

BIOLOGICAL PRODUCTS IN ORGANIC AGRICULTURE

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The review presents data on the creation of complex microbial preparations and their application in agricultural practice. According to economists, the turnover in the field of organic agriculture is worth 85-90 billion US dollars a year. Developers of biological products pay great attention to the creation of complex biofertilizers, which contribute to a stable 20-25% increase in yield, with a significant reduction of plant damage by root rot. Among the considered positive effects of plant growth promoting rhizobacteria (PGPR) on plants are the ability to fix molecular nitrogen from the atmosphere, the synthesis of hormonal and fungitoxic substances, and the mobilization of sparingly soluble soil phosphates. The presented data show promise for the use of these microorganisms in the development of eco-friendly farming technologies in order to increase plant productivity and establish bio-control over the development of plant diseases, reduce the chemical load on the soil, and increase its fertility.

Key words: complex biological preparations, nitrogen-fixing, phosphate and potassium mobilizing microorganisms, bacterium, plant growth stimulation, wheat.

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БИОЛОГИЧЕСКИЕ ПРЕПАРАТЫ В ОРГАНИЧЕСКОМ ЗЕМЛЕДЕЛИИ

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В обзоре рассмотрены данные о создании сложных микробных препаратов и возможности их использования в практике сельского хозяйства. Согласно данным экономистов, оборот средств в области органического, экологически чистого сельского хозяйства насчитывает 85-90 миллиардов долларов в год. Разработчики биологических препаратов придают большое значение созданию комплексных биоудобрений, которые стабильно повышают урожай на 20-25%, при этом значительно снижают уровень поражения растений корневой гнилью. Рассмотрен ряд положительных эффектов действия бактерий PGRP (ризобактерии, стимулирующие рост растений) на растения, в том числе способность бактерий фиксировать молекулярный азот из атмосферы, синтезировать гормональные вещества и соединения, токсичные для грибов растений, а также мобилизовать трудно растворимые фосфаты почвы. Представлены данные, которые свидетельствуют в пользу перспектив использования микроорганизмов в развитии технологий экологически чистого сельского хозяйства с целью повышения продуктивности растений и биоконтроля над развитием болезней растений, уменьшения химической нагрузки на почвы и увеличения плодородия почв.

Ключевые слова: комплексные биопрепараты, азотфиксирующий, фосфат и калиймобилизующие микроорганизмы, бактерия, стимуляция роста растений, пшеница.

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Introduction

Since the middle of the 20th century, and especially during the past 20 years agriculture in economically advanced countries has been focusing on the development of organic agriculture and the production of environmentally sound and full-fledged food. Organic agriculture is based on the reduction or complete prohibition of plants and soil treatment with synthetic mineral fertilizers, chemical protection agents, plant growth regulators, and genetically modified organisms. The management of organic agriculture is aimed at maximizing the use of biological products and biotechnologies at all stages and phases of agricultural production. The following international standards for the organic farm supply and marketing sectors have been adopted: "EC 834/2007 Council Regulation (EC) № 834/2007. On organic production and labeling of organic production and repealing regulation (EC) № 2092/91-2007" and "USDA organic". The international technological Organics platform was created in 2009 to amalgamate several international institutions like IFOAM (International Federation of Organic Agriculture Movements), European organic certified council, Aoel, etc. The global market for organic agricultural produce is currently estimated at \$85 billion (Monastyrsky et al., 2019).

Organic practices in agriculture are currently used in 160 countries of the world. Organic agriculture laws work in 84 countries and, moreover, in dozens of countries such bills are drafted. Economists estimate that based on current gross turnover in organic agriculture, which amounts to \$85 to \$90 billion per year, this amount is projected to reach \$200-250 billion by 2020 (Development organic rural//farms in Kazakhstan, 2018). On November 27, 2015 the Law of the Republic of Kazakhstan "On production of organic products" was adopted. The Act established the legal, economic, social and organizational bases for the production of organic agriculture. This legislation is aimed at the rational use of the soil, promotion of healthy diets and protection of the environment (The Law..., 2015). Currently, Kazakhstan is in the process of adapting the international standards of Codex Alimentarius, as well as those of IFOAM, and is also using the international experience as an organic agricultural producer at the local level.

Kazakhstan has necessary prerequisites and great opportunities for the development of organic agriculture and livestock production: the "2050 Strategy" (The Strategy..., 2003) according to which Kazakhstan has to become a global player on the market of environmentally friendly products has been accepted, the Concept of transition of the Republic of Kazakhstan to "green" economy developed, and the "Agribusiness 2020" program, which specifies the conditions for the development of production and marketing of organic agricultural products, approved.

We already wrote in 1999 about the importance of developing biological agriculture in Kazakhstan. A special part of the monograph focused on biological products developed

with the use of a range of microorganisms either capable of fixing nitrogen, or involved in phosphate and potassium mobilizing activities, and able to stimulate plant growth or to suppress phytopathogenic effects of micromycetes and bacteria (Sadanov, Kurmanbayev, 1999).

In the Republic of Belarus, 75 % of the biological products produced by the Institute of Microbiology of the National Academy of Sciences of Belarus are bio-pesticides (Koloziets, 2018, unpublished).

In the field of biological preparations development for agriculture, the All-Russian Research Institute for Agricultural Microbiology has exclusive experience. The Russian Federation successfully applies the achievements of the Institute's scientists, such as Rhizotorphine and Extrasol biological products. Albit, a biological product developed at the G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms in Pushchino, also has a considerable sales market.

In the CIS (Commonwealth of Independent States) countries, biological products for agriculture are also successfully developed. Noticeable success has been achieved in Ukraine and Uzbekistan.

Creation of complex biofertilizers

Developers of biological products assign greater importance to the creation of integrated biofertilizers. There are two ways of achieving this: either by selecting bacteria with different useful functions, or by searching for one organism with polyfunctional properties.

The theoretical basis for the development of complex biological preparations was already laid by N.A. Krasilnikov and A.I. Korniyako (1944) who showed that the effectiveness of legume bacteria depends in many ways on the condition of soil microbiocenosis. For instance, weakly virulent strains became highly virulent in the presence of activator bacteria, such as those from the genus *Pseudomonas*. On the contrary, bacteria inhibitors delayed the growth of nodule bacteria. It was established that combinations of nodule bacteria with activators ensured the greatest increase in plant yield, if compared to the use of each of those bacteria individually (Krasilnikov, Korniyako, 1944).

The development of complex biological products is a labour-consuming process, nevertheless, intensive research continues.

Microbiologists in Uzbekistan created a multi-component biological product of a very complex structure, namely a complex microbiological fertilizer (CMF), which is a uterine bacterial culture of aerobic, ammonifying and denitrifying bacteria in a nutrient medium. The CMF is based on the aerobic bacteria belonging to the genera *Bacillus*, *Azotobacter*, *Pseudomonas*, *Micrococcus*, *Pseudobacterium*, *Rhizobium*. These microorganisms are involved in the humification and mineral-

ization of organic residues. In addition, the CMF also includes anaerobic bacteria of the genera *Clostridium*, *Methanobacterium* and *Acetobacterium*. These are capable of decomposing almost insoluble organic compounds of phosphorus and have antagonistic activity in relation to phytopathogenic microorganisms. Also, the CMF comprises denitrification, ammonifying and lacto bacteria. Actinomycetes in this preparation are represented by *Streptomyces albus*, *Streptomyces griseus* and *Actinomyces elephantis*. The latter produce biologically active materials in the soil, synthesize vitamins B1, B3, B6, B12, inositol, PP, amino acids, glutamic acid, lysine, alanine, tryptophan and antibiotics. Microalgae were also included in the biopreparation (Patent. IAP 02430. Abduazizov, Baybaev, 2004).

The complex CMF product is very similar to the EM preparation by Teruo Higa ("Effective Microorganisms", Patent US5591634. Higa, 1997). CMF includes more than 80 cultures of microorganisms, microalgae, micromycetes, yeast, actinomycetes, etc., selected from fertile soil to improve the fertility of poor soils. EM consists of the following microorganisms: actinomycetes from the genera *Streptomyces*, *Streptovorticillium*, *Nocardia*, *Micromonospora* and *Rhodococcus*, phototrophic bacteria from the genera *Rhodospseudomonas*, *Rhodospirillum*, *Chromatium* and *Chlorobium*, lactic bacteria from the genera *Lactobacillus*, *Propionibacterium*, *Pedococcus* and *Streptococcus*, micromycetes from the genera *Aspergillus* and *Mucor*, as well as yeast from the genera *Saccharomyces* and *Candida*. In addition, T. Higa included phytopathogenic bacteria in the biopreparation to enhance the immune properties of plants (Higa, Parr, 1994; Patent US5591634. Higa, 1997).

Microbiologists from Taiwan developed a sophisticated biological product based on thermotolerant phosphate-mobilizing microorganisms. *Bacillus coagulans* C45, *Bacillus licheniformis* A3, *Bacillus smithii* F18, one actinomycete of *Streptomyces thermophilus* J57 and one micromycete of *Aspergillus fumigatus* O4 were part of it. All strains listed were selected from various composts. The biological product accelerated the transformation of various organic waste into the organic biofertilizer enriched with mobile phosphorus (Chang, Yang, 2009).

In practice, complex biofertilizers have been shown to contribute to a steady 25-50-plus % increase in yields of or more, as well as to a significant reduction in the incidence of root rot affecting plants (Tereshchenko, 2007). Along with that, the fungistatic effect is most often caused by the fact that soil biodiversity increases significantly due to the use of complex bacterial fertilizers, and the influence of undesirable populations decreases (e.g., populations of pathogenic fungi of plants). This allows plants to overcome their own limitations of the numerous constraints imposed by the Mitscherlich's Law of Diminishing Returns (Tereshchenko, 2007).

With intensive cropland farming, the situation with the multiplication of factors of limitation is quite common. In the same circumstances, even when the significance of each constraint is negligible, the overall effect of the mar-

ginal compensation of each factor can be very high. Thus, even when the efficacy of individual microbial populations is negligible, their combined effect can only be achieved by increasing biodiversity in the soil rhizosphere (Shchedrin, 2010).

Considering the problems related to plant seeds bacterization, like preservation of a high titre of bacteria depending on the preparation form, small shelf-life, processing of seeds in the shade, etc., and a lot of various conditions to be considered when using bacterial preparations, it has to be noted with regret that poor performance of those preps is natural, and their criticism is fair. The simplest statistical treatment of bacterization results generally demonstrates the random nature of rare positive reactions of soil and plants to bacterial fertilizers. The continued so called "introduction" of bacterial fertilizers in collective and individual farms will lead to a total undermining of the very idea of using microbial preparations (Tereshchenko, 2007).

Based on the above considerations, P.A. Kozhevnikov (2014) proposed to activate soil microbial cenosis through readily available carbon sources. The possibility of producing complex microbial fertilizers of different functional use based on natural microbial communities without growing microorganisms on a nutrient medium was shown. For this purpose, soil microbial cenosis was activated by introducing different sources of carbon and nitrogen. The activation of soil actinomycetes resulted in a pronounced effect in such a way that wheat growth increased by 65% compared to the control (Andreeva, Kozhevnikov, 2014).

Meta-analysis of the efficiency of microbial fertilizers based on a complex of microorganisms showed that the best inoculum for plants is a combination of arbuscular mycorrhiza, nitrogen fixators, and phosphate mobilizers. The effectiveness of such a complex was shown in 92% of 112 field experiments. Thus, the contribution to the yield is significant and variability is low (Schütz et al., 2018).

Without diminishing the scientific progress in many countries in this sphere, we believe that an individual approach to biological preparations should be applied in the same way as in medicine, i.e., local biological products have to be developed on the basis of native races of bacteria and micromycetes for a certain field and crops in this field adapted to soil climatic conditions of the particular region.

Northern Kazakhstan is one of the most economically important regions of agroindustrial complex of the Republic, since the production of spring common wheat is concentrated here. This agricultural crop is annually cultivated in the area of 8-10 million hectares and occupies about 80-85% of all acreage, and gross grain harvest averages 8-12 million tons (Shvidchenko et al., 1999). Thus, the spring-sown wheat is the most important crop for Kazakhstan. The Green Economy Development Concept of the Republic of Kazakhstan aims at significantly increasing the yield of this crop. Within the framework of efforts to achieve this goal, the importance of biofertilizers and bio-pesticides cannot be underestimated.

Biopesticides and symbiotic relations between bacteria and plants

A large number of works on microbial fertilizers for wheat are available in modern published scientific literature.

Tests of Rizoagrin and Flavobacterin biological products on 'Dzhangal' winter wheat variety have shown their effectiveness in conditions of Saratov region. Rizoagrin has been developed on the basis of nitrogen fixing bacteria of *Agrobacterium radiobacter* (Beijerinck and van Delden, 1902) Conn 1942 [*Rhizobium radiobacter* (Beijerinck and van Delden, 1902) Young et al., 2001] having signs of bacteria of the PGPR group. Flavobakterin is a biopesticide based on *Flavobacterium* spp. The latter, as part of the biopreparation, produces an antibiotic flavocin that suppresses a wide range of phytopathological bacteria and fungi. The use of Rizoagrin ensured an increase in wheat yield of 0.41 t/ha (13.5%) in comparison with the untreated control, while application of Flavobakterin in combination with humates resulted in an increase of 17.1% (Chekmareva, Nesterova, 2018).

The Rizoagrin preparation used for the inoculation of seeds of spring-sown wheat in the conditions of the Udmurt Republic positively affected the grain productivity, mass of straw and plant residues. Its action can be equivalent to the influence of a dose of $N_{40}P_{40}K_{40}$ fertilizer that gives big economic and power effect to the studied intake. As a result, the abundance of root decay decreased by 17.5%. The increase of a grain yield averaged 0.43 t/ha. Rizoagrin was more effective than the biological products Baikal and Mizorin which ensured an increase of 0.3 and 0.1 t/ha, respectively. Rizoagrin authentically improved nitrogen, phosphorus and potassium nutrition of plants that promoted wheat grain upgrading (Bashkov, 2011).

The application of Elena biofungicide (*Pseudomonas aureofaciens* IB 51) helped to achieve a 6% increase in gluten quality in wheat grain, and turned out to be efficient against a complex of diseases of winter wheat, such as root rot, mildew, septoria spot and brown rust. During tests in Krasnodar, Volgograd and Voronezh regions and also in the Republic of Bashkortostan, it was established that the biological efficiency of biofungicide Elena was 30-50% when the infection backgrounds ranged from 10 to 20% (Kuzina et al., 2013).

Inoculation of seeds of spring-sown wheat with the biological product BP2 based on endophytic bacteria, cultivation of plants in the nitrogen-deficient environment and application of N45 increased the grain yield irrespective of weather conditions. That could possibly happen due to an increase in plant security in the conditions of a lack of nitrogen and rise of plant resistance to stress. Application of Ekstrasol to seeds of spring wheat followed by vegetation under the conditions of nitrogen-free background only promoted an increase in spring wheat productivity of 26% on the average. It has been demonstrated that the complex use of nitrogen fertilizer in a dose of 45 kg/ha and biological products of endophytic bacteria made it possible to increase grain efficiency of spring-sown wheat by 1.6-2.1 times. (Alferov et al., 2017).

Much attention, particularly while developing complex biological products, has been given in recent years to the group of rhizospheric bacteria, the so-called plant growth promoting rhizobacteria (PGPR). This group of bacteria is adapted to live in a rhizosphere of plants, and provide them with the best competitive conditions for growth and development, allowing the plants to overcome environmental stresses.

The Egyptian scientists tested the influence of a three-component PGPR-based biofertilizer on wheat yield. The biological product T11 provided an increase in grain and straw yield by 10.2 and 8.2%, respectively, in comparison with the control that received no treatment. The preparation T11 consisted of bacteria *Azospirillum lipoferum* (Beijerinck, 1925) Tarrand et al., 1979, *Paenibacillus polymyxa* (Prazmowski, 1880) Ash et al., 1994 and *Nostoc muscorum*. In addition to the noted increase in wheat yield, preparation considerably enhanced biological activity of soils in the rhizosphere of wheat (El-Gamal Manal et al., 2015).

The work on the use of lactic bacteria as bacterial fertilizers is of particular interest. D.R. Yarullina with co-authors (2014) showed that *Lactobacillus plantarum* (Orla-Jensen 1919) Bergey et al. 1923 can level an oxidizing stress thanks to positive impact of bacterial NO (nitrogen oxide) on integrated antioxidant capacity and activity of a catalase (Yarullina et al., 2014).

An interesting solution was proposed by researchers from the Russian Federation, who used natural microbial cenosis from koumiss for developing a biological product Microbiovit. The microbiocenosis promoted an increase in yield capacity of wheat, vegetables and potatoes by 26–56%. The maturation period of wheat reduced by 9 days. The treatment of potato tubers with Microbiovit increased their resistance to rot agents, which in turn increased the product shelf-life. Bacteria from the genera *Lactobacillus*, *Streptococcus*, *Rhodopseudomonas*, *Bacillus* and the yeasts belonging to *Kluyveromyces*, *Saccharomyces*, *Torulopsis* dominated in the considered bio-product. The authors emphasize that one of the advantages of the preparation is the ease of cultivation of this biological community and its stability over time, in comparison with artificial compositions (Somova et al., 2017).

Other interesting ways to develop biological products for crop production are to use biologically active bacterial compounds to combat phytopathogens and to improve symbiotic relations between bacteria and plants.

Thus it was shown that biological surfactants produced by bacteria from the genus *Rhodococcus* and other bacteria were capable of suppressing viral infections of potatoes. Those surfactants were proposed as a means of improving crop health in monoclonal reproduction. For example, the preparation KP-2 suppressed potatoes X-virus reproduction. Microbial glycanes, antimetabolites (5-azadihydrouracils, cyanoguanidine) and bio-surfactants were part of the preparation. KP-2 also had anti-neoplastic activity caused by the bacterium *Agrobacterium tumefaciens* [*Rhizobium radiobacter* (Beijerinck and van Delden, 1902) Young et al. 2001] (Kovalenko, Karpenko, 2010 unpublished).

T.P. Pirog with co-authors (2013) showed the possibility of other uses of biosurfactants produced by the bacteria *Rhodococcus erythropolis* (Gray and Thornton, 1928) Goodfellow and Alderson, 1979, *Acinetobacter coaceticus* and *Nocardia vaccinii*, for instance, to monitor the amount of phytopathogenic bacteria. In the presence of surfactants, the growth of phytopathogenic bacteria of the genera *Pseudomonas* and *Xantomonas* was almost completely suppressed (Pirog et al., 2013).

L.M. Babenko with co-authors (2017) used signaling molecules of quorum sensing bacterial cells to stimulate the growth of wheat. Autoinducer N-acyl homoserine lactone activated rhizospheric microflora, which positively affected wheat biomass and grain yield as a result (Babenko et al., 2017).

Recently, scientists have focused on the development of elicitor preparations. Many bacterial fertilizers have elicitor effects, increasing resistance of plants to adverse factors of soil environment and phytopathogens. A number of metabolites and signalling molecules of microorganisms can cause plant immunity and resistance to environmental stresses (temperature, salinity, contamination with heavy metal ions) (Gorovoj et al., 2002; Grineva et al., 2017).

Thus, the analysis of literature showed a variety of approaches in the development of biological products for the increase in productivity of crops and their quality. Bioproducts with fertilizer, elicitor and biopesticide effects are increasingly gaining market share and bringing organic agriculture closer to ideal by reducing the chemical load on the soil. The most promising biological products at the moment are elicitor preparations that increase the immunity of plants and their resistance to adverse environmental factors.

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