm.

Milk-clotting activity. Milk-clotting activity (MCA) was determined according to the Pyatnitsky method: a test tube with a substrate (milk 3.4% volume 10 ml) containing a 0.0015 M solution of calcium chloride was heated to 35 °C and 2 ml of the studied enzyme preparation was added. The preparation activity was evaluated by the time of formation of a dense milk clot. The unit of MCA was the amount of enzyme that clots 100 ml of milk in 40 min at 35 °C [12].

To identify the optimum pH of the enzyme preparation, PA and MCA were determined at 25 °C with gelatin in various pH ranges using the following buffer solutions: 0.2 M acetate (pH from 3.8 to 5.8) and 1M phosphate (pH from 5.8 to 8.0).

To determine the temperature optimum, the enzyme preparation was incubated with gelatin at temperature in the range from 25 to 80 °C. When studying the effect of preincubation on MCA and PA, the enzyme preparation was preincubated at various temperatures in the range from 25 to 80 °C for 1 hour. After that, the determination of proteolytic activity was carried out as described above.

The effect of calcium ions on the milk-clotting activity of the enzyme preparation was determined by adding a solution of calcium chloride to a substrate (milk) in a final concentration from 20 to 500 mM. The samples were incubated at 60 °C, after that milk clotting activity was determined.

The effect of calcium ions on the protease activity was studied as it was explained above for milk except the gelatin that was used as the substrate of the reaction (see 'Gelatinase activity' section).

Electrophoretic analysis was performed in 12 and 10% PAAG by the Laemmli SDS PAGE [13]. Protein zones were identified after Coomassi R-250 staining.

Enzyme electrophoresis was performed to identify protein zones with fibrinogenase activity. Gel was polymerized in the presence of 0.5 mg/ml fibrinogen. After electrophoresis performed by the above method, DS-Na was removed from gel by three times washing in 2.5% solution of Triton X-100. The gel was then incubated in 0.1 M glycine buffer, pH 8.3 for 12 h. The gel was stained with Coomassi R-250 and the areas of proteolytic activity identified by the location of the unstained spots on the gel.

HPLC on phenyl sepharose. Chromatographic system Agilent 1100 was used for the extract analysis with column Dupont Instrument (250 mm long and 4.7 mm over) with ZorbaxSilicogel(20 μm) with phenyl inoculation in pressure of 140 bar and flow 1.5 ml per minute. Two buffer gradients were used: the decreasing one (0.15M TrisHCl pH 6.5, 0.13M NaCl) and increasing one (50% acetonitrile contained buffer with 0.1% trifluor acetic acid).

MALDI-TOF analysis of trypsinolysis products of the main protein component from the cultural liquid of *P. ostreatus* was performed using a Voyager-DE (Applied Biosystems, USA). H+-matrix ionization of polypeptides under sinapic acid (Sigma-Aldrich) was used. The results were analyzed by Data Explorer 4.0.0.0 (Applied Biosystems) [14].

Amidase activity was determined by cleavage of chromogenic substrates: S2238 (H-D-Phe-Pip-Arg-pNa), S236 (pyro-Glu-Pro-L-Arg-pNa), S2251 (D-Val-Leu-Lys-pNa), S2765 (Z-D-Arg-Gly-Arg-pNa), Leu-pNa, Ala-pNa, S2302 (H-D-Pro-Phe-Arg-pNa). The assay was performed in microplates, which wells were successively introduced with 0.05 M Tris-HCl buffer pH 7.4 and a chromogenic substrate in the final concentration 20 μM. The reaction was started by adding an enzyme-containing fraction at 37 °C. Amidase activity was characterized by the rate of release of paranitroaniline (pNa), which was detected at a wavelength of 405 nm using a Multiskan EX reader [15].

Protein concentration was determined according to Bradford [16].

Statistical analysis was performed using STATISTICA 6.0 software ($n = 5$).

Results and Discussion

Total protease and milk-clotting activity

The influence of pH

Milk-clotting activity (MCA) of the enzyme preparation was observed in a narrow pH range from 3.6 to 5.6. The pH optimum of the enzyme preparation with MCA was represented by two peaks at pH 3.6 and pH 5.0. The proteolytic activity of the enzyme preparation from *P. ostreatus* was observed in the entire pH range from 3.6 to 8.0. The pH optimum of proteolytic activity was at pH 7.0 (Fig. 1).

According to the literature, the proteases of some fungi are active in a wide pH range. For proteases derived from *P. ostreatus* fruiting bodies, the pH range, at which proteolytic activity is maintained, was in the range from 4 to 9 [3]. The stability interval of milk-clotting proteases from *P. ostreatus* mycelium was in the pH range from 3.5 to 7.5 [4]. These data are consistent with our results regarding the effect of pH on the activity of proteases from *P. ostreatus* culture liquid. It was shown that with high proteolytic activity of the enzyme preparation, not only the formation of a clot was observed, but also its further hydrolysis. This leads to the appearance of bitter peptides and makes such an enzyme preparation unsuitable for use in the cheese production. Taking into account these data, we recommend for making cheese the use of the enzyme preparation with a pH value of 3.6, since at this pH the ratio of MCA/PA is 74: 1. For example, at pH 5 the ratio of MCA/PA is only 13: 1 (Table 1).

The influence of temperature

In order to investigate the physicochemical properties of milk-clotting proteases from

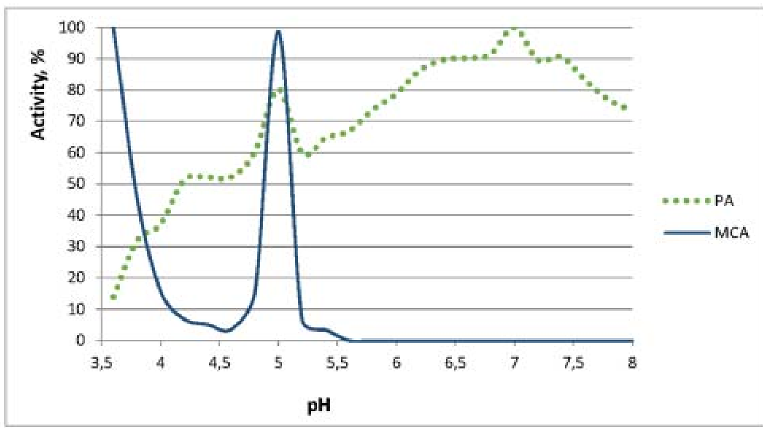

Fig. 1. **The influence of pH on the protease activity (PA) and milk-clotting activity (MCA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

The milk-clotting and protease activity of enzyme preparation at different pH values

pH	Protein mg/ml	Total MCA (U*)	Specific MCA (U/mg of protein)	Total PA (U)	Specific PA (U/mg of protein)	MCA/PA
3.6	1.07	81.08	75.78	1.09	1.02	74:1
3.8	1.07	37.27	34.8	2.5	2.34	16:1
4.0	1.07	12.74	11.9	2.92	2.73	4:1
4.2	1.07	5.57	5.2	4.03	3.77	1.5:1
4.4	1.07	4.12	3.92	4.01	3.75	1.1:1
4.6	1.07	2.89	2.7	4.11	3.8	0.8:1
4.8	1.07	12.35	11.5	4.74	4.43	2.6:1
5.0	1.07	80	74.77	6.32	5.91	13:1
5.2	1.07	5.38	5.03	4.71	4.4	1.2:1
5.4	1.07	2.72	2.54	5.09	4.76	0.6:1

* U-milk-clotting or protease activity unit.

P. ostreatus culture liquid, we studied the effect of temperature on the milk-clotting and proteolytic activity of the enzyme preparation. To study thermostability, we conducted a series of experiments in which the preparations were preincubatedat under various temperatures for 1 hour. The temperature optima for PA and MCA were different.

The maximum milk-clotting activity was observed at 45 °C (Fig. 2). This value coincides with that for *Pleurotus eringii* and is somewhat lower than for the fungi enzymes and *Tricholoma saponaceum* (55 °C) [17, 18]. The milk-clotting activity is maintained up to 55 °C at pH 5. A further temperature increase sharply inactivates the milk-clotting enzymes of the studied fungus. At 4 °C, the milk-clotting activity of the lyophilic powder solution remains at the same level for a month. The obtained data are consistent with the results obtained for the enzyme preparation from the fruit bodies of *P. ostreatus* [4, 5].

The proteolytic activity of the enzyme preparation from *P. ostreatus* was observed in the entire temperature range from 25 to 60 °C (Fig. 2). The temperature optimum of proteolytic activity is at 45 °C. This value is comparable with the temperature optimum, which was previously determined for the enzyme preparation from *P. eringii* [19].

As it can be seen from Fig. 2 and 3, during one hour of pre-incubation of solutions containing enzymes at 35 and 45 °C, an increase in MCA and PA was observed, at least 2 times, respectively. This phenomenon was discovered earlier by other researchers for an enzyme preparation containing MCA from the fruit bodies of *P. ostreatus* [4].

Theinfluence of calcium ions on MCA

There was no influence of calcium ions on protease activity of the enzyme preparation. The concentration range was from 2 till 50 mM. However, the influence of calcium ions on the milk-clotting activity was significant. There is evidence in the literature that calcium stimulates the activity of milk-clotting enzymes. Calcium taken at a sufficiently high concentration was considered as an important component in the formation of the milk clot [18]. The addition of 1.8 μM calcium chloride to milk improved its coagulation and led to an increase in milk clot hardness by 32% [19]. The cheese hardness could be increased to 81% due to addition of 10 mM $CaCl_2$. However, an increase in calcium chloride concentration caused a decrease in cheese hardness [19, 20]. The use of high concentrations of calcium chloride could have negative effects on cheese production.

The use of high concentrations of calcium chloride changed the meltability of the cheese clot that caused a number of problems in cheese production [20]. As can be seen from Fig. 4, the maximum milk-clotting activity in our studies was achieved when calcium chloride was added to the substrate (milk) at a final concentration of 10 mM.

Our data differ from those for the enzyme preparation of microbial origin. Thus, the milk-clotting activity of proteases from *Bacillus amyloliquefaciens* was highest at

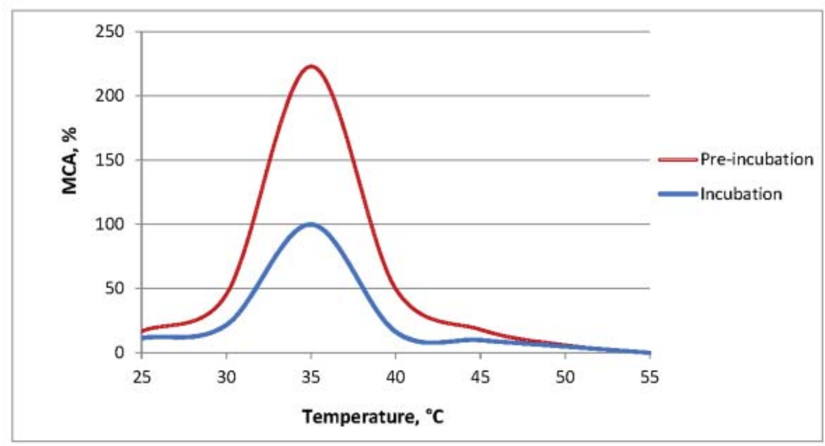

Fig. 2. **The influence of temperature on milk-clotting activity (MCA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

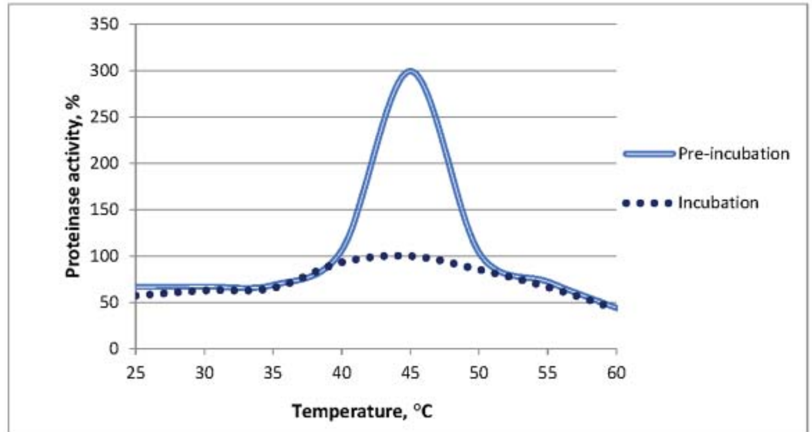

Fig. 3. **The influence of temperature on protease activity (PA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

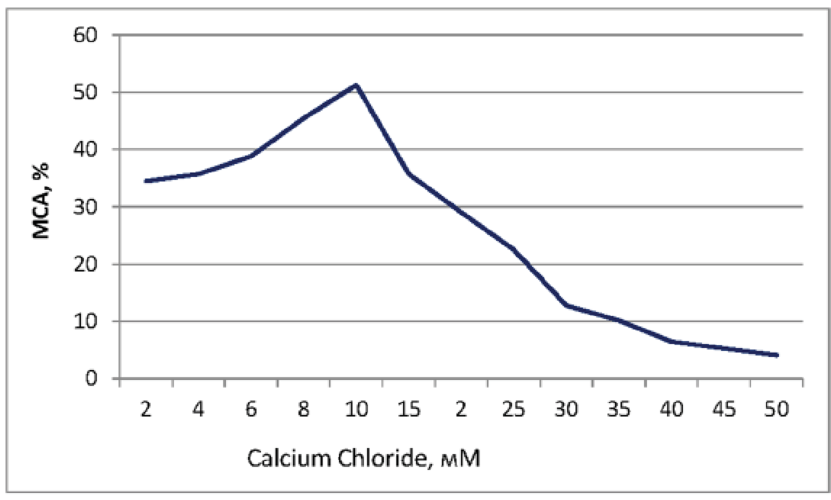

Fig. 4. **The influence of calcium chlorideon MCA of the enzyme preparation from cultural liquid of *P. ostreatus***

a final concentration of calcium chloride of 25 mM. In the range of calcium chloride concentrations from 0 to 20 mM, the milk-clotting rate increased with an increase in Ca^{2+} ions concentration. Meanwhile at a concentration above 25 mM, a decrease in milk-clotting activity was observed [21].

Maximum MCA of the protease from *Enterococcus faecalis* was obtained by adding 50 mM calcium chloride to the incubation medium [14]. The effect of CaCl2 on the aggregation of para-casein micelles is explained by its effect on the average coagulation rate. It was hypothesized that electrostatic repulsions and ionic bonds played an important role in the interaction between chymosin and para-casein [22, 23].

Identification of the protease component from the cultural liquid of P. ostreatus

According to electrophoresis results, main protein component of the fraction had molecular mass 45 kDa (Fig. 5). Using HPLC method it was found the main protein component and some minor proteins (Fig. 6). So, HPLC data proved the electrophoresis results and gave us possibility to concentrate our efforts on identification of the major component of the fraction. To prove the enzyme activity of the protein component, enzyme electrophoresis was applied with fibrinogen as a standard substrate. It was shown that protease activity of the fraction was present in the zone corresponding 45 kDa (Fig. 7).

To identify the enzyme, trypsinolysis of the main protein component was carried out followed by the analysis of its products using MALDI (Fig. 8). Identification of the trypsinolysis products of the main protein component let us to carry out the screening of its amino acid sequence and make the comparison of this sequence with other sequences of known enzymes from different origins. There was no homology with other known proteases.

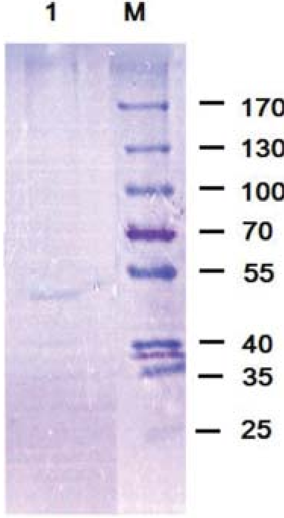

Fig. 5. **SDS-PAGE of the enzyme preparation from the cultural liquid of *P. ostreatus***

Thus, protein with molecular weight of 45 kDa had proteolytic activity in the composition of *P. ostreatus* liquid culture. This enzyme, according to the results of the MALDI-TOF analysis of trypsinolysis products, did not present in publicly available databases and requires further investigation.

The study of hydrolytic activity of a protease from the cultural liquid of P. ostreatus

For a targeted investigation of the substrate specificity of proteases from *P. ostreatus* culture liquid, studies were carried out at pH of 7.4, to exclude the possible contribution of a milk-clotting enzyme. Amidolytic activity was evaluated using several chromogenic substrates: S2238 (H-D-Phe-Pip-Arg-pNa); S236 (pyro-Glu-Pro-L-Arg-pNa); S2251 (D-Val-Leu-Lys-pNa); S2765 (Z-D-Arg-Gly-Arg-pNa); Leu-pNa; Ala-pNa; S2302 (H-D-Pro-Phe-Arg-pNa).

As shown in Fig. 9, the enzyme has the highest specificity for Leu-pNa (among all investigated substrates) – the hydrolysis reaction rate was 0.22 μM/min. Previously, the highest amidase activity with the substrate S2586 (MeO-Suc-Arg-Pro-Tyr-pNA) was determined in the mycelium preparation of *P. ostreatus*. It is also known that chymosin (rennet) has specificity for peptide bonds formed by the C-group of hydrophobic amino acids. Chymosin specifically cleaves the Phe105-Met106 peptide bond in a casein molecule [22, 24–26]. It is known that the most specific substrate for chymosin is a compound with Phe-Met peptide bond. However we recommend Leu-pNa as more available substrate, which also has peptide lond formed by C-group of hydrophobic amino acid.

The effect of various inhibitors on amidolytic activity (with Leu-pNaas a substrate) is shown in Fig. 10. The enzyme from the cultural liquid of *P. ostreatus* was inhibited by 10 mM EDTA, a widely known inhibitor of metalloproteases (Fig. 10).

Moreover, the effect of EDTA had a concentration-dependent nature (Fig. 11). The determined IC50 value for EDTA was 2.5 mM. It

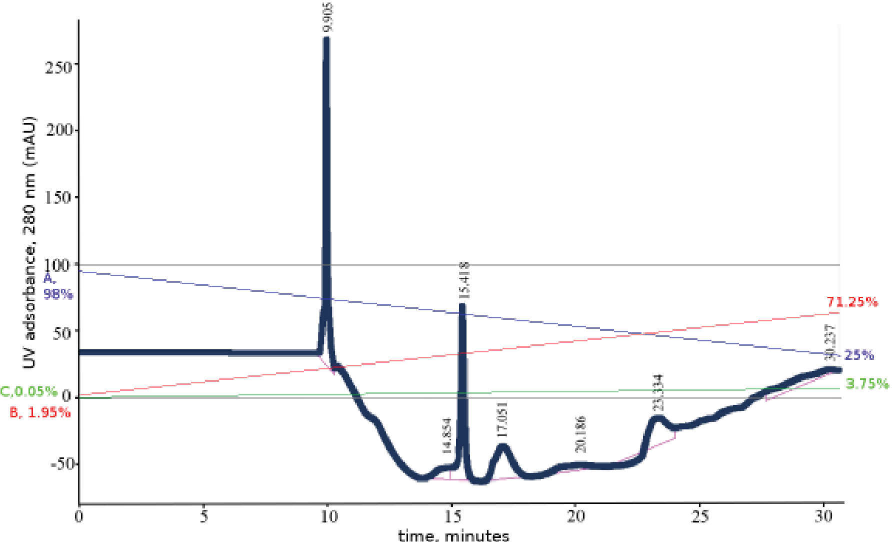

Fig. 6. **HPLC profile of the enzyme preparation from the cultural liquid of *P. ostreatus***

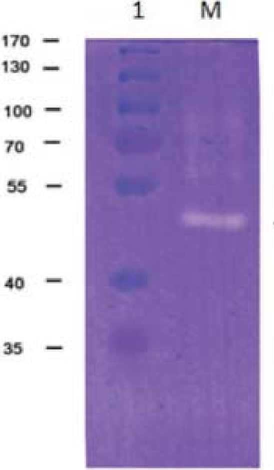

Fig. 7. **Enzyme-electrophoresis of the enzyme preparation from the cultural liquid of *P. ostreatus* using fibrinogen as a universal protease substrate**

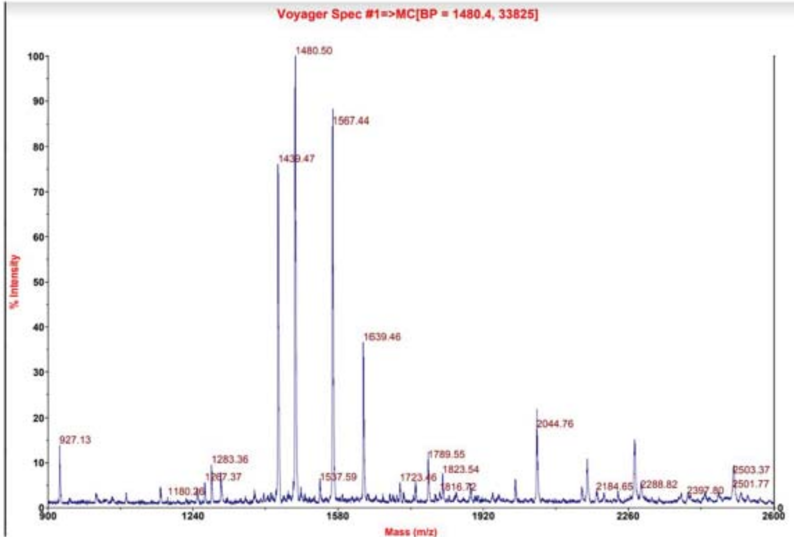

Fig. 8. **MALDI-TOF spectra of the trypsinolysis fragments of the main protein component from the cultural liquid of *P. ostreatus***

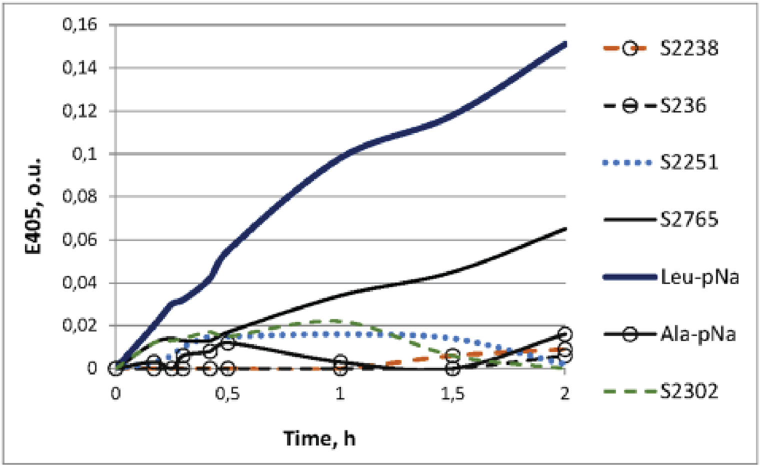

Fig. 9. **Amidase activity of the protease from the cultural liquid of *P.ostreatus* with the following substrates: S2238 (H-D-Phe-Pip-Arg-pNa); S236 (pyro-Glu-Pro-L-Arg-pNa); S2251 (D-Val-Leu-Lys-pNa); S2765 (Z-D-Arg-Gly-Arg-pNa); Leu-pNa, Ala-pNa; S2302 (H-D-Pro-Phe-Arg-pNa)**

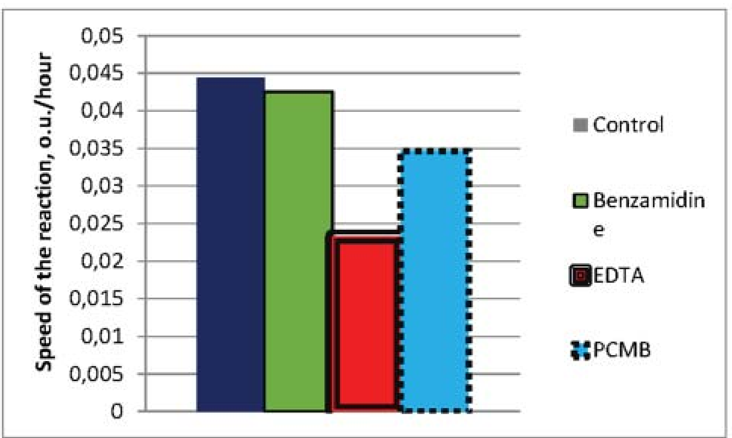

Fig. 10. **The rate of Leu-pNa degradation by protease from *P. ostreatus* cultural liquid in presence or absence (control) of the following inhibitors such as serine proteases (benzamidine), metalloproteases (EDTA), cysteine proteases (PCMB). Each inhibitor was taken at concentration 10 mM**

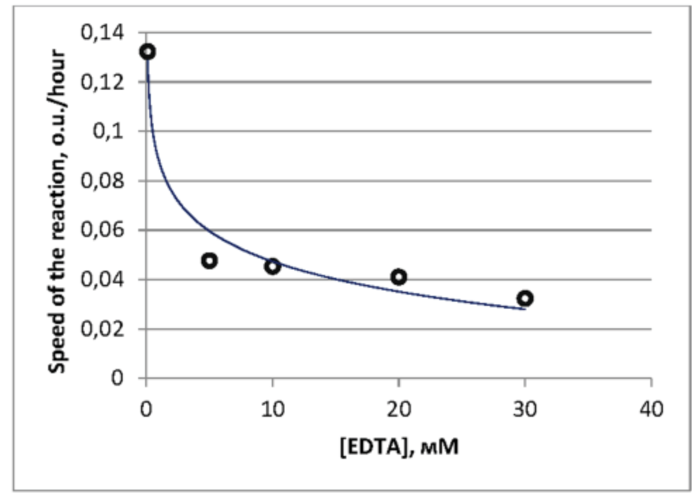

Fig. 11. **The effect of EDTA on the protease activity of the preparation from the cultural liquid of *P. ostreatus* with Leu-pNa as a substrate**

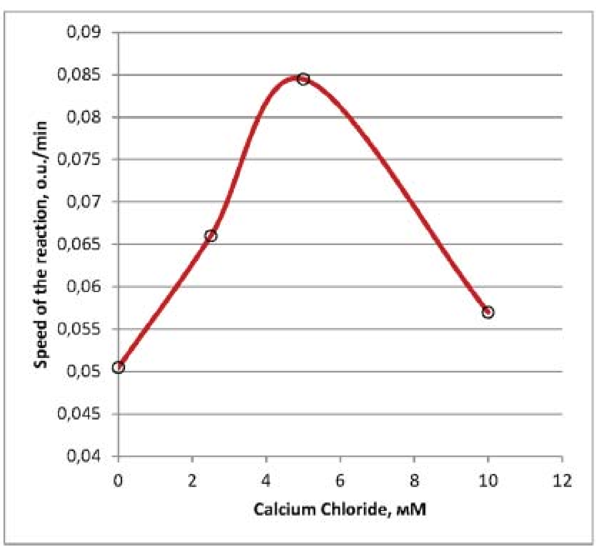

Fig. 12. **The effect of calcium chloride on the protease activity of the preparation from the cultural liquid of *P. ostreatus* with Leu-pNa as a substrate**

should be noted that the marked inhibitory effect of PCMB was not changed with the increasing of the inhibitor concentration and might indicate the presence of disulfide bonds in enzyme. Thus, the protease from the cultural liquid of *P. ostreatus* turns out to be a metalloprotease and, like many other proteases, is calcium dependent (Fig. 12). In particular, 5 mM calcium chloride activates the enzyme more than in 2 times.

Partially purified preparation from the cultural liquid of *P. ostreatus* contained a milk-clotting component, which was characterized for industrial application as a milk-clotting enzyme. According to our results, the recommended pH value was 3.6, optimal temperature was 35 °C. To increase milk-clotting activity we recommend one-hour preincubation of the enzyme preparation.

It was found the calcium-dependent metalloprotease with molecular mass 45 kDa in the cultural liquid of *Pleurotus ostreatus*. The enzyme had no homology with other known proteases and hydrolyzes peptide bonds formed by carboxyl groups of amino acids with hydrophobic side chains.

The work was conducted in the frame of the scholarship of Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine given to Yevhenii Stohnii "Study of hydrolysis of fibrinogen molecule by proteases of different origin".

REFERENCES

1. *Emmons D. B.* Estimating cheese yield losses from proteolysis during cheese making. *Journal of Dairy Science*, 1990, 73(8), 2016–2021. https://doi.org/10.3168/jds.S0022-0302(90)78880-7
2. *Preetha S., Boopathy R.* Influence of culture conditions on the production of milk-clotting enzyme from Rhizomucor. *World Journal of Microbiology & Biotechnology*, 1994, 10, 527–530.
3. *Emmanuel V. Pontual Belany, E. Carvalho Ranilson, S. Ranilson, C. Bezerra, Luana, B. B. Coelho, Thiago H. Napoleão, Patrícia M. G. Paiva*. Caseinolytic and milk-clotting activities from Moringaoleifera flowers. *Food Chemistry*, 2012, V. 135. No. 6, 1848–1854. https://doi.org/10.1016/j.foodchem.2012.06.087
4. *Lebedeva G. V., Proskuryakov M. T.* Purification and characterization of milkclotting enzymes from oyster mushroom (*Pleurotus ostreatus* (Fr.) Kumm). *Applied Biochemistry and Microbiology*. 2009, 45(6), 623–625.
5. *D'jakonova G. V.* The study of some physico-chemical properties of milk-clotting enzymes of oyster mushroom. 03.01.04 VAK RF, *Kazan state University, Rostov-on-don*. 2010, P 44 . (In Russian).
6. *Dahmardeh M., Dahmardeh M., Hossienabadi R., Safarpoor H., Dahmardeh M.* Comparative study on cultivation and yield performance of *Pleurotus ostreatus* (oyster mushroom) grown on different substrates (wheat straw and barley straw) and supplemented at various levels of spawn. *J. Food Agri. Environ*. 2010,

8(3/4), part 2, 996–998. https://www.researchgate.net/publication/224886012

7. *Sakovich V. V., Zhernosekov D. D.* Selection of optimal nutrient media and conditions of submerged cultivation for the cultivation of oyster mushroom (*Pleurotus ostreatus*). C*urrent issues of science, Nezhin*. 2018. P. 88. (In Russian).
8. *Sakovich V. V., Grusha A .M., Zhernosekov D. D.* Guidelines for obtaining a drug with milk-clotting activity from Pleurotus ostreatus. *Veterinary journal of Belarus*. 2018, P. 63–67. (In Russian).
9. *Sakovich V. V., Zhuk O. N.* The effect of mycelium extract and oyster mushroom culture liquid (Pleurotus ostreatus) on the formation of a cheese bunch. *Scientific potential of youth: XI intern. scient. and practical conf. Pinsk*. 2017, P. 340–342. (In Russian).
10. *Rudakova N. L.* Secreted metalloproteinase *Bacillus intermedius*: obtaining a homogeneous preparation of the enzyme and the study of physico-chemical properties. *Scientific notes of Kazan state University*. 2010, T.40, kn.2, P. 145–154. (In Russian).
11. *Leighton T. J., Doi R. H., Warren R. A. J., Kelln R. A.* The relationship of serine protease activity to RNA polymerase modification and sporulation in Bacillus subtilis. *J. Mol. Bio.* 1973, 76(1, 5), 103–122. https://doi.org/10.1016/0022-2836(73)90083-1
12. *Pyatnitsky N. P., Proskuryakov M. T.* Determination of the activity of chymotrypsin at a speed of milk has been curdled and strained-acetate mixtures. *17th century. Conf. physiologists of the South of Russia. T. 2., Stavropol*. 1969, P. 80. (In Russian).
13. *Laemmli U. K.* Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970, 227 (5259), 680–685. https://doi.org/10.1038/227680a0
14. *Chapman J. R.* . Mass Spectrometry of Pro teins and Peptides, *Humana Press*. 2000, 538.
15. *Gilliland G. L., Oliva M. T., Dill J.* Functional implications of the threedimensional structure of bovine chymosin. *Advances in Experimental Medicine and Biology,* 1991, 306, 273–371.
16. *Bradford M. M.* A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976, 72, 248–254.
17. *Rao M. B., Tanksale A. M., Ghatge M. S., Despande V. V.* Molecular and biotechnological aspects of microbial proteases. Microbiology and Molecular Biology Reviews, 1998, 62, 597–635. PMCID: PMC98927
18. *Wang N., Wang.,. Y, Li G., Guo W., Liu D.*. Expression and characterization of camel chymosinin Pichiapastoris. *Protein Expr. Purif*. 2015, 111, 75-81. https://doi.org/10.1016/j.pep.2015.03.012. Epub 2015 Mar 31.
19. *Kim J. H., Kim Y. S.* Characterization of a metalloenzyme from a wild mushroom, Tricholoma saponaceum. *Biosci. Biotechnol. Biochem. 2001 Feb*;65(2), 56–62. https://doi.org/10.1271/bbb.65.356
20. *Neveen M., Mohamed N., Sayed M., Sandy M., Esraa E., Mahmoud M. Mahmoud, Alaa M. Mahmoud, Ali M. Ebrahim, Rania R. Abdel-kader, Arwa S. Mohamad, Wesam S.* Purification and characterization of Milk -Clotting Enzyme from the edible mushroom Pleurotus albidus. *Research Journal of Pharmaceutical, Biological and Chemical Sciencesю* 2018, 9(5) https://doi.org/10.2174/092986610791498966 ·
21. *Poza M, Sieiro C, Carreira L, Barros-Vel zquez J, Villa T. G.* Production and characterization of the milk-clotting protease of Myxococcusxanthus strain 422. *IndMicrobiolBiotechnol*. 2003, 30(12), 691.
22. *McMahon,D.J., Brown R.J., Richardson G.H., Ernstrom C.A.* Effects of calcium, phosphate and bulk culture media on milk coagulation properties. *J. Dairy Sci.* 1984.67:930. https://doi.org/10.3168/jds.S0022-0302(84)81391-0
23. *He X., Ren F., Guo H., Zhang W., Song X., Gan B*. Purification and properties of a milk-clotting enzyme produced by Bacillus amyloliquefaciens D4. *Korean Journal of Chemical Engineering*, 2011, 28(1), 203–208. https://doi.org/10.1007/s11814-010-0347-8
24. *Kumar Sapkota1, Se-eun Park, Bong-Suk choi, Seung Kim, Hyun-Hwa lee, Chun-Sung Kim, Hong-Sung Chun.* Purification, characterization, and cloning of fibrinolyticmetalloprotease from *Pleurotus ostreatus* mycelia. *Microbiol. biotechnol*. 2007, 17(8), 1271–1283.
25. *Gershkovich A. A., Kibirev V. K.* Chromogenic and fluorogenic peptide substrates of proteolytic enzymes. *Bioorganic chemistry*. 1988. 14 (11), 1461–1488. (In Russian).
26. *Raposo S., Domingos A.* Purification and characterization of milk-clotting aspartic proteinases from Centaureacalcitrapa cell suspension cultures. Process Biochemistry. 2008, 43, 139–144. https://doi.org/10.1016/j.procbio.2007.11.003

МЕТАЛОПРОТЕЇНАЗА З КУЛЬТУРАЛЬНОЇ РІДИНИ *Pleurotus ostreatus*

В. В. Сакович[1], *Є. М. Стогній*[2], *Д. Д. Жерносєков*[1], *А. В. Ребрієв*[2], *Д. С. Корольова*[2], *Р. Ю. Марунич*[2], *В. О. Чернишенко*[2]

[1]Поліський державний університет, Пінськ, Білорусь
[2]Інститут біохімії ім. О.В. Палладіна НАН України, Київ, Україна

E-mail: mrs.valeryia@mail.ru

Метою роботи є виявлення і вивчення фізико-хімічних властивостей ензимного препарату, одержаного з культуральної рідини *Pleurotus ostreatus*.

Фракцію, що містить протеїназу, було отримано з культуральної рідини методом осадження хлоридом натрію з подальшим діалізом і концентруванням. Желатиназну і молокозгортальну активність визначали стандартними методами. Зміст протеїнового компонента фракції визначали за допомогою методів HPLC, електрофорезу за Лемлі та MALDI-TOF аналізу. Протеїназну активність вивчали ензим-електрофорезом. Для з'ясування специфічності дії протеїнази використовували низку хромогенних субстратів: S2238, S236, S2251, S2765, Leu-pNa, Ala-pNa і S2302. Інгібіторний аналіз проводили із застосуванням ЕДТА, бензамідином, ФМСФ, ПХМБ.

Отримана фракція виявляла максимальну протеїназну активність за 45 °C. Максимальну молокозсідальну активність спостерігали при 35 °C. Найвищу молокозгортальну активність показано при pH 5,0 і менше 3,0. Найвища протеїназна активність була при pH 6,0. За допомогою методу HPLC було знайдено основний протеїновий компонент і деякі бічні протеїни. Згідно з результатами електрофорезу основний протеїновий компонент фракції мав молекулярну масу 45 кДа. Ензим-електрофорез проведено з використанням фібриногену як стандартного субстрату. Встановлено, що протеїназна активність фракції присутня в зоні, що відповідала масі 45 кДа. При ідентифікації продуктів трипсинолізу не виявлено гомології з іншими відомими протеїназами. Показано, що протеїназа гідролізує пептидні зв'язки, які утворені карбоксильною групою амінокислот з гідрофобними бічними ланцюгами. Ензим інгібували ЕДТА (IC_{50} = 2,5 мМ). Максимальну активність ензиму з желатином і Leu-pNa спостерігали в присутності 5 мМ хлориду кальцію.

У культуральній рідині *Pleurotus ostreatus* виявлено кальційзалежну металопротеїназу з молекулярною масою 45 кДа. Ензим не мав гомології з іншими відомими проїназами і гідролізував пептидні зв'язки, утворені карбоксильними групами амінокислот з гідрофобними бічними ланцюгами.

Ключові слова: базидіоміцети, протеолітичні ензими, молокозгортальна активність, фізико-хімічні властивості.

МЕТАЛЛОПРОТЕИНАЗА ИЗ КУЛЬТУРАЛЬНОЙ ЖИДКОСТИ *Pleurotus ostreatus*

В. В. Сакович[1], *Е. Н. Стогний*[2], *Д. Д. Жерносеков*[1], *А. В. Ребриев*[2], *Д. С. Королева*[2], *Р. Ю. Марунич*[2], *В. А. Чернышенко*[2]

[1]Полесский государственный университет, Пинск, Беларусь
[2]Институт биохимии им. А.В. Палладина НАН Украины, Киев

E-mail: mrs.valeryia@mail.ru

Цель работы — выявление и изучение физико-химических свойств энзимного препарата, полученного из культуральной жидкости *Pleurotus ostreatus*.

Фракция, содержащая протеиназу, была получена из культуральной жидкости методом осаждения хлоридом натрия с последующим диализом и концентрированием. Желатиназную и молокосвертывающую активность определяли стандартными методами. Состав протеинового компонента фракции определяли с помощью методов ВЭЖХ, электрофореза по Лэммли и MALDI-TOF анализа. Протеиназную активность изучали энзим-электрофорезом. Для выяснения специфичности действия протеиназы использовали ряд хромогенных субстратов: S2238, S236, S2251, S2765, Leu-pNa, Ala-pNa и S2302. Ингибиторный анализ проводили с использованием ЭДТА, бензамидина, ФМСФ, ПХМБ.

Полученная фракция обладала максимальной протеиназной активностью при 45 °C. При этом максимальная молокосвертывающая активность наблюдалась при 35 °C. Самая высокая молокосвертывающая активность была при pH 5,0 и менее 3,0. Самая высокая протеиназная активность была при pH 6,0. С помощью метода HPLC были найдены основной протеиновый компонент и некоторые побочные протеины. Согласно результатам электрофореза основной протеиновый компонент фракции имел молекулярную массу 45 кДа. Был проведен энзим-электрофорез с использованием фибриногена в качестве стандартного субстрата. Установлено, что протеиназная активность фракции присутствовала в зоне, соответствующей 45 кДа. При идентификации продуктов трипсинолиза не обнаружено гомологии с другими известными протеиназами. Показано, что протеиназа гидролизовала пептидные связи, образованные карбоксильной группой аминокислот с гидрофобными боковыми цепями. Энзим ингибировался ЭДТА (IC_{50} = 2,5 мМ). Максимальная активность энзима с желатином и Leu-pNa наблюдалась в присутствии 5 мМ хлорида кальция.

В культуральной жидкости *Pleurotus ostreatus* обнаружена кальцийзависимая металлопротеиназа с молекулярной массой 45 кДа. Энзим не имел гомологии с другими известными протеиназами и гидролизовал пептидные связи, образованные карбоксильными группами аминокислот с гидрофобными боковыми цепями.

Ключевые слова: базидиомицеты, протеолитические энзимы, молокосвертывающая активность, физико-химические свойства.

UDC 579.663 https://doi.org/10.15407/biotech12.06.046

POST-HARVEST TREATMENT OF VEGETABLES WITH EXOMETABOLITES OF *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 AND *Rhodococcus erythropolis* IMV AC-5017 TO EXTEND THEIR SHELF LIFE

T. P. Pirog
B. S. Geichenko
A. O. Zvarych

National University of Food Technologies, Kyiv, Ukraine

E-mail: tapirog@nuft.edu.ua

Received 26.09.2019
Revised 02.12.2019
Accepted 20.12.2019

The aim of the work was to study the possibility of *Nocardia vaccinii* IMV B-7405, *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 supernatants usage with various concentrations of surfactants for post-harvest treatment of vegetables.

N. vaccinii IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 were grown on waste sunflower oil and ethanol. For vegetables treatment, supernatants of the culture broth with surfactant concentration of 0.01–0.5 g/l were used. The concentration of surfactants was determined by the gravimetric method after extraction with Folch mixture. The total number of heterotrophic bacteria and fungi on the surface of vegetables was determined by the Koch method on meat-peptone agar and wort agar, respectively.

It was shown that treatment of broccoli, Brussels sprouts, sweet pepper and tomatoes with *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 supernatants was accompanied by 6–17 and 8–50 times decrease of bacteria and fungi number on their surface, respectively, compared with that on the surface of vegetables washed with tap water. The possibility of double use of the same supernatant for various batches of vegetables washing was established. Non-treated and water-washed vegetables rotted faster than those treated with surfactant-containing supernatants.

N. vaccinii IMV B-7405, *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 exometabolites used for treating vegetables to extend their shelf life have the following advantages in comparison with known microbial surfactants: they exhibit high antimicrobial activity when the surfactant concentration was several times lower and in the form of supernatant, which lets you exclude the expensive stage of isolation and purification of the target product from the technological process. In addition, surfactant-containing supernatants are highly effective in their repeated use.

Key words: storage of vegetables, microbial spoilage, surfactants.

Every year, the number of publications on the prospects of the practical use of non-toxic biodegradable surfactants (SAS) of microbial origin in various industries, agriculture, medicine, environmental protection is increasing [1–6].

One of the new trends in the use of microbial surfactants in agro-industrial complex is the post-harvest treatment of fruits and vegetables to extend their shelf life [1, 7–9], which is due to the antimicrobial and anti-adhesive properties of these microbial synthesis products. According to [10], depending on the region, the loss of fruit and vegetable yields in the world is between 15 and 50%, the main cause of such significant losses is microbial spoilage.

Nowadays physical and chemical methods are used for the treatment of fruits and vegetables during transportation and storage, but their side effects, high energy costs and ecological incompatibility [10]

have led scientists to find safe alternative methods, in particular biological ones [9–12]. Thus, the natural biopolymer chitosan or its combinations with aromatic oils, organic acids, and metal nanoparticles are used to protect fruits and vegetables during storage [10–11]. In [12] it is noted that bacteriocins nisin, enterocin AS-48, bovicin HC5, enterocin 416K1, pediocin and bificin C6165 are promising for the treatment of both fruits and fruit concentrates, juices, salads to prevent their microbial deterioration; moreover, nisin and pediocin are allowed as a dietary supplements in many countries. Among microbial surfactants, rhamnolipids are permitted for use in the food industry [13].

Earlier [14] we showed that the treatment of vegetables (squashes, cucumbers, tomatoes) with *N. vaccinii* IMV B-7405 surfactant solutions was more effective than washing with water. In our studies [14], surfactant solutions extracted from the supernatant of the culture liquid with organic solvents were used, which are described in the literature [7–9] as preparations; and such surfactant solutions were used for washing only once, and then were poured out.

In [15–16], we found that surfactants synthesized by *Acinetobacter calcoaceticus* IMV B-7241 and *Rhodococcus erythropolis* IMV Ac-5017 were characterized by high antimicrobial and anti-adhesive activity; and antimicrobial activity was shown not only by surfactant solutions, but also by the corresponding supernatants. It should be noted that after surfactant extraction from supernatants with the Folch mixture, the following lost their surfactant properties, and the aqueous phase remaining after surfactants removal was not characterized by antimicrobial activity. These data may indicate that the major exometabolites with antimicrobial properties in the supernatants of these strains are the surfactants themselves.

In connection with the above, the purpose of the study was to investigate the possibility of use the *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 surfactant-containing supernatants with different concentrations of surfactants for post-harvest treatment of vegetables.

Materials and Methods

Objects of research. The strains identified by us as *Nocardia vaccinii* K-8, *Acinetobacter calcoaceticus* K-4 and *Rhodococcus erythropolis* EC-1 [17] isolated from oil contaminated soil samples were the main objects of study. Strains were registered at the Depository of microorganisms, Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine as *N. vaccinii* IMV B-7405, *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017.

By the chemical nature, *R. erythropolis* IMV Ac-5017 surfactants are a complex of glyco- (trehalose mono- and dimycolates), neutral and phospholipids. Glyco- (trehalose mono- and dimycolates, trehalose mono- and diacelates) and aminolipids are contained in the surfactants of *A. calcoaceticus* IMV B-7241. *N. vaccinii* IMV B-7405 synthesizes a complex of neutral, glyco- and aminolipids. Neutral lipids are represented by mycolic and n-alkanoic acids; glycolipids are represented trehalose diacelates and trehalose mycolates [18].

Cultivation of surfactant producers. *N. vaccinii* IMV B-7405 was grown in the liquid mineral medium of the following composition (g/l): $NaNO_3$ — 0,5; $MgSO_4 \cdot 7H_2O$ — 0,1; $CaCl_2 \cdot 2H_2O$ — 0,1; KH_2PO_4 — 0,1; $FeSO_4 \cdot 7H_2O$ — 0,001; distilled water — up to 1 l, pH 6.8–7.0. Yeast autolysate was added to the medium — 0.5% (v/v). Sunflower oil after potato frying was used as a carbon source (McDonald's fast food restaurant, Kyiv) at a concentration of 2% (v/v).

The cultivation of *R. erythropolis* IMB Ac-5017 was carried out in the medium of the following composition (g/l): $NaNO_3$ — 1,3; $MgSO_4 \cdot 7H_2O$ — 0,1; NaCl — 0,1; Na_2HPO_4 — 0,16; KH_2PO_4 — 0,14; $CaCl_2$ — 0,1; $FeSO_4 \cdot 7H_2O$ — 0,001; distilled water — up to 1 l, pH 6.8-7.0. Ethanol in a concentration of 2% (v/v) was used as the substrate.

For the cultivation of *A. calcoaceticus* IMV B-7241 the following medium was used (g/l): $(NH_2)_2CO$ — 0,35, NaCl — 1,0, $Na_2HPO_4 \cdot 12H_2O$ — 0,6, KH_2PO_4 — 0,14, $MgSO_4 \cdot 7H_2O$ — 0,1; $CaCl_2$ — 0,1; distilled water — up to 1 l, pH 6.8-7.0. Yeast autolysate was added to the medium — 0.5% (v/v) and trace element solution — 0.1% (v/v) containing (g/100 ml): $ZnSO_4 \cdot 7H_2O$ — 1,1; $MnSO_4 \cdot H_2O$ — 0,6; $FeSO_4 \cdot 7H_2O$ — 0,1; $CuSO_4 \cdot 5H_2O$ — 0,004; $CoSO_4 \cdot 7H_2O$ — 0,03; H_3BO_3 — 0,006; KI — 0,0001; EDTA (Trilon B) — 0.5. Ethanol in a concentration of 2% (v/v) was used as a carbon and energy source.

The cultures of exponential growth phase grown on media of the above composition with 0.5% of the corresponding substrate were used as the inoculum. The amount of inoculum (10^4–10^5 cells/ml) was 10% of the volume of

medium. The cultivation of the strains was carried out in 750 ml flasks with 100 ml of medium on a rocker (320 rpm) at 30 °C for 120 h.

Determination of surfactants concentration. The amount of surfactants synthesized was determined by gravimetric method after extraction from culture liquid supernatant with Folch mixture (chloroform and methanol, 2: 1) as described in our previous work [18].

Obtaining of surfactant-containing supernatants. Supernatants obtained by centrifugation of the post-fermentation culture liquid for 25 min (5 000 g) were used as the preparations for vegetable treatment. The supernatants were sterilized for 30 min at 112 °C. Supernatants with a surfactants concentration of 0.01–0.5 g/l were used for the processing of vegetables. To achieve the desired concentration, the supernatants were diluted with sterile tap water.

Selection and processing of vegetables. Green tomatoes (Malachite casket), broccoli (Spring), Brussels sprouts (Long Island), and sweet peppers (Kolobok) were grown in open soil, without pesticides (Gvozdiv, Kyiv region, GPS 50°14′53.5′′N 30°28′41.3′′E). Ripe vegetables with no visible damage and infections were selected.

Vegetables were divided into three groups: the first group (control) was not undergo any treatment, the second one was treated with sterile tap water, and the third one — with surfactant-containing supernatants with different surfactant concentrations. For treatment with water or supernatant, the vegetables were placed in a 500 ml graduated cylindrical beaker, 250 ml of water (supernatant) were added, kept for 5 min, and then water (supernatant) was poured out [19]. In one variant, the surfactant-containing supernatant was not poured out after washing the vegetables, but used again to process a new batch of not yet washed vegetables, then again it was not poured out, and used a third time to wash the next batch of vegetables. When presenting the material, such variants of consecutive application of the same supernatant three times for washing three different batches of vegetables will be referred to as once, double and thrice use of the preparation.

Untreated and washed with water and supernatant, the vegetables were placed in Petri dishes and left for observation at room temperature. Microbiological analysis was performed before vegetables storage.

Microbiological analysis. For microbiological control, several vegetables from each group were collected with sterile tweezers, homogenized for 3 min on a T 10 basic ULTRA-TURRAX device, after which 1 g of homogenate was introduced into a test tube with 9 ml of sterile tap water and stirred. The number of microorganisms (colony forming units, CFU/ml) was determined by Koch' method on meat-peptone agar (MPA) for the detection of heterotrophic bacteria, and on wort-agar (WA) for the detection of fungi. Petri dishes with MPA were incubated for 24 h at 37 °C, with WA — for 48 h at 30 °C.

Vegetables quality assessment. Assessment of the quality of vegetables was carried out visually during the shelf life. The end of the experiment was considered the day when all the fruits were marked with spoilage signs: rot, discoloration and consistency change, cracks and wrinkles.

Statistical data processing. All experiments were performed in 3 replicates, the number of parallel determinations in the experiments was 3-5. Statistical processing of the experimental data was performed as described previously [18]. Mean indices differences were considered significant at the significance level $P < 0.05$.

Results and Discussion

Broccoli is a cruciferous vegetable characterized by high nutritional value, primarily due to high content of antioxidant compounds, vitamins and anticarcinogenic substances [20]. Peculiarities of broccoli harvesting are that ripe inflorescences before transportation and sale are cut off, so this product is especially vulnerable to microbial deterioration as well as phytopathogenic microorganisms and saprophytic microbiota that can get into plant tissues through the cut. Nowadays, the widespread method of post-harvest broccoli treatment is washing with an aqueous solution of sodium hypochlorite (50–150 mg/ml), which, although it has an antimicrobial effect, is dangerous to the environment and humans [21].

In this regard, other variants for the treatment of this vegetable are investigated: washing with water acidified due to electrolysis, washing with calcium chloride solution, UV irradiation, ozone treatment, the use of edible films [20, 22].

Table 1 shows the data about the effect of *R. erythropolis* IMV Ac-5017 supernatant with different surfactants concentration on the total number of microorganisms on the surface of broccoli.

Table 1. **The influence of the treatment method and surfactants concentration in *R. erythropolis* IMV Ac-5017 supernatant on the total number of heterotrophic bacteria and fungi on the surface of broccoli**

Processing variant	**Cell number, 10^2 CFU/ml**	
	Bacteria	Fungi
Without treatment	350±28	1880±120
Water	260±19*	1200±80**
Surfactants (0.01 g / l)	63±8*	108±11**
Surfactants (0.05 g / l)	15±4*	22±4**
Surfactants (0.25 g / l)	30±6*	43±7**

Notes: * — P <0.05 — regarding control (number of heterotrophic bacteria cells on the surface of untreated vegetables); ** — P <0.05 — regarding control (number of fungal cells on the surface of untreated vegetables).

These data indicate that in the case of broccoli treatment with *R. erythropolis* IMV Ac-5017 supernatant with a surfactant concentration of 0.01–0.25 g/l, the number of bacteria and fungi on their surface decreased by two to three orders of magnitude compared to those established for untreated and water-washed vegetables. The maximal decrease in the number of microorganisms was observed for use of supernatant with 0.05 g/l surfactant concentration for broccoli washing.

Note that we could not find any publication in the literature regarding the using microbial surfactants for broccoli treatment to extend their shelf life, although other treatments are available. Thus, in the case of ultraviolet radiation action (6 kJ/m^2) on freshly cut inflorescences, the total bacteria number on the vegetable surface was reduced 16-fold [21], while the treatment with *R. erythropolis* IMV Ac-5017 supernatant with 0.05 g/l surfactants concentration — 23 times, which is more effective than the action of UV rays and traditional disinfectant — sodium hypochlorite solution [21].

Brussels sprouts are another vegetable (except broccoli) with a fine tissue structure and a developed surface that makes them susceptible to microbial spoilage.

Subsequent experiments showed that the treatment of Brussels sprouts with *A. calcoaceticus* IMV B-7241 supernatant with a surfactant concentration of 0.25 and 0.5 g/l was accompanied by a 8-fold decrease in bacteria number, and fungi number — by a 6–9-fold decrease compared with untreated vegetables. It should be noted that, unlike *R. erythropolis* IMV Ac-5017 supernatant, a decrease of surfactant concentration in *A. calcoaceticus* IMV B-7241 supernatant to 0.01–0.05 g/l did not increase its efficiency. Vegetables treated with exometabolites of *A. calcoaceticus* IMV B-7241 did not show any visible signs of deterioration for 21 days, while the untreated ones showed the first signs of rot after 10–12 days of storage.

The next step explored the possibility of three consecutive use of the surfactant-containing supernatant of *A. calcoaceticus* IMV B-7241 for the treatment of three different batches of Brussels sprouts to reduce the bacteria number on their surface. In these studies, the number of fungi was not determined, since Brussels sprouts are insensitive to post-harvest spoilage caused by fungi [https://www.ethylenecontrol.com]. Regardless of the surfactant concentration (0.25 and 0.5 g/l), after once and double use of the supernatant, the bacteria number on the surface of the vegetables was found to be practically the same (63–75 CFU/ml) and almost 8 times lower than after washing with water (Table 2). In the case of the supernatant being used three times, its effectiveness as an antimicrobial agent was slightly reduced; however, the bacteria treatment on the surface of the vegetables was almost 4 times lower than after washing with water (127–130 and 500 CFU/ml, respectively).

Further experiments showed the possibility of repeated use of the *A. calcoaceticus* IMV B-7241 supernatant not only for the treatment of Brussels sprouts, but also for sweet peppers (Table 2 and Fig. 1).

Thus, the number of bacteria on the surface of peppers after one and two times of supernatant use with a surfactant concentration of 0.5 g/l was 110–132 CFU/ml and was 5–6 times lower than the number of bacteria on the surface of washed with water vegetables (Table 2). Two-fold reduction of the surfactant concentration in the supernatant (up to 0.25 g/l) was accompanied by some decrease in its efficiency. However, regardless

Table 2. **The number of heterotrophic bacteria on Brussels sprouts and sweet pepper surface depending on the multiplicity of *A. calcoaceticus* IMB B-7241 supernatant use**

Surfactants concentration in the supernatant, g/l	The multiplicity of supernatant use	The number of bacterial cells (10^2 CFU/ml) on the surface of	
		Brussels sprouts	Sweet peppers
0.25	once	63±8*	170±15**
	twice	75±9*	190±17**
	thrice	127±12*	230±19**
0.5	once	65±7*	110±11**
	twice	72±8*	132±12**
	thrice	130±12*	200±17**
Control (single wash with water)		500±35	640±40

Notes: * — P <0.05 — regarding control (number of bacterial cells on the surface washed with water brussels sprouts); ** — P <0.05 — regarding control (number of bacterial cells on the surface washed with water pepper).

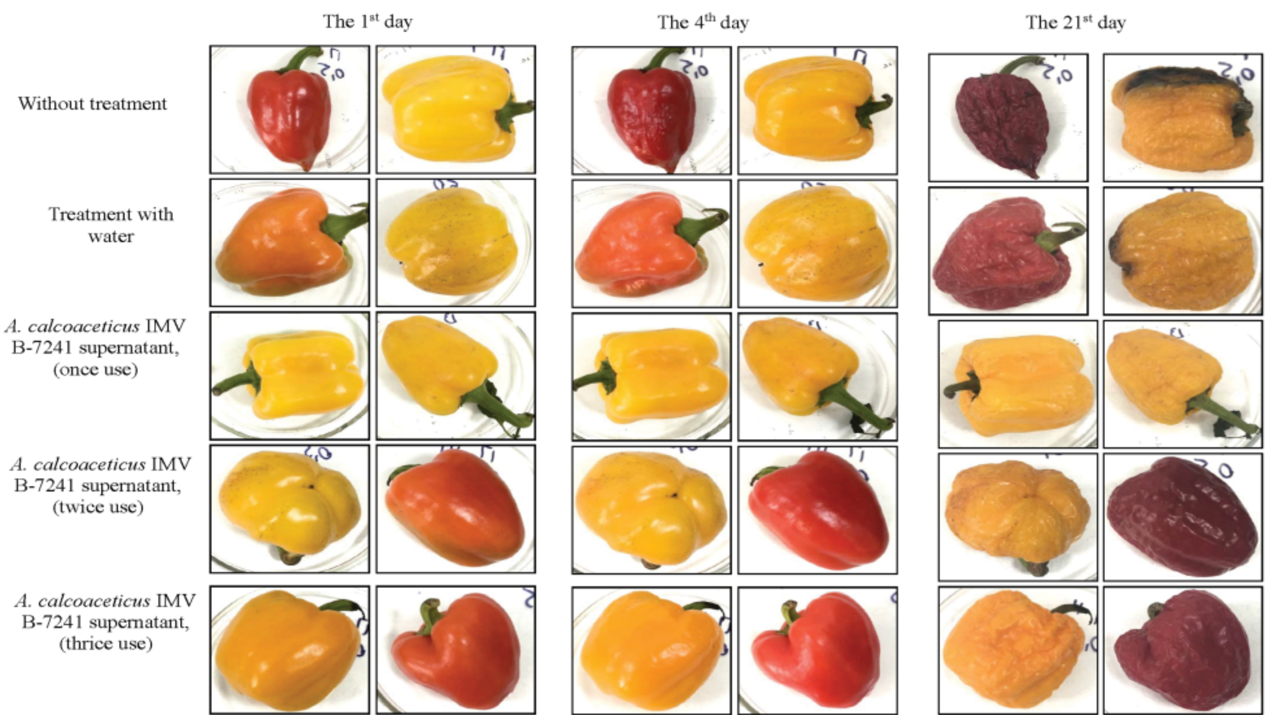

Fig. 1. **The influence of different processing methods on the storage of sweet peppers**

of the surfactant concentration in the supernatant, even after its threefold use, the bacteria number on the surface of the peppers was 3 times lower than after washing the vegetables with water (Table 2). It should be noted that the number of fungi on the surface of sweet pepper in all variants of treatment was 4–5 times lower than bacteria number (data in Table 2 are not shown).

Visual observation of peppers during their storage after treatment with *A. calcoaceticus* IMV B-7241 supernatant showed that even on the 21st day no signs of their spoilage were detected, unlike untreated and water-washed vegetables (Fig. 1).

In [14], we found that the maximum reduction of bacteria and fungi on the surface of tomatoes was achieved with *N. vaccinii* IMV B-7405 surfactants use at a concentration of

Table 3. **The effect of the multiplicity of the use of *N. vaccinii* IMV B-7405 supernatant for the treatment of sweet peppers and tomatoes on the number of microorganisms on the surface of vegetables**

The multiplicity of supernatant use	The number of bacterial cells (10^2 CFU/ml) on the surface of		The number of fungi cells (10^2 CFU/ml) on the surface of	
	pepper	tomatoes	pepper	tomatoes
once	59±8*	13±3**	10±3#	2±1##
twice	83±9*	18±4**	12±3#	2±1##
thrice	190±17*	32±6**	45±7#	3±1##
Control (single wash with water)	520±40	160±15	80±9	17±4

Notes: * — P <0.05 — regarding control (number of bacterial cells on the surface washed with water pepper); ** — P <0.05 —regarding control (number of bacterial cells on the surface of water-washed tomatoes); # — P <0.05 — regarding control (the number of fungal cells on the surface washed with water pepper); ##— P <0.05 — regarding control (number of fungal cells on the surface of water-washed tomatoes); the surfactant concentration in the supernatant is 0.5 g/l.

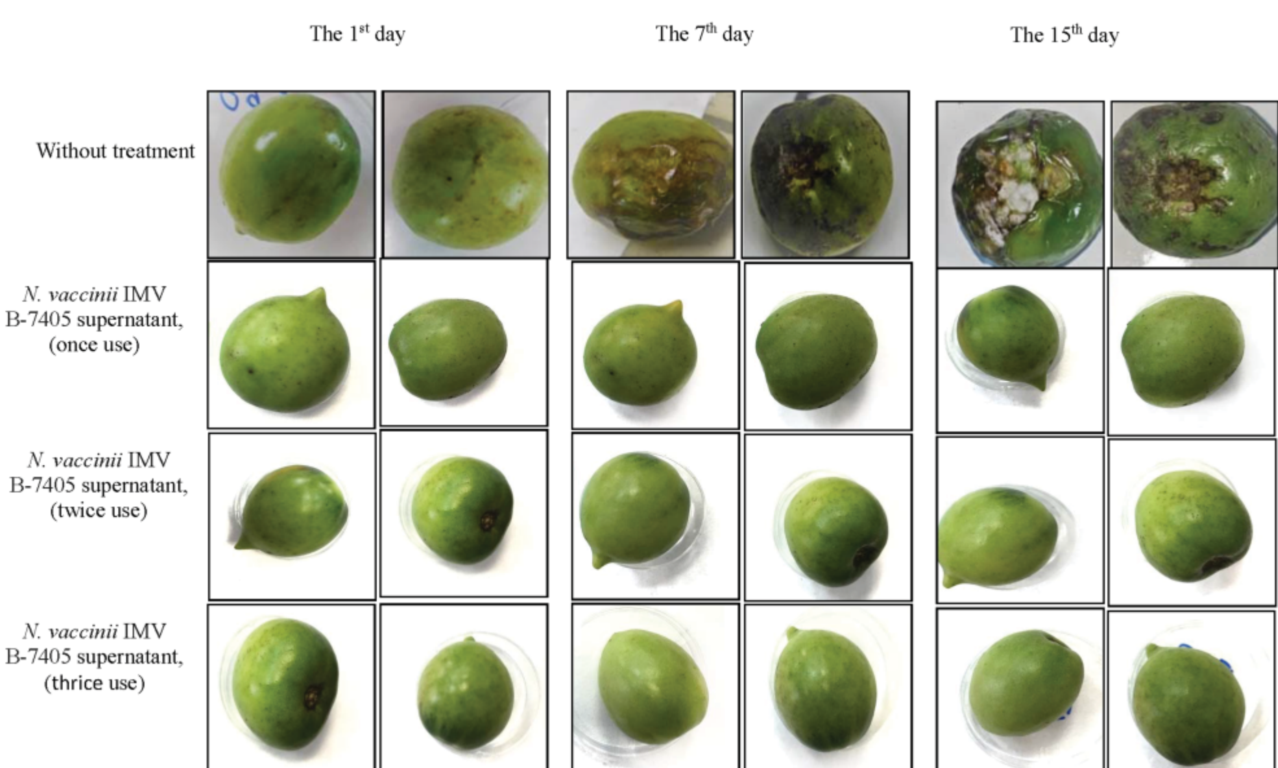

Fig. 2. **The influence of different treatment methods on the storage of tomatoes**

0.5 g/l. In these studies, surfactant solutions extracted from the culture broth supernatant with Folch mixture (chloroform and methanol, 2: 1) were used as surfactant preparations. In the next stage, the possibility of thrice use for the processing of sweet pepper and tomatoes of the *N. vaccinii* IMV B-7405 supernatant with a surfactant concentration of 0.5 g/l was investigated (Table 3, Fig. 2). The results obtained showed the high efficiency of such surfactant preparation for the treatment of these vegetables. Thus, after using the supernatant twice, the number of bacteria on the surface of pepper and tomatoes was 6.3 and 8.9, respectively, and the fungi — 6.7 and 8.5 times lower than after washing vegetables with water. In the case of the supernatant was used three times, the number of microorganisms on the surface of the vegetables was slightly higher than after one and two times of

application, but lower than after washing with water (Table 3).

Visual observation of the tomatoes during their storage after treatment with *N. vaccinii* IMV B-7405 supernatant showed that even on the 15th day no signs of their rotting were detected, unlike untreated vegetables (Fig. 2).

Analysis of the literature showed that publications on the use of surfactants of microbial origin for post-harvest treatment of fruits and vegetables can be divided into three groups.

Articles of the first group [23–27] are devoted to the application for the post-harvest biocontrol of phytopathogens of biological products number based on the microorganisms– antagonists of certain pathogens that affect certain fruits and vegetables. The influence of microbial surfactants in this case is indirect and is considered as one of the possible mechanisms of antagonism, in particular, for preparations based on the biomass of bacteria of *Bacillus* and *Pseudomonas* genus [23–27]. Thus, in [23] it was found that the effect of pre-infected with pathogen *Penicilium digitatum* mandarin treatment with a suspension of *Bacillus subtilis* ABS-S14 endospores, a solution of lipopeptides isolated from the supernatant (10 g/l) and solutions of purified individual surfactants (fengicin and iturin in a concentration of 1 g/l) was almost the same (inhibition of infection development by 60–70% after 5–7 days). The authors of [24] have shown that in the case of spraying of phytopathogenic fungi-affected oranges, apples, grapes and drupaceous fruits with *Bacillus amyloliquefaciens* BUZ-14 supernatant, a faster inhibition of infection compared to the treatment with vegetative or spore cells suspension was observed. The fact is interesting that the antifungal activity of the supernatant against various phytopathogenic fungi depended on the duration of strain BUZ-14 cultivation. The authors explain this phenomenon by the fact that during the cultivation of *B. amyloliquefaciens* BUZ-14 there is a qualitative and quantitative change in the ratio of components in the synthesized antimicrobial complex.

In the publications of the second group [7–8, 28], the compositions containing purified microbial surfactants (mainly rhamno- and sophorolipids) and other components were used as preparations for post-harvest treatment of fruits and vegetables. Thus, treatment of cherry tomatoes with rhamnolipids solution (0.5 g/l) and a suspension of yeast *Rhodotorula glutinis* ($1\cdot10^8$ cells/ml) allowed the infection rate of these vegetables reducing with the agent *Alternaria alternata* by 60% [8]. The authors argue that a solution containing only rhamnolipids proved to be ineffective. In [7] it was reported that in the treatment of tomatoes and cucumbers with germicidal (bactericidal) composition (2.5% of *Candida bombicola* ATCC 22214 sophorolipid in combination with sodium silicate, sodium carbonate and polyethylene glycol) there were not visible signs of microbial spoilage the vegetables for 7 days. It should be noted that the first germicidal composition based on microbial sophorolipids was patented in 1998 [28]. In addition to surfactants (instead of sophorolipids, it may be sodium lauryl sulfate, or a mixture of microbial and chemical surfactants) it contained a mixture of organic acids (citric, glycolic, lactic, malic, tartaric).This composition provided 100% inhibition of *Escherichia coli* bacteria, as well as representatives of the genera *Salmonella* and *Shigella* on the surface of fruits and vegetables [28].

The third group of publications [9, 29–30], to which our research also relates [14], concerns the use of only surfactant solutions for the processing of fruit and vegetable products, without any other ancillary components. Thus, in the patent [29] it is proposed, to prolong the shelf life of citrus fruits, apples, pears, apricots, to spray them with a solution of purified sophorolipids with a concentration of 3 g/l (producer is *Wickerhamiella domercqiae* Y2A). The authors of [9] found that among three studied microbial surfactants (producers are *B. subtilis* 10T, *B. subtilis* 3285 and *Pseudomonas* sp.), only *Pseudomonas* sp. rhamnolipid proved to be effective against *Aspergillus oryzae* MTCC 1846, *Fusarium solani* MTCC 350 and *Curvularia* sp., which cause damage to lemons, potatoes and tomatoes, respectively. In the case of treatment of pre-infected with these pathogens fruits and vegetables with solutions of rhamnolipid at a concentration of 1 g/l, no signs of microbial spoilage during 15 days of storage at room temperature were observed, while the first signs of decay of untreated fruits were appeared as early as in 6–7 days [9]. In [30] found that previously infected with *Botrytis cinerea* grape, tomato and strawberry fruits treatment with a

solution of lipopeptide (8 g/l) synthesized by *Bacillus methylotrophicus* XT1 CECT 8661, was accompanied by an inhibition of infection by 70–100% in 6 days.

The data and results obtained by us earlier show that both surfactant solutions [14] and surfactant-containing *N. vaccinii* IMV B-7405, *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 supernatants used for treatment vegetables to extend their shelf life have the following advantages over microbial surfactants described in the literature [9, 29, 30]: exhibit high antimicrobial activity at much lower surfactant concentrations (0.01–0.5 g/l) and in the form of a supernatant, which lets us to exclude the expensive stage of extraction and purification of the final product from the technological process. In addition, surfactant-containing supernatants are highly effective when reused. It should be noted that at present there is no such information in the literature.

The research was carried out within the framework of the project of the Ministry of Education and Science of Ukraine "Complex microbial preparations of multifunctional purpose: from regulation of biosynthesis and properties to perspectives of practical use" (2019–2021), State registration number GDR: 0119U002575.

REFERENCES

1. *Harjot P. K., Bhairav P., Sukhvir K.* A review on application of biosurfactants produced from unconventional inexpensive wastes in food and agriculture industry. *World J. Pharm. Res.* 2015, 4(8), 827–842.
2. *Mnif I., Ghribi D.* Glycolipid biosurfactants: main properties and potential applications in agriculture and food industry. *J. Sci. Food Agric.* 2016, 96(13), 4310–4320. https/doi.org/10.1002/jsfa.7759
3. *Vecino X., Cruz J. M., Moldes A. B., Rodrigues L. R.* Biosurfactants in cosmetic formulations: trends and challenges. *Crit. Rev. Biotechnol.* 2017, 37(7), 911–923. https/doi.org/10.1080/07388551.2016.1269053
4. *De Graeve M., De Maeseneire S.L., Roelants S. L. K. W., Soetaert W. Starmerella bombicola*, an industrially relevant, yet fundamentally underexplored yeast. *FEMS Yeast Res.* 2018, 18(7). https/doi.org/10.1093/femsyr/foy072
5. *Jimoh A. A., Lin J.* Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicol. Environ. Saf.* 2019, 184:109607. https/doi.org/10.1016/j.ecoenv.2019.109607.
6. *Naughton P. J., Marchant R., Naughton V., Banat I. M.* Microbial biosurfactants: current trends and applications in agricultural and biomedical industries. *J. Appl. Microbiol.* 2019, 127(1), 12–28. https/doi.org/10.1111/jam.14243.
7. *Dengle-Pulate R., Joshi J., Bhagwat S., Prabhune A.* Application of sophorolipids synthesized using lauryl alcohol as a germicide and fruit-vegetable wash. *World J. Pharm. Res.* 2015, 3(7), 1630–1643.
8. *Yan F., Xu S., Chen Y., Zheng X.* Effect of rhamnolipids on *Rhodotorula glutinis* biocontrol of *Alternaria alternata* infection in cherry tomato fruit. *Postharvest Biol. Technol.* 2014, 97, 32–35. https://doi.org/10.1016/j.postharvbio.2014.05.017.
9. *Sharma V., Garg M., Devismita T., Thakur P., Henkel M., Kumar G.* Preservation of microbial spoilage of food by biosurfactant-based coating. *Asian J. Pharm. Clin. Res.* 2018, 11(2), 98–101.
10. *Romanazzi G., Feliziani E., Baños S. B., Sivakumar D.* Shelf life extension of fresh fruit and vegetables by chitosan treatment. *Crit. Rev. Food Sci. Nutr.* 2017, 57(3). 579–601.
11. *Romanazzi G., Feliziani E., Sivakumar D.* Chitosan, a biopolymer with thrice action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties. *Front. Microbiol.* 2018, 9:2745. https/doi.org/10.3389/fmicb.2018.02745.
12. *Barbosa A. A. T., Mantovani H. C., Jain S.* Bacteriocins from lactic acid bacteria and their potential in the preservation of fruit products. *Crit. Rev. Biotechnol.* 2017, 37(7), 852–864. https/doi.org/10.1080/07388551.2016.1262323.
13. *Sinumvayo J. P., Ishimwe N.* Agriculture and food applications of rhamnolipids and its production by *Pseudomonas aeruginosa*. *J. Chem. Eng. Process. Technol.* 2015, 6(2), 223. https/doi.org/10.4172/2157-7048.1000223.
14. *Pirog T., Beregova K., Geichenko B., Stabnikov V.* Application of surface-active substances produced by *Nocardia vaccinii* IMV B-7405 for the treatment of vegetables. *Ukrainian food journal.* 2019, 8(1), 99–109. https/doi.org/10.24263/2304-974X-2019-8-1-11.
15. *Pirog T. P., Konon A. D., Sofilkanich A. P., Iutinskaia G. A.* Effect of surface-active substances of *Acinetobacter calcoaceticus* IMV B-7241, *Rhodococcus erythropolis* IMV

Ac-5017, and *Nocardia vaccinii* K-8 on phytopathogenic bacteria. *Appl. Biochem. Microbiol.* 2013, 49(4), 360–367. https/doi.org/10.1134/S000368381304011X.

16. *Pirog T. P., Lutsay D. A., Kliuchka L. V., Beregova K. A.* Antimicrobial activity of surfactants of microbial origin. *Biotechnologia Acta.* 2019, 12(1), 39–57. https://doi.org/10.15407/biotech12.01.039.
17. *Pirog T. P., Shevchuk T. A., Voloshina I. N., Grechirchak N. N.* Use of claydite-immobilized oiloxidizing microbial cells for purification of water from oil. *Appl. Biochem. Microbiol.* 2005, 41 (1), 51–55. https://doi.org/10.1007/s10438-005-0010-z.
18. *Pirog T., Sofilkanych A., Konon A., Shevchuk T., Ivanov S.* Intensification of surfactants' synthesis by *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* K-8 on fried oil and glycerol containing medium. *Food. Bioprod. Proces.* 2013, 91 (2), 149–157. https://doi.org/10.1016/j.fbp.2013.01.001.
19. *Dilarri G., da Silva V.L., Pecora H. B., Montagnolli R. N., Corso C. R., Biddia E. D.* Electrolytic treatment and biosurfactants applied to the conservation of *Eugenia unflora* fruit. *Food Sci. Technol.* 2016, 36 (3). http://dx.doi.org/10.1590/1678-457X.00516.
20. *Shi J., Gao L., Zuo J., Wang Q., Wang Q., Fan L.* Exogenous sodium nitroprusside treatment of broccoli florets extends shelf life, enhances antioxidant enzyme activity, and inhibits chlorophyll-degradation. *Postharv. Biol. Technol.* 2016, 116, 98–104. https://doi.org/10.1016/j.postharvbio.2016.01.007.
21. *Martínez-Hernández B.G., Artés-Hernández F., Gómez P.A., Formica A.C., Arles F.* Combination of electrolysed water, UV-C and superatmospheric O_2 packaging for improving fresh-cut broccoli quality. *Postharv. Biol. Technol.* 2013, 76, 125–134. https://doi.org/10.1016/j.ifset.2013.11.004.
22. *Ben-Fadhel Y., Ziane N., Salmieri S., Lacroix M.* Combined post-harvest treatments for improving quality and extending shelf-life of minimally processed broccoli florets (Brassica oleracea var. italica). *Food Bioprocess. Technol.* 2017, 11(1), 84–95. https://doi.org/10.1007/s11947-017-1992-2.
23. *Waewthongrak W., Pisuchpen S., Leelasuphakul W.* Effect of *Bacillus subtilis* and chitosan applications on green mold (*Penicilium digitatum Sacc.*) decay in citrus fruit. *Postharv. Biol. Technol.* 2015, 99, 44–49. https/doi.org/10.1016/j.postharvbio.2014.07.016.
24. *Calvo H., Marco P., Blanco D., Oria R., Venturini M. E.* Potential of a new strain of *Bacillus amyloliquefaciens* BUZ-14 as a biocontrol agent of postharvest fruit diseases. *Food Microbiol.* 2017, 63, 101–110. https/doi.org/10.1016/j.fm.2016.11.004.
25. *Zhang B., Li Y., Zhang Y., Qiao H., He J., Yuan Q., Chen X., Fan J.* High-cell-density culture enhances the antimicrobial and freshness effects of *Bacillus subtilis* S1702 on table grapes (*Vitis vinifera* cv. *Kyoho*). *Food Chem.* 2019, 286, 541–549. https/doi.org/10.1016/j.foodchem.2019.02.050.
26. *Dukare A. S., Paul S., Nambi V. E., Gupta R. K., Singh R., Sharma K., Vishwakarma R. K.* Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. *Crit. Rev. Food Sci. Nutr.* 2019, 59(9), 1498–1513. https/doi.org/10.1080/10408398.2017.1417235.
27. *Lastochkina O., Seifikalhor M., Aliniaeifard S., Baymiev A., Pusenkova L., Garipova S., Kulabuhova D., Maksimov I.* *Bacillus* spp.: efficient biotic strategy to control postharvest diseases of fruits and vegetables. *Plants* (*Basel*). 2019, 8(4). pii: E97. https/doi.org/10.3390/plants8040097.
28. *Pierce D., Heilman T. J.* Germicidal composition. *World Patent 9816192.* Publ. 23.04.1998.
29. *Jing C., Bingbing Y.* Sophorolipid fruit preservative and use thereof in fruit preservation. *Chinese Patent CN 101886047.* Publ. 17.11.2010.
30. *Toral L., Rodríguez M., Béjar V., Sampedro I.* Antifungal activity of lipopeptides from *Bacillus* XT1 CECT 8661 against *Botrytis cinerea*. *Front. Microbiol.* 2018, 9, 1315. https/doi.org/10.3389/fmicb.2018.01315.

ПІСЛЯВРОЖАЙНА ОБРОБКА ОВОЧІВ ЕКЗОМЕТАБОЛІТАМИ *Nocardia vaccinii* ІМВ В-7405, *Acinetobacter calcoaceticus* ІМВ В-7241 ТА *Rhodococcus erythropolis* ІМВ АС-5017 ДЛЯ ПОДОВЖЕННЯ ТЕРМІНУ ЇХ ЗБЕРІГАННЯ

Т. П. Пирог, Б. С. Гейченко, А. О. Зварич

Національний університет харчових технологій, Київ, Україна

E-mail: tapirog@nuft.edu.ua

Мета роботи — дослідити можливість використання супернатантів *Nocardia vaccinii* ІМВ В-7405, *Acinetobacter calcoaceticus* ІМВ В-7241 та *Rhodococcus erythropolis* ІМВ Ас-5017 з різною концентрацією поверхнево-активних речовин (ПАР) для післяврожайної обробки овочів.

N. vaccinii ІМВ В-7405, *A. calcoaceticus* ІМВ В-7241 та *R. erythropolis* ІМВ Ас-5017 вирощували на відпрацьованій соняшниковій олії та етанолі. Для обробки овочів використовували супернатанти культуральної рідини з концентрацією ПАР 0,01–0,5 г/л. Концентрацію ПАР визначали ваговим методом після екстракції сумішшю Фолча. Загальну чисельність гетеротрофних бактерій і грибів на поверхні овочів визначали за методом Коха на м'ясо-пептонному агарі та сусло-агарі відповідно.

Показано, що обробка броколі, брюсельської капусти, солодкого перцю і томатів супернатантами *N. vaccinii* ІМВ В-7405, *A. calcoaceticus* ІМВ В-7241 та *R. erythropolis* ІМВ Ас-5017 супроводжувалася зниженням чисельності бактерій і грибів на їхній поверхні у 6–17 і 8–50 разів відповідно порівняно з кількістю на поверхні митих водою овочів. Встановлено можливість двократного використання одного й того самого супернатанту для миття різних партій овочів. Необроблені та миті водою овочі швидше піддавалися гниттю порівняно з обробленими ПАР-вмісними супернатантами.

Екзометаболіти *N. vaccinii* ІМВ В-7405, *R. erythropolis* ІМВ Ас-5017 і *A. calcoaceticus* ІМВ В-7241, використовувані для обробки овочів з метою подовження терміну їх зберігання, порівняно з відомими мікробними поверхнево-активними речовинами мають такі переваги: виявляють високу антимікробну активність за нижчих у кілька разів концентрацій ПАР і у вигляді супернатанту, що дає змогу виключити з технологічного процесу високовартісну стадію виділення та очищення цільового продукту. Крім того, ПАР-вмісні супернатанти характеризуються високою ефективністю у разі їх повторного використання.

Ключові слова: зберігання овочів, мікробне псування, поверхнево-активні речовини.

ПОСЛЕУБОРОЧНАЯ ОБРАБОТКА ОВОЩЕЙ ЭКЗОМЕТАБОЛИТАМИ *Nocardia vaccinii* ИМВ В-7405, *Acinetobacter calcoaceticus* ИМВ В-7241 И *Rhodococcus erythropolis* ИМВ АС-5017 ДЛЯ ПРОДЛЕНИЯ СРОКА ИХ ХРАНЕНИЯ

Т. П. Пирог, Б. С. Гейченко, А. О. Зварыч

Национальный университет пищевых технологий, Киев,Украина

E-mail: tapirog@nuft.edu.ua

Цель работы — исследовать возможность использования супернатантов *Nocardia vaccinii* ИМВ В-7405, *Acinetobacter calcoaceticus* ИМВ В-7241 и *Rhodococcus erythropolis* ИМВ Ас-5017 с различной концентрацией поверхностно-активных веществ (ПАВ) для послеуборочной обработки овощей.

N. vaccinii ИМВ В-7405, *A. calcoaceticus* ИМВ В-7241 и *R. erythropolis* ИМВ Ас-5017 выращивали на отработанном подсолнечном масле и этаноле. Для обработки овощей использовали супернатанты культуральной жидкости с концентрацией ПАВ 0,01–0,5 г/л. Концентрацию ПАВ определяли весовым методом после экстракции смесью Фолча. Общую численность гетеротрофных бактерий и грибов на поверхности овощей определяли по методу Коха на мясо-пептонном агаре и сусло-агаре соответственно.

Показано, что обработка брокколи, брюссельской капусты, сладкого перца и томатов супернатантами *N. vaccinii* ИМВ В-7405, *A. calcoaceticus* ИМВ В-7241 и *R. erythropolis* ИМВ Ас-5017 сопровождалась снижением численности бактерий и грибов на их поверхности в 6–17 и 8–50 раз соответственно по сравнению с количеством на поверхности мытых водой овощей. Установлена возможность двукратного использования одного и того же супернатанта для обработки различных партий овощей. Необработанные и мытые водой овощи быстрее подвергались гниению по сравнению с обработанными ПАВ-содержащими супернатантами. Экзометаболиты *N. vaccinii* ИМВ В-7405, *R. erythropolis* ИМВ Ас-5017 и *A. calcoaceticus* ИМВ В-7241, используемые для обработки овощей с целью продления срока их хранения, по сравнению с известными микробными поверхностно-активными веществами имеют следующие преимущества: проявляют высокую антимикробную активность в более низких концентрациях и в виде супернатанта, что позволяет исключить из технологического процесса дорогостоящую стадию выделения и очистки целевого продукта. Кроме того, ПАВ-содержащие супернатанты характеризуются высокой эффективностью при их повторном использовании.

Ключевые слова: хранение овощей, микробная порча, поверхностно-активные вещества.

UDC 577.21+632.78+57.084.1 https://doi.org/10.15407/biotech12.06.056

DEVELOPMENT OF VECTORS FOR *Agrobacterium*-MEDIATED GENETIC TRANSFORMATION OF PLANTS CONTAINING THE SYNTHETIC *CRY1Ab* GENE ENCODING RESISTANCE TO LEPIDOPTERAN PESTS

A. M. Taranenko[1]
I. O. Nitovska[1]
L. H. Velykozhon[1, 2]
P. D. Maystrov[1]
M. V. Kuchuk[1]
B. V. Morgun[1, 2]

[1]Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, Kyiv

[2]Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine, Kyiv

E-mail: molgen@icbge.org.ua

Received 09.08.2019
Revised 17.10.2019
Accepted 30.12.2019

The research was aimed to develop genetic constructs for *Agrobacterium*-mediated plant transformation, containing the synthetic *cry1Ab* gene, and their testing through the transformation of tobacco, followed by a molecular genetic analysis of the obtained plants to confirm the transformation event. Basic methods of DNA cloning, *Agrobacterium*-mediated transformation of *Nicotiana tabacum* L. by leaf disc method, selection of transformants *in vitro*, analysis of the transgene presence in plant DNA, detection of *cry1Ab* gene expression by PCR with reverse transcription were used. In the course of the study, the vectors pCB182 and pCB241 that contained the synthetic gene *cry1Ab* were constructed. *Agrobacterium*-mediated transformation of tobacco was carried out by created vectors and regenerant plants containing transgenes in their DNA were obtained. Expression of *cry1Ab* transgene in the obtained transformants of tobacco by the RT-PCR method was confirmed. As a result of the *Agrobacterium*-mediated transformation of plants with pCB182 and pCB241 vectors containing the synthetic *cry1Ab* lepidopteran resistance gene it is possible to obtain transgenic plants with expression of the transgene.

Key words: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-mediated transformation, PCR-analysis, transgenesis.

Human agricultural activity is constantly faced with obstacles that lead to significant crop losses. One of these natural factors, and perhaps the most important of it, is the impact of insect pests on crop yields. In particular, current estimates of worldwide productivity losses of wheat, rice, corn, potatoes and soybeans associated with the major 137 pathogens and pests are 8–41% [1] national and global levels. Quantitative, standardized information on crop losses is difficult to compile and compare across crops, agroecosystems and regions. Here, we report on an expert-based assessment of crop health, and provide numerical estimates of yield losses on an individual pathogen and pest basis for five major crops globally and in food security hotspots. Our results document losses associated with 137 pathogens and pests associated with wheat, rice, maize, potato and soybean worldwide. Our yield loss (range. Pest control) is an ongoing process. The use of synthetic insecticides does considerable harm not only to the target species but also to the environment, including humans. The influence of chemical poisons leads to the destruction of ecosystems, also their ability to accumulate in the soil calls into question the possibility of rapid restoration of natural balance. In addition, the development of mechanisms

of resistance to chemical insecticides is a significant problem in populations of insect pests. Thus, from 1938 to 1976, the number of insects insensitive to agricultural poisons increased from 7 to 364, doubling since then every 6 years [2]. This is why the question arises about the creation of new methods of pest control, which will be characterized by the biological origin of the active substances and their ability to rapidly biodegrade in the environment, a narrow range of target species, a harmlessness to other living organisms.

The sought-after remedy are Bt-toxins. The natural source of the toxin is the anaerobic chemoorganotrophic bacterium *Bacillus thuringiensis*, which resides in the soil. A feature of the bacterium is the ability to form peculiar inclusions in the cytoplasm in the process of sporulation which are crystals of prototoxin with a molecular weight of 130–145 kDa with an insecticidal effect [3]. These crystals are unique to each strain of *B. thuringiensis*. The differences between them are revealed at the primary level of organization of protein molecules, as a direct realization of genetic information. The features of the secondary structure directly affect the rate of transition of prototoxin from crystalline form to soluble, that is, the biological activity of the toxin. Some strains of *B. thuringiensis* are able to produce 5–9 varieties of prototoxin instead of one. The peculiarities of the amino acid composition and structural organization of the active toxins formed from prototoxin molecules cause their receptor affinity for the cells of the midgut of a very limited range of insect species. This targeted action makes Bt-toxin an ideal remedy against crop pests [4]. At the very beginning of the use of *B. thuringiensis* as an alternative to chemical insecticides, direct spraying of the bacterial material (in sporulated form) was applied on the fields damaged by the pest. In the end, due to the growing acreage, this method was considered economically inappropriate for mass use. In addition, spraying over large areas of an extraneous bacterial agent alters the composition and balance of the soil biota, which in the long run is capable of disrupting local ecosystems [5]. An alternative to spraying of bacterial material in the fields is the transfer of bacterial Bt-toxin genes to the plant genome by genetic transformation methods, followed by the production of genetically modified plants capable of synthesizing and accumulating the toxin on their own, thereby protecting against pests [6–7]. The data accumulated over recent years have made it possible to characterize a large number of new *B. thuringiensis* strains and specific toxin action for a wide range of insect species. The complex use of bacterial material and genetically modified plants has put Bt-products at the forefront. As of 2015, the worldwide use of Bt-biopesticides is around 97% [8].

Genetic constructs containing *cry1Ab* gene are the most often used for the formation of plant resistance against *Lepidoptera* insects [8]. The obtained transgenic varieties have been industrially grown since 1996, and their use is increasing annually. In particular, as of 2017, the global area allocated for the cultivation of biotechnological crops in 2017 is estimated at 189.8 million hectares [9].

The purpose of this study was to create genetic constructs containing the synthetic *cry1Ab* gene adapted for expression in the plant genome for *Agrobacterium*-mediated transformation of plants. The efficiency of the vectors was determined by transformation of the tobacco model object (*Nicotiana tabacum*), followed by molecular genetic analysis of the plant material obtained.

Materials and Methods

Genetic constructs. When developing constructs containing *cry1Ab* gene, binary vectors pICBV16 and pICBV19 (Icon Genetics GmbH, Germany) were used for *Agrobacterium*-mediated plant transformation. They contained the *uidA* β-glucuronidase reporter gene under the control of the 35S promoter, also selective neomycin phosphotransferase II (*nptII*) gene in pICBV16 vector, or phosphinothricinacetyl transferase (*bar*) gene in pICBV19 vector.

Cloning reactions were performed using the InsTAclone™ PCR Cloning Kit (Thermo Fisher Scientific). Purification of DNA fragments for ligase reaction was performed using the Silica Bead™ DNA Gel Extraction Kit (Thermo Fisher Scientific).

By preparative restriction of the pICBV16 construct, *uidA* gene was removed using restriction enzymes NcoI and BamHI. The isolated and purified DNA fragment with *cry1Ab* gene was embedded to the vector pTZ57R. *Escherichia coli* XL-1 Blue strain was transformed using the resulting structure. Preparative restriction of the recombinant DNA obtained was performed using NcoI and BamHI restriction enzymes, with the isolation of a region containing *cry1Ab* gene. Ligation of the obtained insert fragments (the coding sequence of *cry1Ab* gene) and the

linearized vector pICBV16 was performed (the final construct was named PCB241). Preparative restriction of the recombinant DNA obtained was performed using EcoRI and BamHI restriction enzymes, with the isolation of a region containing 35S promoter and *cry1Ab* gene. By preparative restriction of the pICBV19 construct using the EcoRI and BamHI restriction enzymes, *uidA* gene and 35S promoter were removed. Ligation of the obtained insert fragments (the coding sequence of *cry1Ab* gene and 35S promoter) and the linearized vector pICBV19 led to creation of pCB182 construct. Schemes of final structures are shown in Fig. 1.

Recombinant DNA was transferred into *Agrobacterium tumefaciens* GV3101 cells, resulting in bacterial colonies containing genetic constructs pCB182 or pCB241.

Plant material. The leaves of aseptic tobacco plants (*Nicotiana tabacum* L.) of Petit Havana variety grown on MS nutrient medium [10] without phytohormones, at +25 °C and 16-hour daylighting have been used in the experiments.

Agrobacterium-mediated transformation. Agrobacterium-mediated transformation of *N. tabacum in vitro* plants was carried out by leaf disks method [11]. After transformation, leaf explants were transferred to regenerative selective MS medium containing salts, vitamins and carbohydrates, 1 mg/l 6-benzylaminopurine (BAP), 0.1 mg/l 1-naphthylacetic acid (NAA), 500 mg/l cefotaxime (Cx) to inhibit the growth of bacteria, and 100 mg/l kanamycin, or 5 mg/l phosphinothricin as selective agents, depending on the vector used for transformation. The frequency of regeneration (FR) on selective media was estimated by the percentage of the number of explants formed shoots to the total number of explants transferred. The regenerated plants were grown *in vitro* at phytohormone-free MS medium containing 500 mg/l Cx and a suitable selective agent at the indicated concentration.

Isolation of total plant DNA. Isolation of total DNA from plant leaves was performed by the method [12]. DNA was dissolved in 50 µl of TE buffer pH 8.0. The presence of DNA in the solution was confirmed by 0.8% agarose gel electrophoresis in TBE buffer [13].

The concentration of DNA in the solution was determined by spectrophotometric measurement of the level of adsorption of UV-light with a wavelength 260 nm (A_{260}) [14]. The purity of the DNA was evaluated by the ratio A_{260}/A_{280}. Spectrophotometric measurements were performed using a photometer BioPhotometer v. 1.35 (Eppendorf). The DNA content of the solution was brought to a final concentration of 30 µg/ml by adding TE buffer.

PCR conditions. A Mastercycler® Personal amplifier (Eppendorf) was used to conduct the polymer chain reaction (PCR). The reaction mixture (20 µl) contained 13.9 µl of sterile deionized water, 2 µl of 2 mM dNTP, 2 µl of 10x Green Buffer, 0.5 µl of 10 mM forward primer, 0.5 µl of 10 mM reverse primer, 0.1 µl of DreamTaq™ DNA polymerase (Thermo Fisher Scientific). The primers (Metabion, Germany) used in the study are presented in Table 1.

The PCR reaction for detection of *vir* D1 gene sequence was performed in order to exclude from the list of tested samples those who contaminated with agrobacterial DNA. Confirmation of the fact of agrobacterial contamination allows to reject false positive results of the study. Detection of *vir* D1 gene sequence was performed according to the Lipp João protocol [16].

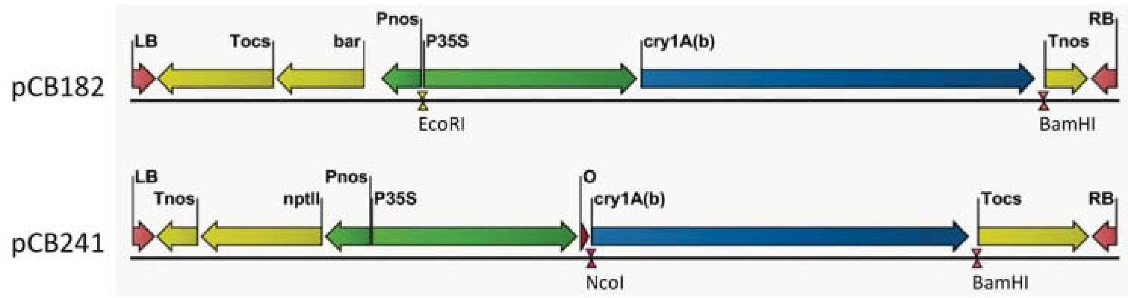

Fig. 1. **Scheme of T-DNA constructs PCB182 and PCB241:**
Tocs — octopinsynthase gene terminator; *Tnos* — nopalinsynthase gene terminator; *bar* – phosphinotricinacetyl transferase gene as selective, the product which confers phosphinothricin resistance; *nptII* — neomycin phosphotransferase II gene as a selective, with product, which confers kanamycin resistance; *Pnos* — nopalinsynthase gene promoter; *P35S* — 35S RNA gene promoter of cauliflower mosaic virus; *OΩ* — omega sequence that enhances the gene expression; *cry1(A)b* — the coding sequence of **cry***1(A)b* gene

Table 1. **The primers used to detect gene sequences**

Primer name	Gene	Sequence	Amplicon length, bp.; reference
Npt1	*nptII*	5′ — GAG GCT ATT CGG CTA TGA CTG — 3′	700; [15]
Npt2		5′ — ATC GGG AGC GGC GAT ACC GTA — 3′	
VirD1-1	*vir D1*	5′ — ATG TCG CAA GGC AGT AAG CCC A — 3′	437; [16]
VirD1-2		5′ — GGA GTC TTT CAG CAT GGA GCA A — 3′	
barpr5	*bar*	5′ — GCG GTC TGC ACC ATC GTC AAC — 3′	551; [17]
rev581		5′ — CAG ATC TCG GTG ACG GGC AGG AC — 3′	
CryO5	*cry1Ab*	5′ — AGG ATT CGC TAC GCT AGC AC — 3′	475
CryO6		5′ — GGA GAT TCC TCT CGT CGC TG — 3′	

Note: bp — base pairs.

The amplification program for the detection of *bar* gene sequence was performed according to Sakhno protocol [17]. Amplification program for detection of *nptII* gene sequence was performed by the TD-PCR method (Touchdown-PCR) [18]. The amplification program for *cry1Ab* gene consisted of: primary denaturation — 4 min at 94 °C; 35 cycles: 30 s at 94 °C, 30 s at 55 °C, 28 s at 72 °C; final elongation — 10 min at 72 °C, with following cooling of reaction mixture to 22 °C.

The amplification products were separated in a 1.2% agarose gel with 0.5 μg/ml ethidium bromide in TBE buffer at an electric field voltage of 6 V/cm during 45 min. The gel image in ultraviolet light was documented on the mLKB Bromma 2011 Macrovue Transilluminator using a Canon EOS 600D digital camera. Electrophoresis images were processed by a GIMP bitmap editor (GNU Image Manipulation Program, www.gimp.org) and a Microsoft PowerPoint text-and-graphic editor.

Reverse transcription. To determine whether transcription of *cry1Ab* gene in the plant genome occurs, total RNA was isolated by the method [19] from the leaf material of rooted regenerants. The cDNA synthesis was initiated by the Oligo dT18 primer (Thermo Fisher Scientific) from mRNA and was performed by Maxima Reverse Transcriptase (Thermo Fisher Scientific) enzyme according to the manufacturer's instructions. The newly synthesized cDNA first strand was analyzed for the presence of *cry1Ab* gene by PCR using the specific CryO5 and CryO6 primers.

Growing transformants in soil. Plants that showed the presence of transgenic sequences in their genome during molecular genetic analysis were transferred to the soil mixture and grown under greenhouse conditions to obtain seed material. The soil mix consisted of peat, sand and turf, in a 2:1:1 ratio. The cultivation was carried out under the conditions of 12-hour light day, at an air temperature of 24 °C.

Results and Discussion

As a result of molecular genetic manipulations, binary vectors pCB241 and pCB182 were constructed. They were containing *cry1Ab* gene under the control of *35S* cauliflower mosaic virus promoter, the selective neomycin phosphotransferase II (*nptII*) gene in case of the vector pCB241, or phosphinothricinacetyl transferase (*bar*) gene for the vector pCB182, under the control of the promoter of bacterial nopalinsynthase gene.

To evaluate the effectiveness of the vectors obtained, the transformation of tobacco was performed using *A. tumefaciens*, which cells contained the binary vector PCB241 or PCB182. As a result of the transformation, the tobacco plants capable to rooting on selective media with 100 mg/l kanamycin or 5 mg/l phosphinothricin were obtained. The beginning of plant regeneration from explants transformed with the pCB241 construct was observed after 5 weeks of cultivation on a selective bacteriostatic medium (Fig. 2), whereas after transformation with the pCB182 vector, plant regeneration occurred after 14 weeks. The percentage of explants that formed plants (regeneration frequency, RF) on a selective medium after transformation with vector pCB182 was significantly lower

Table 2. **Results of Agrobacterium-mediated transformation of *N. tabacum* using vectors pCB182 and pCB241**

Vector	RF,%	Rooting regener-ants, units	Sensitive regener-ants,%	PCR results (+)			
				Analyzed samples, in total	Selective gene, samples	cry1Ab, samples	Selective +cry1Ab, samples
pCB182	22	86	14	54	51	19	19
pCB241	84	123	66	28	25	12	12

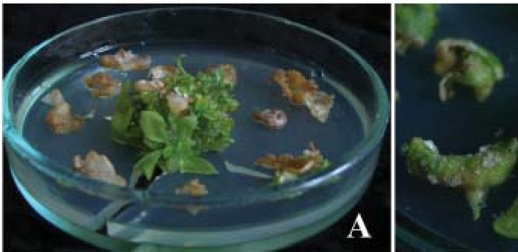

Fig. 2. **Regeneration of tobacco shoots on selective media after *Agrobacterium*-mediated vector t ransformation with pCB182 vector (*A*) or pCB241 (*B*)**

comparing with the transformation using vector pCB241 (Table 2).

After transformation with the pCB241 construct, as well as pCB182, a significant number of regenerants appeared to be sensitive to the selective agent upon further cultivation in banks with selective medium (Table 2). This may be due to a change in transgene expression. Factors that can cause such a phenomenon include: tissue culture, chimerism, transgene integration site (positional effect), copy number (dosing effect), transgenic mutation and epigenetic gene inactivation [20, 21]. Gene silencing, downregulation, or loss of expression can occur at the transcriptional or post-transcriptional level [22]. In the analysis of transgenic lines of rice Chareonpornwattana with co-authors [23] observed that gene silencing correlated more with transgene expression level than with copy number. It has been found that high promoter activity can lead to hypermethylation and, as a consequence, termination of gene transcription in both monocotyledonous and dicotyledonous plant species. The high percentage of plants that showed signs of sensitivity to the selective agent after prolonged cultivation, and the low rate of regeneration on the selective medium observed after transformation with the pCB182 vector, may also be due to the toxic effect of *cry1Ab* gene product on the plant cell, since Cry proteins of GM plants are more solubilized comparing with molecules of the wild type (which are crystalline) and therefore acquire new properties within these plants [24]. In general, up to 23% of plant objects after transformation can show signs of gene silencing [23].

The use of alternative regulatory systems and methods of transformation leads to more stable results. Thus, the adding into the rice genome of *cry1Ab* synthetic gene under the control of corn ubiquitin promoter *Pubi* is characterized by a high (up to 76%) frequency of transformation among the total number of regenerants, stable inheritance [25] and transgene expression [26], a significant level of accumulation of protein product in green parts of plants.

A study by Jabeen [27] demonstrated that the expression levels of *cry1Ab* gene in chloroplasts are significantly higher than those in a nucleus. Thus, the introduction of this transgene into the plastid system is a promising area of work.

DNA of regenerants resistant to selective agents (82 plants in total: 54 plants obtained after transformation with pCB182 construct and 28 plants obtained after transformation with pCB241 vector) was analyzed by PCR. The analysis aimed to identify *bar* gene sequences (for plants transformed with pCB182 construct only), *nptII* (for plants transformed with pCB241 construct only), *cry1Ab*, and *vir* D1 (to control the possibility of agrobacterial contamination).

Among the studied plants, according to the results of PCR analysis to detect the sequence of *vir*D1 gene, only 4 samples showed the presence of the amplicon of the proper size, which may indicate an agrobacterial

contamination of the plant material. The results obtained with these samples were excluded from further research as potentially false positives.

The following PCRs were performed to identify selective genes that are part of the transforming vectors to confirm their transfer to the plant genome: *bar* gene for plants transformed by the pCB182 construct and *nptII* gene for plants transformed by the pCB241 construct.

PCR detection of sequence of *bar* gene in plant DNA obtained after transformation by the pCB182 vector showed that 94.4% of the samples demonstrate the presence of a corresponding 551 bp amplicon. (Table 2, Fig. 3), which indicates the transfer of the gene to the plant genome.

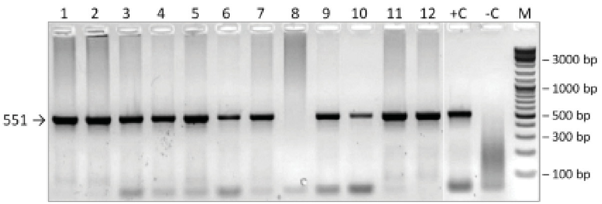

Fig. 3. **Electrophoregram of *bar* gene amplification products from DNA of tobacco regenerants obtained after transformation with pCB182 vector**
Tracks No. 1–7, 9–12 — DNA of *N. tabacum* lines 1-1, 1-2-1, 2-1, 2-2, 1-3-1, 4-1-1, 4-1-2, 2-3, 4-2, 1-4, 1-5; No. 8 — control (DNA of the non-transformed plant *N. tabacum*), +C — positive control (DNA of *N. tabacum* pICBV19); –C — negative control (TE buffer); M – molecular weight marker GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific). Expected amplicon size is 551 bp

Previous attempts to detect the sequence of *nptII* gene using PCR have resulted in a significant number of nonspecific amplicons on electrophoregrams. Therefore, TD-PCR (Touchdown-PCR) was used to achieve a positive result. TD-PCR allows achieving the accumulation of a specific product with a stable composition of the reaction mixture only by optimizing the temperature cycle. The initial primer annealing temperature increases by 10 °C, but gradually decreases in subsequent cycles. Thus, the specificity of the reaction increases [28].

Touchdown-PCR performed to detect the sequence of *nptII* gene in the DNA of plants obtained after transformation by the pCB241 vector showed the presence of the desired 700 bp amplicon in 89.3% of the samples (Table 2, Fig. 4).

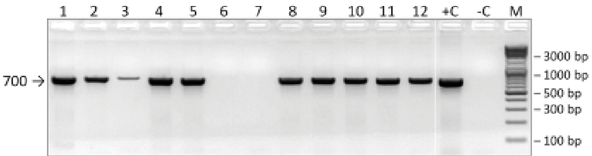

Fig. 4. **Electrophoregram of *nptII* gene amplification products from DNA of tobacco regenerants obtained after transformation with pCB241 vector**
Tracks No. 1–6, 8–12 – DNA of *N. tabacum* lines 24-1, 25-1, 18-1, 12-1, 31-1, 22-1, 38-1, 37-1, 43- 1, 44-1, 46-1; No. 7 – control (DNA of the non-transformed plant *N. tabacum*), +C — positive control (DNA of *N. tabacum* pICBV16); –C — negative control (TE buffer); M — molecular marker GeneRuler ™ DNA Ladder Mix. Expected amplicon size — 700 bp

PCR analysis of the DNA of the analyzed regenerants to detect the sequence of *cry1Ab* gene (Fig. 5) revealed the presence of a corresponding amplicon of 475 bp in length in 35.2% of samples obtained after transformation by vector pCB182 and 42.9% of samples obtained after transformation by vector pCB241. The presence of incomplete T-DNA copies in one or more plant genome sites in transformed plants after *Agrobacterium*-mediated transformation has also been shown by other researchers [29].

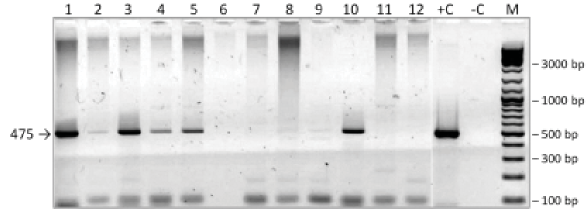

Fig. 5. **Electrophoregram of *cry1Ab* gene amplification products from DNA of tobacco regenerants obtained by transformation with pCB182 construct**
Tracks No. 1–7, 9–12 – DNA of regenerants of *N. tabacum* lines 1-1, 1-2-1, 2-1, 2-2, 1-3-1, 4-1-1, 4-1- 2, 2-3, 4-2, 1-4, 1-5; No. 8 — control (DNA of non-transformed *N. tabacum* plant); +C — positive control; –C — negative control (TE buffer); M — molecular marker GeneRuler ™ DNA Ladder Mix (Thermo Fisher Scientific). Expected amplicon size — 475 bp

For the reverse transcription reaction, total RNA was isolated from the leaf material of 4 rooted plants transformed with the pCB182 construct, and 4 plants transformed with the pCB241 vector. Lines 2-1 (182), 1-3-1 (182), 4-2 (182), 3-2-1 (182), 24-1 (241), 2-4-2 (241), 37-1 (241), 43-1 (241) were used for the study.

PCR analysis of the cDNA synthesized during the reaction showed the presence of *cry1Ab* gene in all samples. A typical electrophoregram is presented in Fig. 6. At the same time, all controls worked in a quality and expected manner.

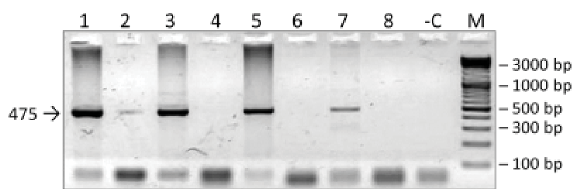

Fig. 6. **Results of the reverse transcription reaction using the transformants obtained by vector pCB182**
Tracks No. 1, 3, 5, 7 — cDNA of transformants of *N. tabacum* plants analyzed; Tracks No. 2, 4, 6, 8 — negative controls (without revertase); −C — negative control (without RNA); M — molecular marker GeneRuler™ DNA Ladder Mix (Thermo Fisher Scientific)

Thus, the expression of *cry1Ab* transgene in the obtained *N. tabacum* transformants was significantly confirmed.

Plants that showed a clear presence of *cry1Ab*, *bar* and *nptII* genes in PCR studies were transferred to the soil for grown and seed production (Fig. 7). Seed generation T1 was obtained after self-pollination, which indicates that there is no influence of transforming vectors on plant fertility.

Fig. 7. **Transformed tobacco plant in soil**
Vector pCB241, line 2-4-2

Conclusions

Thus, in the course of the work, genetic constructs pCB182 and pCB241 were developed. The vectors contained synthetic *cry1Ab* gene, which confers resistance to Lepidopteran pests. Vectors are intended for plant modification by *Agrobacterium*-mediated transformation. The ability of the obtained constructs to transfer *cry1Ab* gene to the plant genome of tobacco, a model object of biotechnology, is demonstrated. Transcriptional activity of *cry1Ab* transgene in the genome of *Nicotiana tabacum* was detected. The expression of a foreign gene did not affect the fertility of the plants.

The work was supported by the financing of the project “Research on molecular-biological and phenotypic manifestations of the functioning of transferred genes and features of their inheritance in biotechnological plants” (State registration number 0118U003663) of the National Academy of Sciences of Ukraine.

REFERENCES

1. *Savary S., Willocquet L., Pethybridge S. J., Esker P., McRoberts N.* The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evolut*. 2019, 3(3), 430–439. https://doi.org/10.1038/s41559-018-0793-y
2. *Rowe G. E., Margaritis A.* Bioprocess developments in the production of bioinsecticides by Bacillus thuringiensis. *CRC Crit. Rev. Biotechnol*. 1987, 6(1), 87–127.
3. *Federici B. A., Park H.-W., Bideshi D. K.* Overview of the basic biology of Bacillus thuringiensis with emphasis on genetic engineering of bacterial larvicides for mosquito control. *Open Toxicol. J.* 2010, 3, 154–171.
4. *Sanahuja G., Banakar R., Twyman R. M., Capell T., Christou P.* Bacillus thuringiensis: a century of research, development and commercial applications. *Plant Biotechnol. J.* 2011, 9(3), 283–300. https://doi.org/10.1111/j.1467-7652.2011.00595.x
5. *Rao K. Y. S., Pattanayak D., Sreevathsa R.* Bt insecticidal crystal proteins: Role in insect management and crop improvement. In: *Biocontrol of Lepidopteran Pests: Use of Soil Microbes and their Metabolites*. Sree K. S., Varma A. (editors). Cham: *Springer International Publishing*. 2015. pp. 53–70. https://doi.org/10.1007/978-3-319-14499-3_3
6. *Ye G. Y., Yao H. W., Shu Q. Y., Cheng X., Hu C.* High levels of stable resistance in transgenic rice with a *cry1Ab* gene from Bacillus thuringiensis Berliner to rice leaffolder, Cnaphalocrocis medinalis (Guenée) under field conditions. *Crop. Protect*. 2003, 22(1), 171–178. https://doi.org/10.1016/S0261-2194(02)00142-4

7. *Buntin G. D.* Corn expressing *cry1Ab* or cry1F endotoxin for fall armyworm and corn earworm (Lepidoptera: Noctuidae) management in field corn for grain production. *Florida Entomol*. 2008, 91(4), 523–530. https://doi.org/10.1653/0015-4040-91.4.523
8. *Valldor P., Miethling-Graff R., Martens R., Tebbe C. C.* Fate of the insecticidal *Cry1Ab* protein of GM crops in two agricultural soils as revealed by 14C-tracer studies. *Appl. Microbiol. Biotechnol*. 2015, 99(17), 7333–7341. https://doi.org/10.1007/s00253-015-6655-5
9. *ISAAA. Global status of commercialized biotech/GM crops in 2017: Biotech crop adoption surges as economic benefits accumulate in 22 years*. Ithaca, NY: The International Service for the Acquisition of Agri-biotech Applications; 2018. http://www.isaaa.org/resources/publications/briefs/53/download/isaaa-brief-53-2017.pdf
10. *Murashige T., Skoog F.* A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*. 1962, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
11. *Curtis I. S., Davey M. R., Power J. B.* Leaf disk transformation. In: *Agrobacterium Protocols*. Gartland KMA, Davey MR (editors). . Totowa, NJ: Springer; 1995. pp. 59–70. https://doi.org/10.1385/0-89603-302-3:59
12. *Somma M*. Session 4. Extraction and purification of DNA. In: *Training Course on the Analysis of Food Samples for the Presence of Genetically Modified Organisms — User Manual*. Querci M, Jermini M, Van den Eede G (editors). . Luxembourg: Office for Official Publications of the European Communities; 2006. p. 229.http://gmo-crl.jrc.ec.europa.eu/capacitybuilding/manuals/Manual%20EN/User%20Manual%20EN%20full.pdf
13. *Sambrook J. F., Russell D. W., editors. Molecular cloning: a laboratory manual*. 3rd. ed. *New York: Cold Spring Harbor Laboratory Press*. 2001.
14. *Ausubel F. M., Brent R., Kingston R. E., Moore D. D., Seidman J. G. Current protocols in molecular biology*. *New York: John Wiley & Sons, Inc*. 2003.
15. *Cannell M. E., Doherty A., Lazzeri P. A., Barcelo P.* A population of wheat and tritordeum transformants showing a high degree of marker gene stability and heritability. *Theoret. Appl. Genet*. 1999, 99(5), 772–784. https://doi.org/10.1007/s001220051296
16. *Lipp João K. H., Brown T. A.* Enhanced transformation of tomato co-cultivated with Agrobacterium tumefaciens C58C1Rif(r)::pGSFR1161 in the presence of acetosyringone. *Plant Cell Rep*. 1993, 12(7–8), 422–425. https://doi.org/10.1007/BF00234705
17. *Sakhno L.* O., *Morgun B. V., Kvasko* O. *Yu., Kuchuk N. V.* Creation of transformed rape plants that express cyp11A1 cytochrome P450 gene of animal origin. *Biotechnology*. 2010, 3(5), 74–82.
18. *Morgun B. V., Stepanenko O. V., Stepanenko A. I., Taranenko A. M., Kuchuk M. V.* Detection of transgenic herbicide resistant rapeseed plants in Kyiv region. *Visnyk ukr. tov. henetykiv i selektsioneriv*. 2014, 12(2), 166–173.
19. *Box M. S., Coustham V., Dean C., Mylne J. S.* Protocol: A simple phenol-based method for 96-well extraction of high quality RNA from Arabidopsis. *Plant Meth*. 2011, 7(1), 7. https://doi.org/10.1186/1746-4811-7-7
20. *Matzke M. A., Mette M. F., Matzke A. J. M.* Transgene silencing by the host genome defense: implications for the evolution of epigenetic control mechanisms in plants and vertebrates. *Plant Mol. Biol*. 2000, 43(2), 401–415. https://doi.org/10.1023/A:1006484806925
21. *Dong J., Kharb P., Teng W., Hall T. C.* Characterization of rice transformed via an Agrobacterium-mediated inflorescence approach. *Mol. Breed*. 2001, 7(3), 187–194. https://doi.org/10.1023/A:1011357709073
22. *Kohli A., Miro B., Twyman R. M.* Transgene integration, expression and stability in plants: Strategies for improvements. In: *Transgenic Crop Plants: Principles and Development*. Kole C, Michler CH, Abbott AG, Hall TC (editors). *Springer Berlin Heidelberg*. 2010. P. 201–237. https://doi.org/10.1007/978-3-642-04809-8_7
23. *Chareonpornwattana S., Thara K. V., Wang L., Datta S. K., Panbangred W.* Inheritance, expression, and silencing of a chitinase transgene in rice. *Theoret. Appl. Genet*. 1999, 98(3), 371–378. https://doi.org/10.1007/s001220051082
24. *Latham J. R., Love M., Hilbeck A.* The distinct properties of natural and GM cry insecticidal proteins. *Biotechnol. Genet. Engin. Rev*. 2017, 33(1), 62–96. https://doi.org/10.1080/02648725.2017.1357295
25. *Wu G., Cui H., Ye G.* Inheritance and expression of *cry1Ab* gene in Bt (*Bacillus thuringiensis*) transgenic rice. *Theoret. Appl. Genet*. 2002, 104: 727. https://doi.org/10.1007/s001220100689
26. *Zhao Q., Liu M., Tan M., Gao J., Shen Z.* Expression of *Cry1Ab* and Cry2Ab by a Polycistronic Transgene with a Self-Cleavage Peptide in Rice. *PLoS ONE* (2014) 9(10), e110006. https://doi.org/10.1371/journal.pone.0110006
27. *Jabeen R., Khan M. S., Zafar Y.* Codon optimization of *cry1Ab* gene for hyper

expression in plant organelles. *Mol. Biol. Rep.* (2010) 37: 1011. https://doi.org/10.1007/s11033-009-9802-1

28. *Rubie C., Schulze-Bahr E., Wedekind H., Borggrefe M., Haverkamp W.* Multistep-touchdown vectorette-PCR — a rapid technique for the identification of IVS in genes. *BioTechn*. 1999, 27(3), 414–418. https://doi.org/10.2144/99273bm03
29. *Kohli A., Twyman R. M., Abranches R., Wegel E., Stoger E.* Transgene integration, organization and interaction in plants. *Plant Mol. Biol*. 2003, 52(2), 247–258. https://doi.org/10.1023/A:1023941407376

СТВОРЕННЯ ВЕКТОРІВ ДЛЯ *AGROBACTERIUM*-ОПОСЕРЕДКОВАНОЇ ГЕНЕТИЧНОЇ ТРАНСФОРМАЦІЇ РОСЛИН ІЗ ЦІЛЬОВИМ СИНТЕТИЧНИМ ГЕНОМ *CRY1Ab*, ЯКИЙ КОДУЄ СТІЙКІСТЬ ДО ШКІДНИКІВ РЯДУ ЛУСКОКРИЛИХ

А. М. Тараненко[1], *І. О. Нітовська*[1], *Л. Г. Великожон*[1, 2], *П.Д. Майстров*[1], *М. В. Кучук*[1], *Б. В. Моргун*[1, 2]

[1]Інститут клітинної біології та генетичної інженерії НАНУ, Київ
[2]Інститут фізіології рослин і генетики НАНУ, Київ

E-mail: molgen@icbge.org.ua

Метою дослідження було створення генетичних конструкцій для *Agrobacterium*-опосередкованої трансформації рослин, які містили б синтетичний ген *cry1Ab*, та їх тестування шляхом трансформації тютюну з подальшим проведенням молекулярно-генетичного аналізу отриманих рослин для підтвердження події трансформації.

Застосовували базові методики клонування ДНК, *Agrobacterium*-опосередковану трансформацію *Nicotiana tabacum* L. методом листкових дисків, селекцію трансформантів *in vitro*, аналіз присутності трансгенів у рослинній ДНК, детекцію експресії гена *cry1Ab* методом ПЛР зі зворотною транскрипцією. У ході дослідження було сконструйовано вектори pCB182 і pCB241 із вмістом синтетичного гена *cry1Ab*. Створеними векторами здійснено *Agrobacterium*-опосередковану трансформацію тютюну та отримано рослини-регенеранти, які містили трансгени у своїй ДНК. Підтверджено експресію трансгена *cry1Ab* в одержаних трансформантах тютюну методом ЗТ-ПЛР. У результаті *Agrobacterium*-опосередкованої трансформації рослин векторами pCB182 і pCB241 із вмістом синтетичного гена *cry1Ab* стійкості до лускокрилих комах можна отримувати трансгенні рослини, у яких відбувається експресія трансгена.

Ключові слова: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-опосередкована трансформація, ПЛР-аналіз, трансгенез.

СОЗДАНИЕ ВЕКТОРОВ ДЛЯ *AGROBACTERIUM*-ОПОСРЕДОВАННОЙ ГЕНЕТИЧЕСКОЙ ТРАНСФОРМАЦИИ РАСТЕНИЙ С ЦЕЛЕВЫМ СИНТЕТИЧЕСКИМ ГЕНОМ *CRY1Ab*, КОДИРУЮЩИМ УСТОЙЧИВОСТЬ К ВРЕДИТЕЛЯМ ОТРЯДА ЧЕШУЕКРЫЛЫХ

А. Н. Тараненко[1], *И. А. Нитовская*[1], *Л. Г. Великожон*[1, 2], *П. Д. Майстров*[1], *Н. В. Кучук*[1], *Б. В. Моргун*[1, 2]

[1]Институт клеточной биологии и генетической инженерии НАН Украины, Киев
[2]Институт физиологии растений и генетики НАН Украины, Киев

E-mail: molgen@icbge.org.ua

Целью исследования было создание генетических конструкций для *Agrobacterium*-опосредованной трансформации растений, содержащих синтетический ген *cry1Ab*, и их тестирование путем трансформации табака с последующим проведением молекулярно-генетического анализа полученных растений для подтверждения события трансформации.

Использовали базовые методики клонирования ДНК, *Agrobacterium*-опосредованную трансформацию *Nicotiana tabacum* L. методом листовых дисков, селекцию трансформантов *in vitro*, анализ присутствия трансгенов в растительной ДНК, детекцию экспрессии гена *cry1Ab* методом ПЦР с обратной транскрипцией. В ходе исследования были сконструированы векторы pCB182 и pCB241, содержащие синтетический ген *cry1Ab*. Созданными векторами осуществлена *Agrobacterium*-опосредованная трансформация табака и получены растения-регенеранты, содержащие трансгены в своей ДНК. Подтверждена экспрессия трансгена *cry1Ab* в полученных трансформантах табака методом ОТ-ПЦР. В результате *Agrobacterium*-опосредованной трансформации растений векторами pCB182 и pCB241, содержащими синтетический ген *cry1Ab* устойчивости к чешуекрылым насекомым, можно получать трансгенные растения, в которых происходит экспрессия трансгена.

Ключевые слова: *cry1Ab*, *Nicotiana tabacum* L., *Agrobacterium*-опосредованная трансформация, ПЦР-анализ, трансгенез.

UDC 631.46.631.445.41:631.84 **https://doi.org/ 10.15407/biotech12.06.065**

IRECTION AND STRENGTH OF MICROBIOLOGICAL PROCESSES IN LAYERS OF GRAY FOREST SOIL UNDER DIFFERENT REGIMES OF MANAGEMENT

Malynovska I. M.

Institute of Agriculture of the National Academy of Sciences of Ukraine, Kyiv

E-mail: irina.malinovskaya.1960@mail.ru

Received 17.09.2019
Revised 29.11.2019
Accepted 20.12.2019

The aim of the work was to study the direction and intensity of mineralization and immobilization processes in different layers of gray forest soil at fallow fields, extensively and intensively used agrosoils. The research included laboratory analysis, microbiological studies, and statistical processing. For the fallow plots, the layers chosen for study were Hd — turf (0–10 cm), He — humus-eluvial (11–40 cm), Hi — humus-illuvial (41–74 cm), Ih — illuvial-humus (75–115 cm), Ip — transitional from the illuvial layer to bedrock (116–156 cm), Pi — bedrock with occasional insertions of illuvial soil (157–191 cm); for the agrosoils of the stationary experiments: He — humus-eluvial (0–10 and 11–40 cm), Hi — humus-illuvial (41–74 cm), Ih — illuvial-humus (75–115 cm), Ip — transitional from the illuvial layer to bedrock (116–56 cm). We found that humus mineralization differed in some layers of the gray forest soil under these management regimes. At fallows, the intensity of humus mineralization tended to decrease with depth, and it was interrupted in the Ih and Ip layers. In the intensively used agrosoil, humus mineralization was more active in Hi- and Ih-layers. Comparatively, the activity of humus mineralization smoothly decreased in the profile of the extensively used agrosoil from the uppermost layer to the lower by 97.2%. The mineralization coefficient of Nitrogen compounds gradually decreased in the fallow ground and extensively used agrosoil, unlike the intensively managed agrosoil, in which the intensity of mineralization-immobilization of nitrogen compounds increased in the He and Hi layers. It was shown that the fallow ground had the more efficient system to transfer substrates and mineral ions down the profile to the lower layers. The difference in biologic activity between the upper and lower layers was maximum in the fallow ground, intermediate in the intensively used agrosoil and minimum in the extensive agrosoil.

Key words: soil horizons, index of pedotrophy, nitrogen mineralization coefficient, humus mineralization activity, fallow ground, agrosoil.

Research of the microbial distribution along the soil profile gains weight considering that the microbial biomass is relatively evenly distributed across the whole cross-section of the gray forest soil, turf podzol, chernozem, kastanozem [1] and peat [2, 3]. Vertically layered microbial communities are viewed as integral components of ecosystems [4]. For every layer of the ecosystem, there are specific ranges of both quantity and taxonomic diversity of the microorganisms. For every type of soil, the maximum quantity and taxonomic diversity of the bacterial complexes are seen in the litter, minimum in the mineral horizon. The main factor determining the quantities and diversity of prokaryotes is type of substrate (layer position). Biotope features and seasonal characteristics have much lower influence [3, 4].

Materials and Methods

The study was done on gray forest soil at closely situated plots: 1 — soil excluded from agriculture use in 1987; 2–3 — agrosoils of the stationary experiment that was started in 1987 on the territory of the experimental station “Chabany” in Kyiv-Svyatoshynskyi district of Kyiv region. We studied versions with a ground development scheme that is traditional for Forest-Steppe: 2 — control, crop rotation without additional mineral and organic fertilizer (extensively used agrosoil); 3 — crop rotation with additional mineral

fertilizer N_{96} P108 K112.5 and conventional tillage of agricultural side products (intensive agrosoil).

Soil samples were taken from such horizons of the fallow ground: Hd — turf (0–10), He — humus-eluvial(11–40 cm), Hi — humus-illuvial (41–74 cm), Ih — illuvial-humus (75–115 cm), Ip — transitional from illuvial to bedrock (116–156 cm), Pi — bedrock with insertions of soil from the illuvial horizon (157–191cm). For agrosoils, the sampled horizons were: He — humus-eluvial (0–10 and 11–40 cm), Hi — humus-illuvial (41–74 cm), Ih — illuvial-humus (75–115 cm), Ip — transitional from the illuvial horizon to bedrock (116–156 cm).

The mineralization intensity of nitrogen compounds was calculated after Ye. N. Mishustin and E.V. Runov [5], the index of pedotrophy after D. I. Nikitin and V. S. Nikitina [6], the activity of humus mineralization — after I. S. Demkina and B. N. Zolotaryova [7]. To make a general assessment of the biologic state of soil, we calculated the parameter of overall biologic activity (OBA) using the method of relative valuation [8, 9].

Results and Discussion

Having analyzed the direction of microbiological processes in soil by the mentioned parameters and indices, we found that the intensity of mineralization processes changes depending on the position of the sample along the profile (Tables 1–3). Thus, indices of pedotrophy are somewhat lower for soil from the upper horizons of fallow ground and intensive agrosoil than from the He horizon, perhaps due to lesser humidity of the soil. The intensity of the organic matter mineralization decreased by 47.1% in the upper horizon of fallow field. For the intensive agrosoil it decreased in 4.18 times. For extensive agrosoil the trend was reversed: the mineralization activity was higher precisely in the upper horizon. This provides additional evidence about the relatively more similar soil processes in the intensive agrosoil and the fallow ground.

The activity of the organic matter decomposition sharp decreased at the edge of the Hi horizon by 4.92 times for the fallow field and by 3.03 times for the intensive agrosoil. In the extensive agrosoil the index of pedotrophy conversely increased by 87.4%. Such difference underscores the significant consequences of different intensity of soil management. The highest mean activity of the organic matter utilization in the arable horizon was seen in the intensive agrosoil (0.989), then the fallow field (0.546) and extensive agrosoil (0.434).

Nitrogen mineralization coefficient gradually decreased in fallow soil from 0.254 (upper horizon) to 0.072 (lowest of the studied horizons) (Table 1), the same tendency was seen in extensively used agrosoil (Table 3). However, in the intensively used agrosoil the intensity of mineralization-immobilization of nitrogen compounds was higher in horizons He and Hi, perhaps due to migration of Nitrogen mineral compounds to the layer of

Table 1. **Intensity of mineralization processes and phytotoxic properties of gray forest soil used for fallow since1987**

Horizon / depth	Index of pedotrophy	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity , %	Total bioactivity, %	Kr	Mass of 100 plants of the test culture, winter wheat, g		
							stem	root	total mass
Hd (0–10)	0.221	0.037	0.254	21.2	2264.0	0.352	8.08	13.9	22.0
He(11–40)	0.325	0.039	0.242	20.1	2249.8	0.341	8.46	7.34	15.8
Hi (41–74)	0.066	0.005	0.109	9.26	1184.0	0.140	9.57	10.4	20.0
Ih (75–115)	0.029	0.007	0.139	27.2	382.6	0.018	7.87	9.53	17.4
Ip (116–156)	0.014	0.003	0.025	49.8	344.8	0	6.80	6.90	13.7
Pi (157–191)	0.029	0.010	0.072	6.10	100	0	8.08	8.32	16.4
HIP05							0.11	0.12	

Table 2. **Intensity of mineralization processes and phytotoxic properties of gray forest soil managed as intensively used agrosoil since 1987**

Horizon / depth	Index of pedotrophy	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity, %	Total bioactivity, %	Mass of 100 plants of the test culture, winter wheat, g		
						stem	root	total mass
He (0–10)	0.191	0.100	0.160	25.7	1460.0	8.73	4.84	13.6
He (11–40)	0.798	0.744	0.621	23.8	962.0	6.54	4.77	11.3
Hi (41–74)	0.263	0.173	0.048	59.6	466.0	4.65	4.76	9.41
Ih (75–115)	0.119	0.299	0.155	42.6	147.9	4.31	5.57	9.88
Ip (116–191)	0.202	0.166	0.274	24.0	100.0	4.62	5.58	10.2
HIP05						0.13	0.10	

Table 3. **Intensity of mineralization processes and phytotoxic properties of gray forest soil managed as extensively used agrosoil since 1987**

Horizon / depth	Index of pedotrophy	Oligotrophy coefficient	Nitrogen mineralization coefficient	Humus mineralization activity, %	Total bioactivity, %	Kr	Mass of 100 plants of the test culture, winter wheat, g		
							stem	root	total mass
He (0–10)	0.252	0.16	0.37	21.1	864.2	4.28	6.5	5.89	12.4
He (11–40)	0.182	0.12	0.17	18.8	611.7	1.51	8.0	5.10	13.1
Hi (41–74)	0.341	0.22	0.045	17.5	159.7	4.28	4.33	4.66	8.99
Ih (75–115)	0.081	0.65	0.14	16.8	125.4	3.78	4.41	5.19	9.60
Ip (116–191)	0.390	0.33	0.46	10.7	100.0	2.80	4.44	4.01	8.45
HIP05							0.07	0.08	

11–74 cm (Table 2). The nitrogen compounds mineralization coefficient increased in the lowest of the studied horizons in the extensively agrosoil to 0.456. It is impossible to explain by vertical migration of these compounds, because this agrosoil was not fertilized since 1987. Though, the ions may have migrated from the regularly fertilized plots of adjacent stationary experiments.

Humus mineralization activity gradually decreases down the profile; the trend is all the more clear in old fallow ground and extensively used agrosoil. Thus, the microbiological destruction of humus decreases between the upper and the lowest studied horizons by the factor of 3.5 in the fallow soil, and by the factor of 1.97 in extensively used agrosoil. Meanwhile, the decomposition of humus substances increased by 24.4–65.2 % in the layer of 41 to 115 cm compared to the upper and lower horizons in the fallow soil and intensive agrosoil. It could be caused by accumulation of mobile humus compounds in these soil layers (41–115 cm).

Agrochemical analysis showed that in fallow soil, the percentage of humus was

1.43% at He-horizon, 0.041% at Ih-horizon, and 0.018% at Pi-horizon [10]. This means that humus content between the horizons decreases by the factor of 34.9 and 79.4, respectively. The indigenous microflora decreased 10.5 and 200-folds, respectively, though the physiologic-biochemical activity of microorganisms in different horizons remained almost the same (11). Therefore, humus decomposition depends on many factors: humus content, concentration of easily utilized substrates and of macroelements which delay the mineralization of humus compounds, availability of oxygen, abundance of indigenous microorganisms and their physiologic and biochemical activity, etc.

Long-term research showed that the activity of humus mineralization was minimum in the first 20 cm of fallow soil and maximum in the extensive agrosoil [12]. In 2014, the humus mineralization activity decreased in the extensive agrosoil, perhaps due to overall lesser amounts of humus in it. For 27 years, no organic fertilizer has been added neither cover crop plowed under; the organic matter has been also taken out with the main crop leading to a significant decrease in humus content [13].

The comparison of different types of soil by microbial biomass and the comparison of soil horizons which strongly differ in humus do not confirm the dependence of microbial biomass on the amount of organic matter [1]. It can be caused by the microbial biomass not being wholly active, a large part staying dormant until the favorable stage of succession [14].

In the vegetation period of 2014, the phytotoxicity of fallow soil (horizon He) was the lowest, while that of the intensive agrosoil was 74.3% higher and of the extensive agrosoil — 92.7% higher than the in fallow soil. That agrees with long-term data [15[. In the arable layer, the difference was 64.7 and 61.4% (Tables 1–3). In the lowest of studied horizons (Ih) it was 84.9 and 68.8%, respectively, which also confirms that the fallow soil contains a system of capillaries which is absent (or imperfect) in agrosoils.

The total bioactivity, which is an integral parameter, was minimum in the lowest of studied horizons in all cases (Tables 1–3). The difference in bioactivity between the higher and the lower horizons is highest in the fallow soil (22.6 times). In the intensive agrosoil it was higher by 14.6 tomes, and in the extensive agrosoil the difference was minimum (by 8.64 times).

In this study, the total bioactivity of the gray forest soil was highest in the fallow soil. It exceeded the bioactivity of the intensive and extensive agrosoils in the upper horizon by 1.55 and 2.62 times, respectively. The total bioactivity in the fallow soil gradually decreased from the upper horizon Hd to He by 16.7%, from He to Hi by 90.0%, from Hi to Ih by 218.0%, from Ih to Ip by 8.44%, from Ip to Pi by 3.44 times (Table 1). In the extensive agrosoil, total bioactivity decreased between horizons by 44.6, 97.8, and 99.6%, respectively, and in the intensive agrosoil, by 23.7% between He (0–10 cm) and He (11–40 cm) and 3.39 times between He and Hi layers. The three lowest horizons all have almost identical total bioactivity (Table 3).

Same trend was observed at the arable layer (0–40 cm): the highest activity was seen in the fallow soil (2330.7), then the intensive agrosoil (2139.0) and the extensive agrosoil (2061.3), which agrees with previous data [12].

Hence, the direction and intensity of mineralization processes change across the soil profile. The specifics and scale of the change depend on soil use.

Conclusions

1. The fallow soil has the highest total bioactivity of all studied examples of management regimes of gray forest soil. The bioactivity in the upper horizon of fallow soil exceeds the values for the intensive and extensive agrosoils by the factors of 1.55 and 2.62, respectively. The difference in bioactivity between the upper and the lower horizons is highest in the fallow soil (22.6 times), moderate in the intensive agrosoil (14.6 times) and lowest in the extensive agrosoil (8.64 times).

2. The mineralization coefficient of nitrogen compounds gradually decreases in fallow soil and extensive agrosoil, but not in intensive agrosoil. In the last case, the intensity of mineralization-immobilization of nitrogen compounds increases in the He and Hi horizons, perhaps because of migration of mineral nitrogenous compounds to the 11–74 cm deep soil layer.

3. Humus mineralization activity is the highest in the two uppermost horizons of intensive agrosoil among the studied management regimes of gray forest soil. It does not decrease downwards along the soil profile unlike humus mineralization activity in the extensive agrosoil, where it gradually decreases along the profile.

4. In the fallow soil and intensive agrosoil, humus substances decomposition are more actively decomposed in the layer of 41–115 cm compared to higher and lower horizons by 24.4–65.2% , perhaps because of accumulation of mobile humus compounds in these specific soil layers.

5. The system of transport of substrates and mineral ions downwards to lower horizons is more efficient in the fallow soil, which is reflected by the change in phytotoxicity values.

The work was carried out according the budget topics of the Institute of Agriculture of the National Academy of sciences of Ukraine.

References

1. *Polianskaya L. M., Heidebrecht V. V., Stepanov A. L., Zvyagintsev D. G.* The distribution of the number and biomass of microorganisms according to the profiles of zonal soil types. *Pochvovedeniye*. 1995, No 3, 322–328. (In Russian).
2. *Golovchenko A. V., Polyanskaya L. M., Dobrovolskaya T. G.* Features of the spatial distribution and structure of microbial complexes of swamp-forest ecosystems. *Pochvovedeniye*. 1993, No 10, 77–90. (In Russian).
3. *Golovchenko A. V., Dobrovolskaya T. G., Chernov I. Yu.* The structure of bacterial complexes in protected spruce forests. *Pochvovedeniye*. 1995. No 9, 1121–1124. (In Russian).
4. *Dobrovolskaya T. G., Chernov I. Yu., Lukin S. M.* Bacterial diversity of virgin and arable I. I.u. soils of the Vladimir region. *Pochvovedeniye*. 2001, No 9, 1092–1096. (In Russian).
5. *Mishustin E. N., Runov E. V.* Successes in developing the principles of microbiological diagnosis of soil conditions. *Uspekhi sovremennoy biotekhnolohii. Moskva, AN SSSR*. 1957.V. 44, 256–267. (In Russian).
6. *Nikitin D. I., Nikitina V.* S.. Self-cleaning processes and parasites. *Moskva: Nauka*. 1978, 205 P. (In Russian).
7. *Demkina T. S., Zolotareva B. N.* Microbiological processes in soils at various levels of intensification of agriculture. *Vilnus*. 1986, V. 103, 101. (In Russian).
8. *Kariahina L. A.* Microbiological fundamentals of increasing soil fertility. Mn .: Science and technology. *Minsk*: *Nauka i tekhnika*. 1983, 181 p. (In Russian).
9. *Rusakova, I. V.* Biological properties of sod-podzolic sandy loam soil with prolonged use of straw for fertilizer. *Pochvovedeniye*. 2013, 1493(12), 1485. (In Russian).
10. *Gamalay B. I., Korsun S. G.* Particular processes of land settlement on land for processing lands. *Zbirnyk nauk. prats Instytutu zemlerobstva*, *Kyiv: Norra Print*. 2003. 25(4), 25. (In Ukrainian).
11. *Malinovska I. M., Tkachenko M. A.* The number and physical activity of microorganisms of the genetic horizons of the gray fox area for different purpose. *Zbirnyk naukovykh prats Instytutu zemlerobstva*. 2015, No 4, 13–24. (In Ukrainian).
12. *Malinovska I. M., Soroka O. P.* Rebooting microbial processes at fallow lands and agrozems. *Hruntosnavstvo*. 2011, 12(3–4), 84–91. (In Ukrainian).
13. *Gorodetska S. P.* Diya Dobriv i crops on the balance of humus in the grain-saponny sivozmini. *Zbirnyk naukovykh prats Instytutu zemlerobstva*, UAAN, *Kyiv*. 2003, Issue 1–2. 55–64 p. (In Ukrainian).
14. *Polyanskaya L. M. Zvyagintsev D. G.* Microbialsuccessioninsoil. *Soviet scientist reviews. Horwood Academic Publ.Gmbh*. 1994, No 1. P.1–65.

СПРЯМОВАНІСТЬ ТА НАПРУЖЕНІСТЬ МІКРОБІОЛОГІЧНИХ ПРОЦЕСІВ У ГОРИЗОНТАХ СІРОГО ЛІСОВОГО ҐРУНТУ ЗА ЙОГО РІЗНОЦІЛЬОВОГО ВИКОРИСТАННЯ

І. М. Малиновська

ННЦ «Інститут землеробства НААН», Київ, Україна

E-mail: irina.malinovskaya.1960@mail.ru

Метою роботи було дослідження спрямованості та напруженості мінералізаційних процесів у ґрунті горизонтів сірого лісового ґрунту за його використання як перелогу, екстенсивного та інтенсивного агроземів. У дослідженні використовували лабораторно-аналітичний, мікробіологічний, статистичний методи. З'ясовували перебіг мінералізаційних та іммобілізаційних процесів у горизонтах сірого лісового ґрунту на перелогових ділянках: Hd — дернина (0–10 см), He — гумусово-елювіальний (11–40 см), Hi — гумусово-ілювіальний (41–74), Ih — ілювіально-гумусовий (75–115), Ip — перехідний від ілювіального горизонту до породи (116–156), Pi — порода із вкрапленнями ґрунту ілювіального горизонту (157–191 см) і на агроземах стаціонарного досліду: He — гумусово-елювіальний (0–10 і 11–40 см), Hi — гумусово-ілювіальний (41–74), Ih – ілювіально-гумусовий (75–115), Ip — перехідний від ілювіального горизонту до породи (116–56). Встановлено, що варіанти використання сірого лісового ґрунту відрізняються за активністю мінералізації гумусу в окремих горизонтах: у ґрунті перелогу простежується тенденція до зниження інтенсивності мінералізації гумусу вглиб профілю, що порушується у горизонтах Ih і Ip. В інтенсивному агроземі спостерігали підвищення активності мінералізації гумусу в Hi- і Ih-горизонтах, у профілі екстенсивного агрозему — плавне зниження активності мінералізації гумусу від верхнього горизонту до нижнього на 97,2%. Коефіцієнт мінералізації сполук азоту поступово знижувався в ґрунті перелогу й екстенсивного агрозему, на відміну від інтенсивного агрозему, де інтенсивність мінералізації-іммобілізації сполук азоту в горизонтах He і Hi підвищувалась. Показано, що ефективнішою системою транспортування субстратів і мінеральних іонів вглиб профілю до нижчих горизонтів характеризується ґрунт перелогу. Різниця в біологічній активності між верхнім і нижнім горизонтами була максимальною у ґрунті перелогу, середньою – в інтенсивному агроземі та мінімальною — в екстенсивному агроземі.

Ключові слова: горизонти ґрунту, індекс педотрофності, коефіцієнт мінералізації азоту, активність мінералізації гумусу, переліг, агрозем.

НАПРАВЛЕННОСТЬ И НАПРЯЖЕННОСТЬ МИКРОБИОЛОГИЧЕСКИХ ПРОЦЕССОВ В ГОРИЗОНТАХ СЕРОЙ ЛЕСНОЙ ПОЧВЫ ПРИ ЕЕ РАЗНОЦЕЛЕВОМ ИСПОЛЬЗОВАНИИ

И. М. Малиновская

ННЦ «Институт земледелия НААН», Киев, Украина

E-mail: irina.malinovskaya.1960@mail.ru

Целью работы было исследование направленности и напряженности минерализационных процессов в почве горизонтов серой лесной почвы при ее использовании как перегноя, экстенсивного и интенсивного агроземов. В исследовании использовали лабораторно-аналитический, микробиологический, статистический методы. Выясняли протекание минерализационных и иммобилизационных процессов в горизонтах серой лесной почвы на перегнойных участках: Hd — дерн (0–10 см), He — гумусово-элювиальных (11–40 см), Hi — гумусово-илювиальный (41–74), Ih — илювиально-гумусовый (75–115), Ip — переходный от илювиального горизонта до породы (116–156), Pi — порода с вкраплениями почвы илювиального горизонта (157–191 см) и на агроземах стационарного опыта: He — гумусово-элювиальных (0–10 и 11–40 см), Hi — гумусово-илювиальный (41–74), Ih — илювиально-гумусовый (75–115), Ip — переходный от илювиального горизонта к породе (116–56). Установлено, что варианты использования серой лесной почвы отличаются по активности минерализации гумуса в отдельных горизонтах: в почве перегноя прослеживается тенденция к снижению интенсивности минерализации гумуса вглубь профиля, которая нарушается в горизонтах Ih и Ip. В интенсивном агроземе наблюдали повышение активности минерализации гумуса в Hi- и Ih-горизонтах, в профиле экстенсивного агрозема — плавное снижение активности минерализации гумуса от верхнего горизонта к нижнему на 97,2%. Коэффициент минерализации соединений азота постепенно снижался в почве перегноя и экстенсивного агрозема, в отличие от интенсивного агрозема, где интенсивность минерализации-иммобилизации соединений азота в горизонтах He и Hи повышалась. Показано, что более эффективной системой транспортировки субстратов и минеральных ионов вглубь профиля к нижележащим горизонтам характеризуется почва перегноя. Разница в биологической активности между верхним и нижним горизонтами была максимальной в почва перегноя, средней — в интенсивном агроземе и минимальной — в экстенсивном агроземе.

Ключевые слова: горизонты почвы, индекс педотрофности, коэффициент минерализации азота, активность минерализации гумуса, перегной, агрозем.