

# MARKET ANALYSIS AND MICROBIAL BIOPREPARATIONS CREATION FOR CROP PRODUCTION IN UKRAINE

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A microbial biopreparations, particularly for the leguminous, grains, industrial and other agricultural crops of Ukrainian as well as foreign production are analyzed. All biopreparations on the market of Ukraine may be divided into basic groups — growth-activating preparations, phytoprotective preparations, preparations for the accelerated decomposition of plant wastes, preparations for restoration and preservation of soil fertility (depending on the functional activity of a biological agent) as well as into mono- and complex biopreparations (depending on the number of components within them). Approaches to the creation of complex biopreparations and compositions based on biological agents — microorganisms with agronomically valuable properties, fungi, algae, biologically active metabolites of plants and microorganisms as well as microelements in chelated forms are considered to solve the following environmental goals: getting organic crop production, restoration and preservation of soil fertility. The results of original research on the complex compositions based on nodule bacteria, rhizobacteria, microorganisms — destructors of xenobiotics, microbial (exopolysaccharides, exometabolites) and plant (phytolectins, extract of blue-green algae) metabolites for growth of legumes and grain cultures are presented. It is established that the complex biopreparations and compositions have more effective and stable action as compared to monoculture. The examples of biopreparations of a new generation which are created using the innovative technologies such as IGET Technology (Induced Gene Expression Triggers), LHO-promoter (lipohitooligosaccharides-promoter) technology, TOP-technology (technology osmoprotector) involving achievements of molecular and cell biology, nanotechnology as well as the modern solid carriers, adhesives, lipkogenes, formularies that provide high titer of microbial cells in biopreparations, close contact the bacterial cells with seeds when inoculated and continued prolonged viability of microorganisms on the seeds are given.

**Key words:** microbial biopreparations, agrarian market of Ukraine.

Microbial Biotechnology are an integral part of modern innovative technologies that have found their application in industry, medicine, pharmacy, water management, agro-industrial production. According to Frost and Sullivan [1], the volume of the global biotechnology market in 2013 is estimated at 270 billion US dollars and projected growth rate until 2020 will be up to 10–12% per year, i.e. the global market for biotechnology will approach 600 billion dollars. According to experts, the global biotechnology market in 2025 will reach 2 trillion United States dollars, and the growth of individual segments of the market will be up to 30% [2]. Segmentation of the global biotechnology market is as follows (Frost and

Sullivan, 2014) [1]: the main share (60%) is in biopharmaceuticals and biomedicine (the so-called “red biotechnology”), the share of industrial biotechnology and bioenergy (“white biotechnology”) is 35%. The agricultural and environmental (“green”) biotechnologies are 5%. The last segment of the market is actively developed in the US, Europe (France, Germany, Denmark, Switzerland, Sweden), Canada, Australia, Japan and Israel. Growing in the last 5 years, biotech markets, including agrobiotechnological market, are typical for China, India, Brazil, Argentina.

A significant part of agricultural biotechnology is associated with microbial biopreparations for crop production that is one of the components of ecological (organic)

agriculture [3]. These biopreparations contain soil microorganisms, fungi with agronomically valuable properties, such as an ability of molecular nitrogen fixation, poorly soluble phosphorus compounds transformation, hormones, vitamins, amino acids, substances of bactericidal and fungicidal action synthesis, pollutants splitting and etc. [4]. They are used for seeds pre-inoculation or plants spraying during the growing season in order to improve nutrition, increase plant resistance to pathogens, increasing productivity and improving the quality of agricultural products, as well as bioremediation, restoration and preservation of soil fertility [4–8].

Domestic [9–15] and foreign [16–20] microbial biopreparations for crop production on the basis of microorganisms with an agronomically valuable properties are developed. Analysis of microbial biologics market in Ukraine indicates the presence of a wide range of products for different groups of agricultural plants (legumes, cereals, industrial crops, fruit and others). Depending on the number of components included in their composition, there are distinguished mono and complex biologics [5–7] i.e. in their composition can be included as one component — the microbiological agent — an alive culture of microorganisms, fungi and products of its metabolism, and several producing their metabolites, as well as biologically active substances (BAS), microelements chelated forms exogenously introduced into the composition. Depending on the functional activity spectrum of the main bioagents, microbial biopreparations may be divided into three blocks:

- *Biologics that stimulate plants growth and development, contributing to the improvement of their nitrogen and phosphorus nutrition.* Their agents are diazotrophic bacteria (fix molecular nitrogen and convert it into a form available to plants) of the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azotobacter*, *Azospirillum*, *Agrobacterium*, *Clostridium*, *Enterobacter*, *Flavobacterium*, etc., phosphate mobilizing microorganisms (mobilize phosphorus from poorly soluble mineral and organic compounds, making it available to the plant) (bacteria — *Achromobacter*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, fungi — *Penicillium*, *Glomus*), as well as bioagents producing a variety of biologically active compounds (hormones, vitamins, amino acids, enzymes, etc.).

Examples of this group of biologics for legumes are products based on monoculture

of nodule bacteria — Rhizobin [9] Rhizoactiv, Rhizobofit [11], Bioinoculant BTU [12], Nitrofix and its modifications [13, 16], Hi Coat Super, Hi Stik, Rhizup [19] and complex preparations — line of products of ABM company (ABM-inoculant, Excalibre Encapsulated Inoculant, Excalibre SA, Mega Pack) [16], alliance Novozymes — Monsanto BioAg (Optimize, Optimize Pulse) [17], Ecobact, Ecovital [9], Rhizostim [14], Rizoliq TOP [18], Rhizohumin [10]. All biologics for legumes are based on specific for plants, active in nitrogen fixation, competitive and effective strains of nodule bacteria. The strains of rhizobia — legume symbionts are selected by domestic microbiologists and protected by patents of Ukraine № 2901, 1994; № 3324, 1994; № 13298, 1997; № 39545, 2001; № 50851, 2002; № 51890, 2002; № 54591, 2003; № 65227, 2004; № 104211, 2004; № 7954, 2005; № 85089, 2008, № 43516, 2009; № 85943, 2009; № 91635, 2010; № 72367, 2012; № 78755, 2013; № 101388, 2013; № 103991, 2013; № 95714, 2015 [21].

For non-leguminous crops on the market of Ukraine monobiologics Agrofil, Albobacterin, Diazofit, Diazobacterin, Rhizoenterin, Polimixobacterin, Rhizobrazin, Flavobacterin [10, 11], Azotofit [12], Nitromais [13], Jamp Start [17], Rizofos Liq [18] and complex preparations Azotobacterin, Azogran, Azophosphorin, Bactophosphorin, Phosphobacterin, Ecophosphorin [9] Azohetomik, Biogran, Microhumin [10] Azolec, Agrinos [14], BTU complexes [12], Nitro Zlak, Rhizomix [13] are presented. Strains of rhizosphere diazotrophic bacteria, forming the basis of nitrogen-fixing preparations for non-leguminous culture, are produced in specialized research institutes of Ukraine and are covered by patents of Ukraine № 40548, 1993; № 40542, 1993; № 1524, 1994; № 5703, 1994; № 17694, 1997; № 48836, 2002; № 56032, 2005; № 72856, 2006; № 46103, 2009; № 59561, 2011; № 95554, 2011; № 102914, 2013; № 104212, 2014 [21]. Phosphate mobilizing microorganisms strains of microorganisms (biological agents for preparations that improve phosphorus nutrition of plants) patents of Ukraine № 54923, 2003; № 3203, 2004; № 12536, 2006; № 12537, 2006; № 50344, 2010; № 97198, 2012; № 98052, 2012 are obtained [21].

- *Biologics of phytoprotector action.* Their agents are microorganisms (bacteria, fungi) that produce substances of bactericidal, fungicidal, insecticidal activity.

Phytoprotector preparations application provides reduction of plants damage by various fungal, bacterial, viral diseases, and also inhibits the development of insects and rodents. In some cases, biofungicides and bioinsecticides can completely replace chemical analogues. To protect legumes the Respecta preparations based on *Pseudomonas aureofaciens* and metabolic products of bacteria [11], the Hetomik preparations (biological agent is *Chaetomium cochliodes* 3250 — biogenic elicitor of arachidonic acid producer) [10] in conjunction with specific for plants rhizobia inoculation are developed. For non-legume crops — preparations based on monoculture and products of its metabolism, in particular of streptomycetes (Averkom) [9], of fungi of *Aschersonia* genus (*Aschersonia*) [15] and of *Trichoderma* genus (Fungistop) [13], of the fungus *Beauveria bassiana* (Bals) Vuill. (Boverin) [15], of different species of spore bacilli — *Bacillus subtilis* (Bactofit) [13] (Phitocid) [12], (Phitosporin) [9], *Bacillus amyloliquefaciens* (Phytodoctor) [9], *Bacillus thuringiensis* (Bitoxibacillin) [10], (Lepidocide) [10, 15], of the spore-forming bacteria *Paenibacillus polymyxa* (Biopolicid) [11], of the bacteria *Pseudomonas aureofaciens* (Pseudobacterin, BioZlak) [13], of the murine typhus bacteria *Salmonella enteritidis* var. Issatchenko (Rodenta, Bacteroncid gel, Antimyshin) [10, 11, 13], of the nuclear polyhedrosis virus (Virin and its modifications) [15] are developed. Azostrept, Gaupsin, Averkom-Nova [9], Sabrex Root Inoculant may serve as the examples of complex biologics of phytoprotector action [16]. Biological agents for phytoprotector preparations are selected by Ukrainian microbiologists and covered by patents of Ukraine № 49390, 2002; № 56033, 2004; № 49390, 2004; № 69639, 2006; № 49158, 2010; № 77141, 2013; № 79741, 2013; № 107972, 2015 [21].

• *Biologics for accelerating the decomposition of plant stubble remains and restoration of soil fertility.* Agents are microorganisms with a high cellulolytic, xylanolytic, pectolytic, hydrolase activity that promotes deeper and more efficient decomposition of stubble remains and are characterized by the enzyme complexes stability in a wide range of temperature and pH, the ability to produce biologically active compounds as growth activating and protective action. On agrarian market of Ukraine following preparations of this group are presented — Azotofit based on *Azotobacter chroococcum* [12], Biopreparation for stubble remains utilization based on aerobic

spore-forming bacteria *Bacillus subtilis* [9], CD (cellulose destructor) based on a set of spore and non-spore bacteria, microscopic fungi: bacteria *Paenibacillus polymyxa* 6M, *Azotobacter vinelandii* 87S, fungi *Trichoderma harsianum* 15S (patent of Ukraine № 92030, 2014) [11, 21], Organic-balance based on concentrate of viable and inactivated microorganisms of different taxonomic groups and their active metabolites: the cells of bacteria *Bacillus subtilis* 221, *Paenibacillus polymyxa*, *Azotobacter*, *Enterococcus*, *Lactobacillus*, macro- and microelements, BAS — nicotinic and pantothenic acids, pyridoxine, biotin, heteroauxins, gibberellins, cytokinins, enzymes, fungicides and bactericides [12], Effect Bio — based on fungi *Trichoderma viride*, *Trichoderma lignorum* mycelium and chlamydospores, and also live bacterial cells *Bacillus acidocaldarius* [13], Embiko — based on effective microorganisms with different functional activity such as *Lactobacillus casei* strain 21, *L. lactis* strain 47, *Rhodospseudomonas palustris* strain 108, *Saccharomices cerevisiae* strain 76 as well as their metabolites [10].

At the same time, biological agents — components of biopreparations due to their inherent sets of functions can provide a comprehensive (growth regulative and phytoprotective) action, as well as improve the soil constitution.

Microbial biopreparation created on the basis of bacterial monoculture, is characterized by action stability in the range of 60–70% of its potential realization [6], as monoculture is more sensitive to the effects of environmental factors. Therefore, along with monoinoculants usage in agricultural production for seeds bacterization the development of complex biological preparations or compositions based on several types of microorganisms under condition of their ecological and physiological compatibility, as well as fungi, algae and growth regulative and phytoprotective action BAS [5–7, 22, 23] is promising. Such works are started by Ukrainian microbiologists about 20 years ago [24] and actively carried out today [4–7].

Assessing the level and the state of biotechnological research in Ukraine, aimed at ecological crop production obtaining, it should be noted the presence of a powerful scientific potential of human resources capable of creating necessary for agricultural production microbial products. In recent decades, scientists from Zabolotny Institute of Microbiology and Virology of the National

Academy of Sciences of Ukraine (IMV NASU), the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (IPPG NASU), the Institute of Agricultural Microbiology and Agricultural Production of the National Academy of Agrarian Sciences of Ukraine (IAMAP NAASU) and its South Branch, Institute of Agroecology and Environmental Management of the National Academy of Agrarian Sciences of Ukraine (IAEM NAASU), the Institute of Plant Protection of the National Academy of Agrarian Sciences of Ukraine (IPP NAASU) developed competitive biotechnological products for crop research [9–11, 14, 15].

The main requirements for microbial biopreparations used in the cultivation of crops and for crop production, are their environmental friendliness, efficiency and economic efficiency. In this regard, the microbial preparations and compositions should be based on different types of agriculturally useful soil microorganisms, which are obtained by analytical selection from natural sources (soil, rhizosphere, root nodules of leguminous plants, etc.: Ukrainian patent № 59033, 2005) [4, 21], which makes them environmentally friendly as biotechnological elements in crop production. According to our results, the inoculation of leguminous plant seeds, such as soybeans and peas, by specific species of highly active, competitive, efficient nodule bacteria *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* bv. *viciae* ensure by 10–15% higher crop yields [7]. Bacterization of wheat seeds by nitrogen-fixing active strain of *Azotobacter chroococcum* T79 improves the grain productivity of spring wheat up to 10% with the improvement of the basic elements of crop structure: the length (by 13%), the weight of wheat spikelet (by 8%), the number and weight of grains per spikelet (by 16 and by 9%, respectively), as well as microbiological indicators of soil due to the introduction and development in the rhizosphere of the nitrogen-fixing microorganisms populations [4, 7, 25].

Stabilization of the action and increase of the effect of bacterial monocultures are achieved by the introduction of additional agents meeting the requirements of three “E” (environmental friendliness, efficiency, economic efficiency) and also by the creation of complex biological compositions.

In the selection of additional agents of the compositions one should proceed from the following objectives:

- improvement of nitrogen and phosphorus nutrition of plants;

- ensuring of plants by substances of growth regulatory and phytoprotector action to enable the growth and development of plants, increase of yield productivity and protection from disease;

- bioremediation of soils, soil fertility restoration and preservation.

Analysis of currently available experimental results in this area allowed defining a number of approaches to solve these problems.

1. Improvement of plants nitrogen nutrition can be achieved by expanding the range of phyto-bacterial systems nitrogen-fixing ability during plants growing season due to the introduction of new species or strains of diazotrophic microorganisms (as nodule bacteria and rhizobacteria), as well as nitrogen-fixing algae or natural metabolites that activate the functioning of nitrogenase complex [4–7, 23].

2. Improvement of plants phosphorus nutrition — by introduction of rhizobacteria or fungi, which transform poorly soluble phosphorus compounds in the soil [4–7, 22].

3. Providing of plants by growth regulative and phytoprotective substances is achieved by introducing into the microbial composition of rhizobacteria, algae, fungi — producers of biologically active substances, mainly hormones of auxin, cytokinin and gibberellin nature, vitamins, microbial antibiotics and fungicides, insecticides, antioxidants, as well as plant and microbial metabolites with this activity introduction into the composition [4–7].

4. Bioremediation of soil — due to introduction into the composition of microorganisms carrying plasmids for xenobiotics degradation or using pollutants as energy substrates [6, 7].

5. Restoration of soil fertility with the usage of microorganisms-destructors of cellulose, pathogens’ antagonists, biologically active substances producers [4–7].

As additional agents while creating compositions based on nodule bacteria (for legumes) or rhizosphere diazotrophic microorganisms (for non-legumes crops), we used [7]:

- PGPR-bacteria of the genera *Azotobacter*, *Enterobacter*, *Agrobacterium*, *Pseudomonas*, obtained by analytical selection and have complex of positive effects on the system “plant — soil — microorganisms” (nitrogen-fixing activity, phosphates and xenobiotics disintegration, growth regulative and phytoprotective substances synthesis);

- blue-green algae of *Spirogyra* sp., characterized by the ability to produce

biologically active metabolites with growth activating and antioxidant effect;

- bioactive microbial metabolites (exopolysaccharides, exometabolites) and plant metabolites (lectins), possess a wide range of physiological actions, including activation the function of diazotrophic bacteria nitrogenase complex.

Evaluating the effectiveness of complex compositions in a greenhouse and field experiments showed [17] the presence of:

- growth stimulation, formation of plants vegetative mass (by 11–47%) and the grain yield (by 12 to 22%) of spring wheat of sort Rannya 93 using binary bacterial composition (agrobacteria + azotobacter) based on rhizobacteria of genera *Azotobacter*, *Agrobacterium* and ternary composition Koktail based on bacteria of the genus *Enterobacter* for pre-sowing seed inoculation, and also increase of seed productivity of soybean of sort Maryana in the field conditions on average by 4.6 cwt/ha and of sort Annushka on average by up to 14% in the greenhouse experiments by bacterial binary composition rhizobia + azotobacter of soybean seed bacterization compared with seeds rhizobia monoinoculation;

- full development of clover plant of sort Darunok and functioning of phyto-bacterial symbiotic systems with the 1.6–2.3 times increased nitrogen fixation level on the xenobiotics contaminated substrate (sodium benzoate) at seeds inoculation by binary bacterial compositions based on clover nodule bacteria and pseudomonades carrying the degradation of xenobiotics plasmid RP4: TOL;

- gain of nodulation (1.2–2.3-fold) and nitrogen-fixing (1.3–6.5-fold) ability of root nodule bacteria of soybean and lupine in symbiosis with soybean plants of sort Chernoburaya, Maryana and lupine of sort Soyuz when microbial exopolysaccharides or exometabolites specific for rhizobium culture entering into rhizobia inoculation suspension;

- activation of rhizobia symbiotic properties and increasing productivity of soybean – rhizobium symbiosis using blue-green algae *Spirogyra* sp. extract having growth regulative and antioxidant activity. Nodulation capacity increased 1.2–2.9 times and nitrogenase capacity — 1.6–4.1 times. Soybean plants of Maryana sort actively grown (1.1–1.3-fold faster) and formed above ground mass (1.2–1.7-fold), and also the grain crop in the field was by up to 11% higher as compared to seeds inoculation with rhizobia monoculture;

- enhance the implementation degree of symbiotic and productive potential of rhizobium-legume symbiosis of soybean of Maryana, Annushka and pea of Vostok using the specific lectins as additional agents: (nodulation activity — 1.4–2.6-fold and 1.3-fold, nitrogen-fixing activity — 1.5–3.0-fold and 1.2-fold, respectively), the intensification of plant photosynthetic activity (1.2–1.3-fold), stimulation of vegetative growth (1.1–1.4-fold) and increasing the grain productivity in the field an average up to 10% in comparison with rhizobia monoculture and up to 17% with uninoculated control. The application of lectin composition for wheat seeds treatment contributed the increasing of functional (nitrogenase) activity of rhizosphere nitrogen-fixing microorganisms (1.4–3.6 times), of seeds field germination (1.3-fold), of chlorophyll content in the leaves of vegetating plants (1.1–1.3-fold), as well as the growth stimulation, vegetative mass accumulation and grain formation of plants. Yields of spring wheat of Rannya 93 in the field increased by 17.4% or 3.4 cwt/ha, of two-handle wheat of sort Zymoyarka in growing it as a spring form — up to 21% or 8 cwt/ha, winter wheat of sort Podolyanka — by 3 to 16% or 0.6 to 6.1 cwt/ha as compared with uninoculated control. At the same time, the increase in grain yield was due to the positive changes in the main elements of its structure — increasing the length of the spikelet by 10–15%, the number of spikelets per plant by up to 7%, grains in spikelet — by 16–26%, the mass of grains in spikelet- by 25–33%, the mass of 1000 grains — by 6–7%. The improvement of bread bakery properties of winter wheat of Podolyanka, in particular, the gluten quality due to the index deformation by 5 points and the bread volume by 23 cm<sup>3</sup> increase;

- activation of seeds germination (1.1–1.2-fold), the formation of vegetative mass (1.1–1.7-fold) and yield (by 11–17%) of soybean plants Annushka by increasing the basic elements of its structure: the number of fruit units on the plant (by 6–15%), number and weight of seeds per plant (by 22–27 and 20–25% respectively), the mass of 1000 seeds (by 5–8%), and also the gain on the roots nodule formation (1.2–2.5-fold) and the functional activity of nodules (1.3–3.2-fold) using polycomponent compositions based on nodule bacteria, rhizobacteria of *Azotobacter* genus and phytolectins (soybean seeds lectin, wheat germ agglutinin) as compared to rhizobia monoculture.

Economical use of new complex composition exhibits in production cost reducing, its profitability and net income of soybeans and wheat growing improving [7]. The novelty of the approaches and of complex biological compositions development is confirmed by patents of Ukraine (№ 55620A, 2003; № 62819A, 2003; № 62820A, 2003; № 41723, 2009; № 59561, 2011) [25–29].

Thus complex compositions on the basis of soil diazotrophic microorganisms and additional biological agents (plant and microbial metabolites, algae, rhizobacteria) may be promising biotechnological elements for use in crop production.

Numerous experimental investigations in various scientific institutions of Ukraine and abroad indicate a variety of approaches allowed to create a complex microbial biopreparations and compositions for crop production, in particular with the usage of:

- the several strains of one nodule bacteria species adapted to different types of soil and agro-climatic conditions with an extended range of functional activity. Moreover, they can be the natural strains consortia and man-made mixes of microorganisms. Examples are preparations for soybean seeds inoculation of company ABM, Imperial Agro [16]: Excalibre — a proprietary mix of three strains of soybean rhizobia (ABM201 — for moist and cold soil, ABM202 — for hot and dry soil, ABM203 — for soils with low humus content), ABM Inoculant on the basis of peat and three strains of soybean rhizobia, Graph-Ex — a mix of three strains of soybean rhizobia with innovative carrier from graphite and talc composition, ABM Inoculant Plus Extender 30 — a mix of three strains of soybean rhizobia and formular Extender 30, preserving the effectiveness of the preparation on the seeds up to 30 days;

- the mix of rhizobia with nitrogen-fixing rhizobacteria or some species (strains) of nitrogen-fixing rhizobacteria for the growth activity of microbial cultures, bacterial nitrogenase activity enhance and the level of plants nitrogen nutrition increase (patents of Ukraine № 1524, 1994; № 48836, 2002; № 62820, 2003; № 79361, 2013; № 103966, 2013) [21]. Azotobacterin, Ecoriz (IMV NASU) [9], Biogran (IAMAP NAASU) [10] can be referred to as such preparations;

- the nitrogen-fixing (rhizobia, rhizobacteria) and phosphate mobilizing (rhizobacteria, fungi) microorganism compositions to improve nitrogen and

phosphorus supply (patents of Ukraine № 89120, 2009; № 101388, 2013; № 105276, 2014) [21]. The preparations of this series Ecobact with xanthan and lipkogen EPAA and also Ecovital developed by IMV NASU for legumes are represented [9]. The preparations Azogran, Azophosphorin, Bactophosphorin, Ecophosphorin, BTU — peat organic fertilizer (IMV NASU) [9], Biocomplexes BTU (BTU center) [12], Nitrozlak, Rhizomix (BIONA) [13] for non-leguminous crops on the market of Ukraine;

- the compositions of nitrogen-fixing bacteria (rhizobia or rhizobacteria) and microorganisms (fungi, bacteria) and also microorganisms — pathogens' antagonists and producers of metabolites with bactericidal, fungicidal, insecticidal action (patents of Ukraine № 77141, 2013; № 78164, 2013; № 79741, 2013; № 106034, 2014) [21]. In order to protect soybean against root rot, Moldovan scientists have developed biologics based on *Trichoderma virens* [30] that contribute to the plants active development during the vegetation period and to the high yields formation. Company ABM [16] presented the preparation Graph-Ex SA — three strains of soybean rhizobia — in combination with the preparation Sabrex Root Inoculant based on the mix of multifunctional races of fungi *Trichoderma microbias* for soybean protection against diseases, and for active symbiosis in different agroclimatic conditions. When using soybean root nodule bacteria with the preparation of the protective action Hetomik [10], the degree of plants infestation decreasing, the amount of fungi *Fusarium* in rhizosphere reducing and the amount of fungi *Trichoderma* and *Acremonium*, having antagonistic properties with respect to phytopathogens, increasing are noted [5]. Preparation Azohetomik to protect of non-legume crops is designed using this approach [10];

- compositions of nitrogen-fixing, phosphate mobilizing microorganisms, fungi and BAS of growth-stimulating and phytoprotective action (patents of Ukraine № 47304, 2002; № 55620, 2003; № 62819, 2003; № 37579, 2008; № 46103, 2009; № 95554, 2011; № 97198, 2012; № 107972, 2015) [21]. On the market of Ukraine the following preparations developed using this approach are presented: Rhizostim, Azolec [14] — based on diazotrophic microorganisms (soybeans and peas nodule bacteria and rhizobacteria azotobacter respectively) and specific plant lectins (SSL — soybean seeds

lectin, WGA — wheat germ agglutinin); Optimize 200, Optimize 400, Optimize Pulse (Novozymes — Monsanto alliance BioAg) [17] — based on soybeans and peas nodule bacteria and synthetic analogue of rhizobia lipohitooligosaccharide molecules; Rhizohumin, Microhumin [10] — based on nitrogen-fixing bacteria (rhizobia and rhizobacteria) and BAS of vermicompost for legumes, vegetables, cereals, buckwheat; Agrinos (company “Agrinos”, Norway) [14] — based on nitrogen-fixing bacteria *Azotobacter vinelandii*, *Clostridium pasteurianum*, free amino acids, chitin, chitosan, glucosamine; phytoprotective biologics for plant treatment Averkom-nova [9] with the ethanolic extract of the biomass of the strain *Streptomyces avermitilis* IMB Ac-5015 with avermectin level of 100 µg/ml and the culture supernatant of the same strain (1:1), and the chitosan biopolymer (0.01 mM) (patent of Ukraine № 107972, 2015) [21]. Compositions combining microorganisms and flavonoids, hormones, polysaccharides, seed extracts, as well as natural plant growth regulators (Agrostimulin, Emistim, Biosil et al.) are at the stage of research [6, 7].

The biologics of new generation created by methods of cell and molecular biology, as well as with nanotechnology usage, are of particular interest. The basis for such preparations is alive culture of active microorganisms and their metabolites, which determines preparation efficiency and tropism of action. Additional introduction of components — metabolites of plants and microorganisms involved in the formation and functioning of phyto bacterial systems and also chelated forms of macro- and microelements allows creating preparations of a new level and submitting them to the market of agricultural products. New biotechnological approach yields positive results in crops growing.

IGET Technology (Induced Gene Expression Triggers) for the creation of modern microbial biopreparations deserves special attention and more detailed consideration. This technology is based on the usage of signal-triggers that activate genes in plants or microorganisms. Drugs produced using IGET Technology, — it is mostly the product of foreign companies, in particular — the ABM, as well as of alliance BioAg, which combined the two leading companies in the area of innovative agricultural technologies and microbiology — Novozymes and Monsanto. The result of 30 years of research of scientists from Cornell University (USA) with the assistance of several

international research programs and about one hundred field trials was biologics creation by the company ABM [16], presented on the Ukrainian market, — Sabrex Root Inoculant, Excalibre SA and Graph-Ex SA. The biological agent of the first preparation is a mix of fungi *Trichoderma reesei* multifunctional races on innovative media based on talc and graphite. The preparation is intended for maize and wheat; it ensures the development of a strong root system, improves the wheat bushiness and the resistance of plants to stress factors, creates conditions for effective water and nutrients usage by plants. Preparations Excalibre SA and Graph-Ex SA are inoculants for soybeans.

Examples of biologics created by alliance BioAg Monsanto [17] using IGET Technology are:

- the line of drugs Optimize: Optimize 200, Optimize 400, Optimize Pulse — inoculants for soybean and pea based on homologous for host plants nodule bacteria, and synthetic promoter Lho — analogue of rhizobia Lho. Rhizobium Lho is a signal molecule, *Nod*-factor of the bacteria, synthesized as a result of rhizobium *nod*-gene induction by plant metabolites — flavonoids. *Nod*-factor participates in the early stage of the nodules on the plant roots formation as trigger of numerous morphological and physiological processes in plants — the deformation of root hairs, root nodule structures ontogeny, cortical cells division, organization of actin microfilaments in root hairs cells changing, plant nodulin genes expression, as well as a number of intracellular processes — intra — and extracellular alkalizing, membrane potential depolarization, ion fluxes changing [28–31, 32]. It is known about the use of flavonoids and synthetic *Nod*-factors — rhizobium Lho analogues to enhance the realization degree of the symbiotic and the productive potential of legumes, such as soybeans [33], as well as about the activation of the plants lateral roots formation and arbuscular — mycorrhizal symbiosis by Lho-molecules obtained from mycorrhizal fungi [34]. Lho promoter technology (alliance BioAg Monsanto-Novozymes) is created on the basis of synthetic Lho — analogue of soybean nodule bacteria *Nod*-factor usage. The advantage of Lho technology is earlier nodulation due to Lho-signaling use, during which a cascade of macrosymbiont responses runs, aimed at enhancing the formation and functioning of symbiotic systems, thereby providing an increased nodule formation level (2.4–2.5-fold according the results of IPP NAASU research

held in Kyiv and Cherkasy regions of Ukraine), nitrogen fixation, the activity of soil nutrients intake increasing, expanding the host plant varieties range, which are responsive to inoculation, the productivity of grain legumes increasing (yield increase was as follows: up to 2 cwt/ha of peas — based on the results of 83 trials in 2004–2013 in United States and Canada, up to 0.9–1.1 cwt/ha of soybeans — based on the research findings of PPI NAASU held in 2010 in Kyiv and Cherkasy regions of Ukraine).

- the preparation Tork RT with Lho-technology is designed for corn seeds processing with a solution of rhizobia Lho synthetic analogue, resulting in roots active mycorrhization and bacterization with spontaneous soil microflora, increasing the availability of nutrients for plants and improved absorptive capacity of roots, plant growth and development, efficiency formation. The average yield increase of corn according to the research of (IAEM NAASU) in 2013 in three regions of Ukraine (Kyiv, Vinnytsia, Odesa) was 5-8 cwt/ha, 4-6 cwt/ha, 4-7 cwt/ha with protein content in grain increase by 1.1–1.3% 1.2–1.4% 1.3–1.6%, respectively.

- the preparation Ratchet RK (soluble concentrate) with Lho-technology — leaf biostimulator for soybean and maize foliar fertilizing (both separately and together with the majority of post-emergence preparations). The results of field investigations in 2012–2014 (IAEM NAASU) carried out in three regions of Ukraine (Vinnytsia, Odesa, Mykolaiv), witnessed an increase of corn yield by 3 to 13 cwt/ha and soybean yield by 2 to 5 cwt/ha simultaneously with protein content in grain increase by 0.7 to 2.3%.

Domestic biotechnology product obtained using innovative IGET Technology is a line of preparations containing protein components — plant lectin. Preparations Rhizostim and Azolec (IPPG NASU) [14] on the basis of nitrogen-fixing microorganisms — nodule bacteria of soybeans, peas (Rhizostim), rhizobacteria of genus *Azotobacter* (Azolec) and plant lectins — lectin of soybean seeds and peas (Rhizostim), wheat germ agglutinin (Azolec) are registered in Ukraine up to 2022. Preparation for potatoes (IAMAP NAASU) based on natural consortium of the strains of the genus *Azotobacter* and potatoes lectin is at the stage of research [5]. Phytolectins are plant signal molecules (triggers) causing responses of symbiotic bacteria that lead to the formation of legume-rhizobium symbiosis and natural plant associations with bacteria.

These proteins are also characterized by a wide spectrum of biological activity (bioeffector, communicative, adaptogenic, protective, growth regulative) [7, 35]. The results of phytolectins biological activity in complex inoculants are increasing titer of microorganisms culture, earlier and more active nodules formation on the plant roots, high nitrogenase activity of symbioses, the development in plants rhizosphere of nitrogen-fixing microorganisms strong population with high functional (nitrogenase and growth activating) ability, plants active development, vegetative mass and grain formation, photosynthesis activation, increased photosynthetic pigments, phytohormones of auxin and cytokinin nature, RNA and proteins (endogenous lectins, enzymes) synthesis in the leaves of growing plants, and also plants adaptogenic plasticity under biotic and abiotic stress factors due to antioxidant defense system activation and flavonoid compounds rising [7, 26, 29, 35].

The biotechnological products, that are represented on the market of Ukraine by Scientific and Production Company “Agrosvit”, are created on the basis of innovative TOP technology — osmoprotectant technology allowing thicken bacterial cell walls by osmotic hardening, by the Company Rhizobacter (Argentina) [18], which is a world leader in the production of microbiological products for agriculture since 1977. One of these products is an inoculant for soybeans Rizoliq TOP based on nodule bacteria strains *Bradyrhizobium japonicum* Semia 5079 and Semia 5080 with thickened cell walls that protect the bacteria from negative stress factors and ensure the high level of nitrogen fixation in symbiosis.

Innovative approach in complex preparations for crop production creating is agronomically useful microorganisms and microelements in chelated forms (necessary for the development and vital functions of both plants and soil microflora and also lipkogenes based on natural raw materials) association in complexes. Examples of such a product represented on the market of Ukraine may be domestic products of Agro Bio Complex technology the ABC-biocomplex (OOO SPC Avatar) [36], as well as complex preparation Niva 2B (BIONA) [13], and the product of foreign production Myrazonit (Stuttgart: Germany, Debrecen: Hungary Agricultural Universities) [19].

ABC-biocomplex includes nanoactivator Avatar-1, Biocomplex AT, biocolloids-



lipkogene. Avatar-1-ecologically ultrapure preparation based on natural acids carboxylates that act as chelators, is prepared using nanotechnology. The feedstock — ultrapure biogenic metals based on citrate or succinate in deionized water (water purity 99,99999%) [36]. Preparation has membranotropic action that promotes rapid digestibility by plants and allows its use in small doses. Biocomplex AT is characterized by phytoprotective properties relative to bacterial, mycoplasma and viral infections. The main active ingredients of the complex are nitrogen-fixing, phosphate and potassium-mobilizing bacteria, microorganisms-antagonists of phytopathogens. Biocolloid-lipkogen is polysaccharide component used to prevent the loss of the ABC-complex components on the seeds or plants when spraying. It does not change its structure and lipkogene-agglutinative ability in a wide temperature range (-2 up to +80 °C). The use of ABC-biocomplex (pre-sowing seeds treatment, plants foliar feeding) helps to improve plants nutrition, enhance crop yields and quality of agricultural products, seeds sowing properties, plant resistance to diseases of various etiologies, improve soil fertility and reduce their phytotoxicity and also effects on the succeeding crops [36]. According to the results of field studies [37], simultaneous usage of nodule bacteria and nanoactivator Avatar for soybean seeds pretreatment has led to an increase in nodulation rhizobia activity (quantity and weight of nodules in plants increased in comparison with seeds monoinoculation with bacteria respectively 1.7–3.3 and 3.0–3.5 times). Nitrogen-fixing activity of root nodules depending on the preparation Avatar dose (1 l/t or 2 l/t) increased 2.7 to 7.7 times. Grain yield increased respectively — by 13 and 22% in comparison with the strain-control and by 23 and 33% in comparison with absolute control (without inoculation of seed). The obtained results allowed the authors to conclude that the use of a comprehensive pre-sowing treatment of soybean seed by rhizobia and fertilizer “Avatar 1,” containing microelements in aqua chelates form, contributes to more efficient formation of symbiotic apparatus in soybeans, increases its nitrogen-fixing activity and has a positive effect on the formation of this culture grain productivity. The most effective dose was found 2 l per 1 ton of seeds [37].

Niva 2B [13] — multifunctional biological preparation based on alive cells

*Pseudomonas aureofaciens* producing natural antibiotic piolyuteorin and auxin growth hormone IAA and also chelated microelements (zinc, cobalt, bromine, molybdenum, iron, manganese, copper), humic and fulvic acids. The preparation is intended for crops spraying in the phases of 2–3 leaves, of budding — flowering for protection against fusariosis, anthracnose, septoriosi, ascochyti, gray decay and bacterial infections.

Myrazonit [19] — a preparation on the basis of soil bacteria *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas putida* with agronomically valuable properties (nitrogen fixation, phosphorus transformation, auxins, cytokinins and gibberellins synthesis, high cellulase activity, pollutants cleavage) and also microelements and group B vitamins. The preparation has complex action, in particular contributes to early germination, improves the nitrogen, phosphorus and potassium nutrition, micronutrients absorption, improves the plant growth, the root system formation, the vegetative mass, increases the chlorophyll content, photosynthetic efficiency, crop yields, improves the structure and restores soil fertility, promotes the decomposition of plant residues, increases plant resistance to biotic and abiotic factors.

In addition to the search for new effective and active species and strains of microorganisms as the main preparation biological agents and to the development of new approaches to the creation of preparations, an active work to improve and enhance the processability of microbial agents, in particular the search for new carriers, lipkogenes, formularies, extending the viability of the bacteria when applied on seeds, et al. is underway.

Liquid preparation forms as well as preparations on the bases of peat are developed in the twentieth century. Nowadays new carriers for microbial cultures in preparations discovery takes place, the followed are mainly used: vermiculite, defecate, biohumus, clay materials (patents of Ukraine №47304, 2002; №53187, 2003; №95376, 2011) [21], activated carbon (preparation Rhizoactiv) [9], which has a large adsorption surface (1 g of carbon has a surface of 800 m<sup>2</sup>), which allows to keep the bacteria-bioagents in the preparation and increase their titer, and also innovative carriers graphite and talc (preparation Graph-Ex of ABM company) [16], providing excellent sowing seeds fluidity and sowing elements lubrication.

One of the innovative technological methods is the use of modern lipkogenes, adhesives — sticky film formers, providing a higher level of inoculants with seeds contact when pre-sowing bacterization or with leaves of plants when spraying on phases of vegetation. Urea resins, cellulose and starch water-soluble forms, microbial exopolysaccharides (patent of Ukraine №54847, 2010) are used as lipkogenes [21, 38]. On the market of Ukraine biological gel preparation EPAA developed by domestic scientists on the basis of microbial polysaccharides is presented [38, 39] (IMV NASU, Ukraine patent №60637 2011; №96883, 2011) [9, 21], which is used, in particular, when creating preparation Ecobact (IMV NASU, Ukraine patent №89120, 2009) [9, 21], as well as Adyugreyn (BIONA) [16] on the basis of biologically active substances of natural origin, surface-active polymers, phyto hormones, nutrients, humektants, natural cryoprotectants; bio-glue Liposam (BTU center) [12]; Biocolloids-lipkogen (OOO SPC Avatar) [36].

Another one of the innovative elements in microbial preparations for agriculture creation is the use of formularies, mostly of polysaccharide nature, that contribute to maintaining the viability of microorganisms in the seeds (patent of Ukraine №54847, 2010) [21]. On the market of Ukraine, in particular, the formulary Extender 30 (company ABM) [16], preserving the effectiveness of the inoculant on the seed up to 30 days is presented; as well adhesive-stabilizer Extender (Argentina) on the basis of polysaccharides of natural origin, preserving the viability of nodule bacteria cultures on the seeds and prevents the effect of environmental factors such as soil drought and over wetting. The last formulary is used to create the preparation Nitrofix Pre Noctin A (company BIONA) [13], and is intended for preliminary (21 days) seed treatment. Formularies included in a line of preparations Optimize (Novozymes — Monsanto alliance BioAg) [17] and ensuring the viability of inoculants within the space of week (for pea) up to 120 days (for soybeans) without reducing the activity of microorganisms — bioagents of preparations, are also active. Biological protector Premax (Rhizobacter, Agrosvit [18]) provides seeds uniform coating, keeps the bacteria on the seeds, feeds the bacteria, keeping them active during the period from inoculation to germination, supports the

required number of bacteria on the seeds upon their contact with the soil during seeding, and protects inoculated seeds.

All microbiological preparations contain living microorganism cells, so you must strictly adhere to the following conditions of their use.

1. Drugs adding to the soil or spraying the plants should be done in the morning or after rain, but in any case not in the sun, because the sun's rays detrimental effect on microorganisms and can reduce the effectiveness of preparations.

2. The development of microorganisms is influenced by temperature so the preparation introduction into the soil or into seeds should be done preferably in a warm, but not in a hot weather.

3. Plant spraying should be finely dispersed since large drops easily roll off the leaf surface.

4. The effectiveness of any microbial preparations is increasing, while the use of organic fertilizers and crop rotation.

*Microbiological preparations application method includes:*

1. Seeds pre-sowing treatment, which is carried out the day before or in a day of sowing (other than preparations with extenders, adyugreynes, etc. preserving viable microorganisms on seeds 7 to 120 days). Seedlings are treated when planting. The seeds were sprayed, stirred (manually or in machines for etching purified from pesticides) and gently dried in air. The treatment is carried out in a place protected from direct sunlight.

2. Plants spraying during the growing season, which is carried out by fine spray droplets in the absence of direct sunlight and rain in certain phases of plant development.

3. The adding to the soil carried out by fine spray droplets in the absence of direct sunlight and rain.

The cost of domestic preparations is significantly lower than the cost of foreign counterparts. According to the results of the IAEM NAASU [40], in average the cost of a microbiological preparation is about 3–5% of the prime cost of one or another agricultural crop production, on the basis of that the cost of inoculants is economically inadvisable, exceeding these indicators. At observance of all the recommendations on the use of microbial agents, domestic products are not inferior to foreign on effectiveness since biological agents — microorganisms which

they are based on, are isolated from the soil and are adapted to soil and climatic conditions of Ukraine.

Thus, on the market of Ukraine in 2015 a wide range of microbiological preparations for plant growing of multi-vector orientation of action are created with the involvement of a variety of approaches and technologies,

including the latest innovative technology. Compliance with the conditions of these preparations application in growing of different crop groups (legumes, cereals, industrial crops, fruit, etc.) will provide environmentally friendly crop production, reduce anthropogenic (chemical) pressure on ecosystems, and restore soil fertility.

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## АНАЛІЗ РИНКУ ТА СТВОРЕННЯ МІКРОБНИХ БІОПРЕПАРАТІВ ДЛЯ РОСЛИННИЦТВА В УКРАЇНІ

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Проаналізовано мікробні біопрепарати для рослинництва, зокрема під бобові, зернові, технічні та інші сільськогосподарські культури, як вітчизняного, так і закордонного виробництва. Наявні на ринку України біопрепарати, залежно від функціональної активності біологічного агента, можна розділити на основні групи: рістстимулювальної; фітопротекторної дії; для прискореного розкладання рослинних залишків, відновлення і збереження родючості ґрунтів, а також залежно від кількості компонентів, що входять до їхнього складу, — на моно- і комплексні біопрепарати. Розглянуто підходи до створення комплексних біопрепаратів і композицій на основі біологічних агентів — мікроорганізмів з агрономічно цінними властивостями, грибів, водоростей, біологічно активних метаболітів рослин і мікроорганізмів, а також мікроелементів у хелатованих формах, для вирішення основних екологічних завдань — отримання органічної продукції рослинництва, відновлення і збереження родючості ґрунтів. Наведено результати досліджень автора зі створення комплексних композицій для вирощування бобових і зернових культур на основі бульбочкових бактерій, ризобактерій, мікроорганізмів-деструкторів ксенобіотиків, мікробних (екзополісахариди, екзометаболіти) і рослинних (фітолектини, екстракт синьо-зелених водоростей) метаболітів. Установлено, що комплексні біопрепарати і композиції виявляють більш ефективну і стабільну дію порівняно з монокультурою. Наведено приклади біопрепаратів нового покоління, створених з використанням інноваційних технологій: IGET Technology (Induced Gene Expression Triggers), ЛХО-промоутер (ліпохитоолігосахарид-промоутер)-технологій, ТОП-технологій (технологія осмопротектора) із залученням розробок молекулярної та клітинної біології, нанотехнологій, а також сучасних твердих носіїв, адгезивів, ліпкогенів, формулярів, що забезпечують високий титр мікробних клітин у біопрепаратах, тісний контакт бактерій із насінням за інокуляції та пролонговане збереження життєздатності мікроорганізмів на насінні.

**Ключові слова:** мікробні біопрепарати, аграрний ринок України.

## АНАЛИЗ РЫНКА И СОЗДАНИЕ МИКРОБНЫХ БИОПРЕПАРАТОВ ДЛЯ РАСТЕНИЕВОДСТВА В УКРАИНЕ

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Проанализированы микробные биопрепараты, в частности под бобовые, зерновые, технические и другие сельскохозяйственные культуры, как отечественного, так и зарубежного производства. Представленные на рынке Украины биопрепараты, в зависимости от функциональной активности биологического агента, можно разделить на основные группы: ростстимулирующего; фитопротекторного действия; для ускоренного разложения растительных остатков, восстановления и сохранения почвенного плодородия, а также в зависимости от количества компонентов, входящих в их состав, — на моно- и комплексные биопрепараты. Рассмотрены подходы к созданию комплексных биопрепаратов и композиций на основе биологических агентов — микроорганизмов с агрономически ценными свойствами, грибов, водорослей, биологически активных метаболитов растений и микроорганизмов, а также микроэлементов в хелатированных формах, для решения основных экологических задач — получения органической продукции растениеводства, восстановления и сохранения почвенного плодородия. Представлены результаты исследований автора по созданию комплексных композиций для выращивания бобовых и зерновых культур на основе клубеньковых бактерий, ризобактерий, микроорганизмов-деструкторов ксенобиотиков, микробных (экзополисахариды, экзометаболиты) и растительных (фитолектины, экстракт сине-зеленых водорослей) метаболитов. Установлено, что комплексные биопрепараты и композиции обладают более эффективным и стабильным действием по сравнению с монокультурой. Приведены примеры биопрепаратов нового поколения, созданных с использованием инновационных технологий: IGET Technology (Induced Gene Expression Triggers), ЛХО-промоутер (липохитоолігосахарид-промоутер)-технологій, ТОП-технологій (технологія осмопротектора) на основе разработок молекулярной и клеточной биологии, нанотехнологий, а также современных твердых носителей, адгезивов, липкогенов, формуляров, обеспечивающих высокий титр микробных клеток в биопрепаратах, тесный контакт бактерий с семенами при инокуляции и пролонгированное сохранение жизнеспособности микроорганизмов на семенах.

**Ключевые слова:** микробные биопрепараты, аграрный рынок Украины.