
How to Increase the Productivity of the Soybean-Rhizobial Symbiosis

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1. Introduction

Fixation of molecular nitrogen is one of the processes determining the biological productivity of our planet, which is why its study is one of the primary tasks of modern biology. The nitrogen cycle in nature is one of the key components of biogeochemical cycles of the Earth. The atmosphere consists of almost 80% (by volume) of the chemical element and is its main source. Nitrogen makes up a part of proteins and other molecules that form the basis of the structural organization of all levels of life. Humans and animals need it in the form of animal and vegetable protein. Plants need it in the form of salts of nitric acid and ammonium ions. [1-3].

The economic and environmental crisis, the decline in the quality of crop production, and the deterioration of the natural fertility of soil - are all reasons for increased attention to biological farming, which focuses on using the potential of natural ecosystems, in particular, nitrogen-fixing microorganisms.

Microorganisms which assimilate molecular nitrogen in the atmosphere are diazotrophs and they have a similar biochemical mechanism of nitrogen fixation. There are two main groups of nitrogen-fixing microorganisms, those that enter into a symbiosis with plants and those living freely in the soil. The second group includes associative nitrogen fixers and microorganisms, more adapted to a free existence in the soil. The division of nitrogen fixers into freeliving and associative is conditional, since the ability to live freely in the soil is typical to all nitrogen-fixing bacteria, whilst only symbiotic nitrogen fixers are able to assimilate molecular nitrogen very closely with plants. Links of cyanobacteria with other organisms can be quite diverse: there are phycobionts in lichens which live in the air-cells of mosses, in the leaves of water ferns, and in other places. It should be noted that the potential of symbiotic nitrogen fixers is significantly higher than of those that are free living [4-7].

The symbiotic and associative systems of plants and diazotrophs are an example of the evolution of the interaction of living organisms. Their study is particularly relevant with the implementation of highly productive and environmentally friendly farming. The biological fixation of molecular nitrogen from the air is a process of fixation and assimilation of nitrogen by microorganisms. It is of great practical importance, since the industrial production of chemical nitrogen fertilizer requires significant use of costly energy resources, which by themselves can be harmful for the environment. A comprehensive study of this problem is necessary due to the need to create new and effective biological preparations. The creation and use of biological agents on the basis of nitrogen-fixing microorganisms is the most justifiable method of increasing the productivity of plants and the quality of their harvest, which allows maintenance of the natural fertility of soils and the ecological balance of the environment. Their use makes it possible to regulate the number and activity of beneficial microflora in the rhizosphere of crops, and to provide plants with nitrogen fixed from the atmosphere. For example, in addressing the shortage of high-grade protein the key role belongs to the soybean. However, the soils on which the crop is grown for the first time [8] usually do not have nodule bacteria compatible with soybean or bacteria number is small (up to 20 per gram of soil).

2. Legume Lectins as a Factor in an Effective Symbiosis

The full establishment and functioning of the legume-rhizobial symbiosis depends significantly on a number of abiotic, biotic and anthropogenic factors. In particular, the effectiveness of symbiotic nitrogen-fixing is affected by temperature, aeration, pH level and moisture content of the substrate, the presence of pesticides, the content of nitrogen and other macro- and micronutrients. Of further importance is the virulence and activity of root nodule bacteria. The study of the influence and the establishment of the degree of importance of different factors to the effectiveness of symbiosis, will determine the conditions, and help to develop methods of optimizing the functioning of the symbiotic systems.

Wide dissemination of lectins in a variety of plants and the presence of these proteins in virtually all organs and tissues of plant organisms demonstrate the importance of their role in life processes. Initially it was assumed that the presence of lectins was a distinctive feature of seeds from the legume family. However, the number of organisms in which lectins have been found is increasing every day and there is reason to believe that all the above mentioned plants as well as algae, lichens and fungi contain lectins [9-11]. Nevertheless, in the present day researchers focus much of their attention on lectins of leguminous plants, the increased study of which leads us to understand the activity of these proteins in relation to the structure of the latter.

Legume lectins are a large group of carbohydrate binding proteins derived mainly from seeds. Lectins contained in the seeds of legumes, are localized in the proteins and make up approximately 10% of soluble protein extract. In recent years it has been found that these

proteins are present in other parts of plants, including stems, leaves, bark, roots, and root nodules. Lectins accumulate in the vacuoles of cells and can come to the surface of the plants. They can also be associated with membranes and cell walls [12-15].

The basis of the biological activity of lectins is a reversible reaction with carbohydrates, which defines the different types of biological reactions [16] and their physiological significance. Lectins do not have uniform structural characteristics. In legumes, these proteins are generally composed of two or four subunits with a molecular mass of 25 000 - 30 000 and each having one carbohydrate binding site [15, 17]. Lectins can interact with both mono- and oligosaccharides, as well as the remnants of carbohydrates present in the complex organic substances - glycoproteins, polysaccharides, and glycosides. In the early 1980's there was established a carbohydrate specificity for many legume seed lectins. However indisputable physiological carbohydrate ligands for any of these proteins have not been identified [14].

Over the past half-century many hypotheses were developed regarding the role of lectins in the life of legumes, but so far none of them have been fully confirmed. These proteins were detected due to their ability to bind carbohydrates, and most hypotheses about their functions are based solely on this wonderful property. Since it is known that the plant may contain a significant number of lectins, which are localized in different tissues and may form a gene duplication, while carrying out a variety of functions, it is believed that any attempt to dedicate a specific role to these proteins is doomed to failure [14].

An important feature of most of the representatives of the family *Fabaceae* (Legumes) is the ability to enter into symbiotic relationships with rhizobia. They belong to the family of *Rhizobiaceae* (bacteria genera *Rhizobium*, *Bradyrhizobium*, *Mezorhizobium*, *Sinorhizobium*, *Azorhizobium*), and form special structures on the roots called nodules. Lectins are considered as a component of the molecular and chemical interactions underlying the formation of symbiotic structures [6, 9, 11]. The symbiosis between rhizobia and legumes is based on a complex sequence of morphophysiological changes in the cells of both partners. Stages in the development of symbiosis are: preinfection (chemotaxis of bacteria, the exchange of signals, the adsorption of microsymbionts on the root surface), infection of the roots and development of nodules (penetration, formation of infection threads, the transformation of rhizobia into bacteroids), as well as the functioning of the nodules of nitrogen fixation [9, 18, 19].

Plants throughout their lifespan release various matter into the environment. The presence of lectins has been found in the soil [20], which are secreted during the germination of seeds together with other biologically active substances [21]. It is believed that in the first stages of interaction between rhizobia and legumes an important role is played by the chemotactic response to bacteria. Nodule bacteria and other soil microorganisms, in response to plant exudates, stimulate the reproduction and active movement of bacteria towards the roots, colonizing the rhizosphere and rhizoplane. A positive chemotaxis of rhizobia on the root exudates and germinating seeds can be either nonspecific (due to the excretion of organic acids, carbohydrates, vitamins), or specific (induced by flavonoids and lectins) [9, 18, 22].

The initial stage of many plant-microbe interactions is recognition of partners, which is largely due to the exchange of molecular signals [23, 24]. This process begins with the exu-

dation by the host plant of chemicals such as flavonoids and betaines that induce the gene expression of nodulation in rhizobia [25]. Flavonoids induce the transition of bacteria from free-living to those in a symbiotic state [26]. The first chemically-characterized inducer of the expression of nod-gene was luteolin, drawn from the extract of lucern seeds [27]. For each type of rhizobia, plants produce individual flavonoid signals that can stimulate or inhibit the nod-genes of rhizobia [28]. The identification of factors by microorganisms which are released by plants initiates physiological processes required for the infection of the host plant. In turn, the microbial signals induce the plant to express the genes required for the formation of responses [5, 18, 25, 29]. Amongst the signals produced by the bacteria, the most studied are the lipochitooligosaccharide Nod-factors [14, 24]. During the formation of symbiotic relationships lectins on the plant recognize the nod-factor signal released by the bacteria. It is believed that lectins binding to the bacteria with the surface of root hairs are able to identify the rhizobial signaling molecules, through surface polysaccharides on the rhizobia [14, 30]. It has been shown that the lectin of dolichos roots, which is localized on the surface of root hairs, is able to connect with some of the Nod-factors [14]. Furthermore, from the soybean root tissue there has been found and characterized a chitin-binding protein of the plasma membranes of cells. The presence of this protein and the specificity of the induction of the biological response to the binding of ligand *Bradyrhizobium japonicum* indicates its importance in the initiation of response to the binding of chitin in soybean [31].

Therefore Nod-factors play the role of a trigger mechanism for initiating bacterial invasion and nodulation of the plant [24]. Lipopolysaccharides inform the plant about the transition to a symbiotic interaction and the formation of the so called functional areas [32]. Legume plants are unique in their ability to form a response to Nod-factors, which consist of a preparation for the penetration of bacteria into the roots. Unfortunately, we do not know how the representatives of this family developed the ability to recognize such signals and how they improved upon these mechanisms [26].

An important stage of preinfection is the adsorption of bacterial cells on the surface of root hairs [33, 34]. The process of attachment of the bacteria from the *Rhizobiaceae* family consists of several stages with the participation of bacterial surface proteins in the first stage (rickadhesin, porin) and polysaccharide fibrils in the second stage. The first stage, involving proteins, is more decisive for the success of infection than the second stage, mediated by specific cellulose fibrils. The latter promote the retention of bacterial cells on the surface of the plant, but are not necessary for the infection [35].

The adsorption of rhizobia has also been associated with the ability of legumes to synthesize specific glycoproteins called lectins, which bind to polysaccharides on the surface of rhizobia cells [36, 37]. It is believed [33, 34] that the ability of rhizobia to be adsorbed on the surface of the roots of the host plant (like the chemotaxis) may be either specific or nonspecific. The initial binding of rhizobial cells to the surface of root hairs occurs by the means of exopolysaccharides (EPS). The degree of affinity between EPS microsymbionts and host plant lectin determines the degree of homology between the symbiotic partners and provides an advantage over other homologous strains in the process of plant infection.

Dazo and his co-writer [38] proposed a model of the attachment of rhizobia to the surface of the roots of dicotyledonous plants. The first phase is non-specific attachment which is characterized by the fact that the polyvalent host plant lectin binds carbohydrate receptors on the cell surface of nodule bacteria. This results in an intercellular "bridge" between lectin and polysaccharide. The second phase is the "anchoring" of bacteria on the surface of plant cells. This is the phase of specific attachment. The bacterial cells attach to the plant cell, which is the signal for further stages of infection. At this point microfibrils are formed between the contacting surfaces which consist of cellulose [35].

The "Lectin" hypothesis which explains the specificity for formation of nodules as resulting from the complementary interaction of the surface structures of bacterial and plant cells. This forms an "antigen-antibody" complex, which was first recognized in the early 1980's. Although initially a controversial subject, Diaz and co-writer [39] provided strong genetic evidence that the root lectins of legumes determine the specifics of symbiosis development. They injected special strains of *Agrobacterium* into the roots of clover through transfer of the *psl* gene (codes for synthesis of pea lectin). This resulted in clover acquiring the ability to form nodules with pea rhizobia. A similar study ten years later had the same result [40]. Transgenic lucerne plants carrying genes encoding soybean or pea lectin generated a structure similar to that of the root nodule in response to inoculation with pea and soybean nodule bacteria producing the specific Nod-factor. However not all of the structures created were able to form complete infection threads. The results confirm the importance of the presence of lectin in establishing contact between the symbionts and demonstrates the unique role of exopolysaccharides (EPS) in the formation of nodules. *Galega officinalis* and *orientalis* are other examples demonstrating the importance of lectins in legume-rhizobial symbiosis. The validity of this result is confirmed by sequencing the amplifiers of DNA extracted from the seeds of the mentioned crops from different geographical locations [41].

Therefore numerous experimental data confirms the important role of lectins in the early stages of the symbiosis and suggests the involvement of these proteins in a variety of physiological processes of plants.

Once they have prepared each other with molecular signals, the partners begin to form the structural basis of symbiosis - a nodule. This leads to a morphophysiological differentiation of bacterial and plant cells [18, 25]. Nod-factors control the phenotypic changes that occur in the roots of the host plant (the initial stage of symbiosis.) In addition there is also the deformation and proliferation of root hairs [25], the expression of early nodule genes [5], the induction of mitotic divisions in the cortex, followed by the onset of the first stages of histogenesis of nodules [42, 43].

Based on the assumption regarding the participation of lectins in various physiological processes in the formation of symbiosis, much attention was paid by researchers to the study of the direct effects of plant lectins on the manifestation of the symbiotic properties of nodule bacteria. In particular, it has been shown that the incubation of nodule bacteria *Bradyrhizobium japonicum* with soybean root exudates and lectin from its seeds increased the activity of nodule formation in the mutated soybean HS 111, characterized by delayed nodulation, and in doubling the number of nodules formed by wild strain USDA 110 [44]. Later it was

shown that treatment of rhizobia by specific plant lectin increases their virulence and competitiveness [34], and also increases the quantity of infectious threads in the roots of leguminous plants [45]. The particular modulating effect of the plant lectin on the formation and functioning of symbiosis has been established, which is manifested in the stimulating effect on partners, homologous data of the proteins, and the neutral or suppressive reaction of lectin which is not in accordance with the symbionts [46]. Moreover it is shown that the incubation of nodule bacteria with homologous lectin has a positive effect on the symbiotic properties of the active strain *Bradyrhizobium japonicum* 634b, and the intensity of the basic physiological processes in plants - nitrogen fixation and photosynthesis. The introduction of the same protein into an inactive strain of rhizobia suspension *Bradyrhizobium japonicum* 604k has a suppressive effect on the symbiotic system which it took part in forming as well as the stated processes [47]. It has also been found that the nature of the influence of homologous lectin on the growth of nodule bacteria in a axenic culture and the biosynthesis of extracellular carbohydrates [48], the ability of rhizobia to form nodules and their nitrogenase activity, as well as its impact on the productivity of host plants depends on the concentration of this protein in the bacterial suspension [49].

Co-incubation of nodule bacteria with lectin enhances soybean growth processes in the early stages of ontogeny as well as in the functioning of the symbiotic system, increases the nitrogen-fixing activity and, consequently, the productivity of plants [47, 49, 50]. This is why in recent years work was done on the selection of the optimal concentration of the homologous lectin, and the length of time of its co-incubation with the culture of *Bradyrhizobium japonicum* in the production of bacterial fertilizers both in liquid and solid form. Lectin is used as a growth stimulating biologically active substance, which is introduced into the culture of bacteria under certain conditions [51].

In the bacterial model *Bacillus subtilis* where antibiotics were used as metabolic inhibitors, which block the processes of replication, transcription or transmission, there was established an ability of carbohydrate-binding proteins, lectins of plant origin, to have various effects on intracellular processes. Among the presumed processes affected by lectins were reparative functions [52]. It was also found that lectins from the seeds of leguminous plants that have a high molecular weight (> 100 000 Da) can stimulate the respiration of some *Rhizobium*. According to the authors [53], this effect is induced by the lectins, and significantly increases the interaction of lectin-*Rhizobium* due to the physiological properties of the bacteria.

The presented data gives cause to consider the homologous lectin, not only as a receptor or a signaling molecule in the initial stages of symbiosis, but also as a molecular signal that changes the metabolism of rhizobia, which significantly affects their symbiotic properties and the physiological status of the host plant.

In the process of development of nodules in legume plants, lectins are localized at least in three different places. Their possible functions are related to areas of infection on the surface of the roots. In the nodule primordia lectins can stimulate mitotic activity reducing the threshold of sensitivity to the rhizobial Nod-factors. At the same time, in the central part of the mature nodule, lectins can function as spare nitrogen compounds [54].

It is thought that legume lectins are involved in the formation of nodules. The activating effect of lectin on the synthesis of extracellular and capsular polysaccharides of rhizobia is shown, which in turn induces the formation of infection threads [55]. Data for the study of lectin gene expression during the development and functioning of root nodules of lucerne provided evidence supporting the involvement of these proteins in the early stages of the ontogenesis of nodules [56]. A study has been made of a number of genes and plant proteins encoded by them, which play a role in the formation and functioning of root nodules. Among them is a lectin related to the pea *ps1* gene with the presumed function as a mitotic stimulator and soybean lectin *le1*, which takes part in the attachment of cells [19].

It is established that the proteins extracted from lupine and soybean nodules are capable of hemagglutinating activity. A comparative analysis of the nitrogen-fixation of soybean nodules and the lectin activity of the protein extracts from these nodules revealed a link between these rates in ontogenesis [57]. This data suggests that the proteins which are capable of lectin activity may be directly involved in the processes of nodule functioning.

A hypothesis was made by Antoniuk and his co-writer that the lectin of wheat germs acts as a signal for *Azospirillum brasilense*, which changes the metabolism of the bacteria in a direction favorable to the growth and development of the host plant [58, 59]. According to the authors, the level of lectin in the plant depends on a number of conditions and is one of the factors responsible for the variability in the results of wheat inoculation.

Nodule bacteria penetrate the plant cell (as opposed to *Azospirillum*) and transform into bacteroids, which do not divide, but only increase in size. Nonetheless a number of analogies can be made between the influence of the wheat germ lectin on *Azospirillum* and the influence of a specific legume lectin on *Rhizobium*. In the first as well as in the second case, the homologous lectin has a positive effect on the symbiotic properties of bacteria, which promotes a more efficient interaction between the partners in the different symbiotic systems that they create. There remains the little-studied question regarding the participation of lectins in the functioning of the symbiotic apparatus of legumes, but it is possible that the presence of proteins with lectin activity in the nodules is associated with the biosynthesis of proteins (including nitrogenase) in bacteroids and in ensuring conditions for the effective functioning of their symbiotic system.

Legume lectins may influence the receptor and signaling molecules during the stages of the symbiosis. Hemagglutinating activity of the proteins contained in the nodules, varies depending on the efficiency of their functioning. Specific lectins are able to modify the symbiotic properties of the nodule bacteria which positively affect the physiological status of the host plant and, ultimately, the effectiveness of the symbiotic system. Uncovering the role that lectins have in the functioning of the symbiotic apparatus of legumes requires further research. At the same time these proteins can be regarded as one of the factors for the effective symbiosis, which must be considered when developing and implementing new approaches to the management of production process in legume plants.

3. Biotechnology of Diazotrophs

Microorganisms which are the basis of biological preparations must comply with certain requirements, as well as have certain properties including virulence, activity and effectiveness, specificity, competitiveness, and technological properties (the ability to accumulate titer in the standard and production environments). Among the general requirements for the development of new biological preparations are the following: a high titer of active bacteria cells, the desired length of shelf life, transportability, technological properties (solubility, the ability to stay on the seeds, etc.), as well as the economic efficiency of their production.

The efficiency of symbiotic systems, "plant - microorganism" is defined by the virulence and level of activity of microsymbionts. Virulence of nodule bacteria for example is the ability to penetrate into the soybean root through root hairs and lead to the formation of nodules by complex morphophysiological changes. The first stage of virulence is tumorigenic activity which is the ability to form tumors on the roots. Truly virulent strains are characterized by nodulating activity (nodulation), the ability to form complete nodules. A nodule is a complex organ of the plant, the main structure of which is made up of: tissue infected with bacteria where molecular nitrogen is fixed; conductive tissue through which photosynthates are delivered and products of nitrogen fixation are taken out; and the meristem, due to which the growth of nodules occurs [1, 5, 18].

At certain stages of the formation of associative rhizocenosis and legume-rhizobial symbiosis, other important properties of bacteria develop such as: nitrogen-fixing activity - the recovery rate of which is N_2 in NH_3 , and symbiotic effectiveness - the ability of plants to develop intensively, using symbiotrophic feeding on nitrogen. Symbiotic efficiency is to a great extent determined by the nitrogen-fixing activity of nodules, especially in conditions where there is a shortage of fixed nitrogen, but the efficiency of the symbiosis also depends on a number of factors not directly linked to nitrogen fixation. An important role in determining the productivity of plant-bacterial interactions can be played by the compatibility of the metabolic systems of the partners (for example, ways of transporting nitrogen and carbon), and the absence of active defense reactions in plants in response to the presence or the penetration of microorganisms. Bacteria found in the rhizosphere or nodules can synthesize substances that stimulate (phytohormones, vitamins) or inhibit (rhizobiotoxins) the development of the host plant. It has been found that the effective and ineffective strains of nitrogen-fixing bacteria differ on a number of biochemical parameters. Effective strains most probably have a far richer metabolic fund, and their acidifying- restoring processes occur more actively [1, 3, 6, 18].

Specificity is the ability of bacteria to selectively interact with a particular species or group of plants. Specificity is one of the important systematic characteristics of nodule bacteria and is closely linked to their activity. *Rhizobium* for example is divided into: active, low-active, and non-active. It should be noted that the virulence of the bacteria and the activity may depend on the certain strain, species, varietal specificity of the plant, soil, climatic conditions and other factors [5, 6, 18].

Different races of nitrogen-fixing bacteria compete with each other. The more virulent strains will more actively colonize or inoculate relative to other strains, the root system of plants specific to them. Researchers have different interpretations of the concept of bacteria competitiveness. Some consider it to be the ability to compete with the strains which spontaneously inoculate the plants; while others consider it to be resistance to the local saprophyte microflora and to displace the local strains [60, 61].

There are several methods for application of microorganisms to plants: a liquid culture, preparations on gel substrates (bacterial exopolysaccharides, silica gel, highly dispersed materials) and applications on solid carriers (vermiculite, lignin, perlite, peat) [6, 62]. Obtaining the production-strains of nitrogen-fixing bacteria and the creation of biological preparations based on them - is a long process of research and production (Fig. 1), in which we can distinguish the following stages:

1. Research:

- reisolation of the nitrogen-fixing microorganism from its natural habitat;
- an introduction into the culture and analytical selection of perspective strains;
- generation of new highly effective strains using genetic engineering methods;
- the study of physiological and biochemical characteristics, symbiotic properties, competitiveness, efficiency and technological properties of nitrogen-fixing bacteria;
- conducting research trials, registration, depositing and patenting of the strains;
- cultivating of axenic cultures of microorganisms in museum conditions.

2. The preparatory stage of production:

- preparation of nutritional medium, and nutrient supplements;
- revival of the physiological activity of nitrogen-fixing bacteria after storage (reseedling of culture, reactivation on a shaker at constant temperature and aeration);
- establishing a system of air purification, and other activities (if necessary) predetermined by the specifics of the production.

3. Production process:

- the cultivation of bacteria on industrial shakers, in flasks or in fermenters (bioreactors in the form of specially constructed chambers, in which occurs the process of growing microorganisms and fermentation);
- selection of the final product (obtaining a liquid culture of the microorganism);
- preparation of the carrier (packing and sterilization of the substrate, the introduction nutrient supplements), or containers for the liquid substance (sterilization of containers);
- inoculation of the used carrier.

4. Storage or incubation of the biological preparations under certain temperature conditions.

- 5. Quality control (titer of bacterial cells / 1g of the preparative form, the presence of foreign microflora).
- 6. Treatment of wastewater and gas emissions, recycling waste.

The general scheme of production (see figure 1) includes the above mentioned stages; however each case has its peculiarities. This is due to the degree of complexity of each separately organized biotechnological process (laboratory conditions, the use of shakers, industrial rockers, different fermenters), and the technological requirements of cultured organisms and choice of the preparative form of the final product (liquid culture, different carriers). For example, a kit for the fermentation of microorganisms in liquid media may consist of inoculation and production fermentors, air purification systems for fermentation, a set of connecting tubes, as well as the compressor and the gas meter.

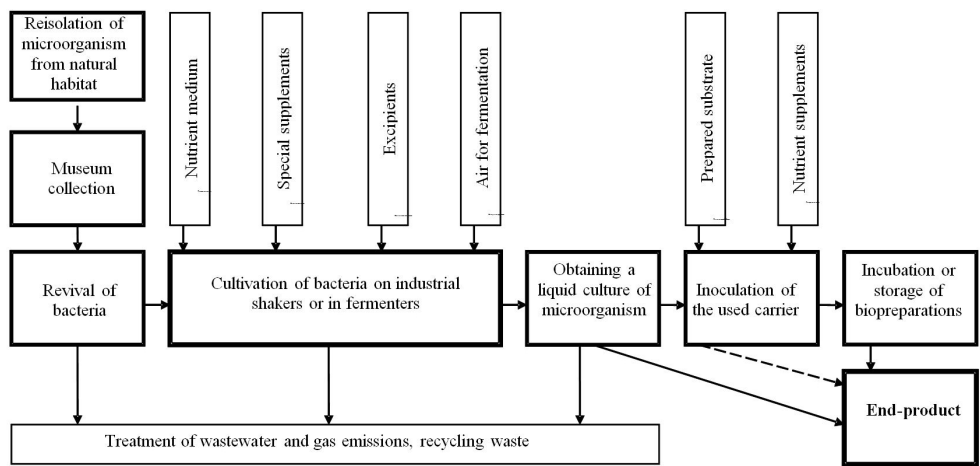


Figure 1. The scheme of creation and production biopreparations, based on nitrogen-fixing bacteria (Sytnikov, 2012).

After establishing a positive impact of the soil microflora on plant productivity, the question arose about the practical application of micro-organisms, in particular those fixing atmospheric nitrogen. The creation of biological products on the basis of nitrogen-fixing bacteria was dictated by the need to preserve their life and functional activity in a specific preparative form (nutrient medium or substrate) aiming to widen its practical application.

The first biological preparation based on the nitrogen-fixing nodule bacteria - Nitragin, was produced in Germany in 1896 [63]. In the Soviet Union the preparation Rizotorfin was created and widely used. It is a peat substrate with nutrient supplements containing highly active and competitive strains of rhizobia for a specific type of legume plants. Bacterial fertilizers for legume plants based on symbiotrophic nitrogen fixers are the most commonly

used biological preparation of diazotrophs. For example, in the United States Nitragin and Double-noktin were produced and used on hundreds of thousands of hectares of crops. In other countries the following compounds were used: Nitrosoil and Nitrum in Argentina and Uruguay, Rhizoctonia in New Zealand, Tropical-inoculum, Nodulite and Nitrogerm in Australia, Ariss Agro in India, and Okadin in Egypt [64]. In Ukraine, up to 20% of crop cultures (predominantly soybean) are inoculated by biological preparations [3].

To inoculate the seeds the most commonly used preparations are based on nodule bacteria from the families of *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium* for the legume plants on the basis of vermiculite or perlite under the general name of Rizobofit. Symbiotrophic nodule bacteria provide nitrogen fixation of up to 350 kg / ha in lucerne during the second year of vegetation [65], as well as up to 280 kg / ha for soybean [66] and 70 kg / ha in pea [9]. As a result of many years of research it has been found that the inoculation of plants with high efficiency strains of rhizobia increases the productivity of legumes by an average of 10-25% [67]. On the basis of associative nitrogen-fixing bacteria, there has been developed a technology of production of a whole line of biological preparations. The list of biotechnological products and microbial preparations for crop production, has increased considerably over the past decade and includes products that are based on free-living, associative, symbiotrophic nitrogen-fixing bacteria, and phosphate-mobilizing bacteria, as well as products of binary action obtained from a combination of various microorganisms [6, 62, 64].

4. Ways to Improve the Productivity of the Soybean-Rhizobial Symbiosis

One of the objects with prospects in biotechnology are cyanobacteria because of their ability in photosynthesis, nitrogen fixation, the synthesis of biologically active substances and growth activating substances. These substances positively influence on soil fertility and the activity of soil biota. Cyanobacteria are closely linked to the bacteria such as *Rhizobium*, *Agrobacterium*, *Pseudomonas*, and are capable of forming a new stable association that opens the perspective for the creation of efficient microbial consortia and preparations based on them. Positive results were obtained when using in agricultural biotechnology artificial algal-rhizobial associations to inoculate the seeds of lotus, peas and clover. To date, it has been proved that under the influence of artificial consortia on the basis of *Nostoc* and various *Rhizobium* the effect of nitrogenation is enhanced on leguminous plants [6, 68-70].

The co-treatment of lucerne seeds by nitrogen-fixing bacteria and cyanobacteria *Nostoc punctiforme*, as well as by their binary compositions stimulates plant growth and development. It was found that the most effective method was co-inoculation by cyanobacteria with certain Tn5-mutants of *Rhizobium* when compared to inoculation by monocultures [71]. We have also studied the reaction of soybean *Glycine max* (L.) Merr. to inoculation by algal-rhizobial compositions based on the nodule bacteria *Bradyrhizobium japonicum*, and cyanobacteria *Nostoc punctiforme* [72]. It is shown that the inoculation of soybean seeds by algal-rhizobial compositions enhances germination and positively influences seedling formation. The inclusion of cyanobacteria into the inoculation suspension of rhizobia and Tn5-mutants in certain combi-

nations can stimulate the growth and development of soybean, the accumulation of photosynthetic pigments in the leaves and the protein content in seeds. However, it does not have a significant effect on the activity of nitrogen-fixing nodules, and plant productivity. This data indicates the need to find effective algal-rhizobial complex compositions to inoculate soybean plants by the optimal selection of the strains of bacteria and the ratio of inoculating agents. In our opinion further study is also required of the algal-rhizobial compositions created on the basis of microorganisms with genetically modified properties.

Creation and selection of compatible algal-rhizobial associations, including the axenic cultures of cyanobacteria and nodule bacteria as well as their Tn5-mutants may be one of the methods of biologically stimulating legume-rhizobial symbiosis, which enhances the significance of the interaction of rhizobia with plants and the efficiency of bacterial preparations based on them.

In the formation of legume-rhizobial symbiosis the essential components of the interaction of the symbiotic partners are polysaccharides which are synthesized by nitrogen-fixing bacteria. Perhaps it was under the influence of these substances, acting as elicitors in the early stages of the morphogenesis of nodules, that gene activation occurs for a number of plant genes that "sit in silence" in the uninoculated plant roots.

It has been suggested that the polysaccharides of non-rhizobial origin, as well as glycopolymers of rhizobia, are able to mimic the action of phytohormones and stimulate the processes of nodulation and morphogenesis in the legume-rhizobial symbiosis [73]. It is shown that the cells of nodule bacteria during the action of exogenous polysaccharides (Baktozol) increased in growth, produced more biomass and changed the activity of some enzymes of the nitrogen exchange [74, 75]. The stimulating effect of the synthetic polysaccharide (PS MOD-19) on the growth of rhizobia, biomass accumulation and changes in their metabolism during the growth of bacteria on solid and liquid media in the presence of the biopolymer was later revealed. During the processing of the seeds of peas (*Pisum sativum* L.) prior to sowing with PS MOD-19 there was found an increased rhizogenesis in plants, an increased peroxidase activity in plant cells, as well as the increase of the effectiveness of symbiosis as a whole due to secondary formation of nodules on side roots and the prolonging of the period of their active nitrogen fixation [76]. In this regard, synthetic polysaccharides may be of interest as biologically active compounds for practical application, in particular, for the expansion of the range of substances that can stimulate the growth activity of rhizobia and, to a greater extent, enhance and prolong the activity of nitrogen-fixing nodules formed on roots of leguminous plants. The latter circumstance is of particular importance for legumes with a short vegetation period, of which the most vivid representative is the pea [73]. In our opinion, further study of the stimulating effect of polysaccharides of different origin is a promising direction of improving the efficiency of legume-rhizobial symbiosis.

Our studies also show the effectiveness of biological products of nodule bacteria modified by homologous lectin, and the economic feasibility of their use [47, 49-51, 77-79]. In accordance with the existing concepts about the mechanisms of interaction of plants with rhizobia, polysaccharides of the latter are a factor which provides a "recognition" by the bacteria of the corresponding host plant through complementary binding to the plant lectin. Lectins are

proteins that have the ability to reversibly and selectively bind to carbohydrates and carbohydrate parts of biopolymers without changing the covalent structure of the latter [77, 79]. Along with other biologically active substances legume lectins during the germination of seeds secrete themselves into the environment [21]. These proteins stimulate the proliferation and active movement of soil microorganisms to the roots, and influence the growth of microsymbionts and the synthesis by them of exoglycans [48]. Plant lectins are regarded as one of the factors of effective symbiosis, which is also proposed for consideration when developing and implementing new approaches to the management of production processes in legume plants [77]. It is known that treatment of rhizobia by plant lectin specific to them has a positive effect on their virulence and competitiveness [34], and also increases the nitrogen-fixing activity of root nodules. Lectin acts on the biosynthesis of nitrogenase [58, 59] in the bacterial cell. As a consequence, pre-incubation of rhizobia with homologous lectin enhances the growth processes of plants and increases the productivity of the symbiosis [47].

The results of our studies indicate to the prospects of using bacterial agents modified by homologous lectin, both liquid and manufactured on a solid carrier (Table 1).

The use of this protein makes it possible to improve the efficiency of the symbiotic system of soybean *Glycine max* (L.) Merr. and increase its productivity. The tests showed that the concentration of homologous lectin of 100 mcg / ml of bacterial suspension *Bradyrhizobium japonicum* is the optimal dose in both physiological and economical aspects in the manufacture of rhizobia preparations using perlite as a solid carrier (Table 2). In addition, the effectiveness has been established of using bacterial preparations modified by homologous lectin, on the basis of active production-strains of rhizobia and some Tn5-mutants (T66 and T3-11) [78].

Carrier	Lectin concentration, µg/ml	Seed yield, cwt/ha	Yield increment relative to control	
			cwt/ha	%
Liquid	0	29,3 ± 2,7	–	–
	100	36,6 ± 1,5	7,3	24,9
	300	32,5 ± 1,8	3,2	10,9
Perlite	0	34,6 ± 1,2	–	–
	100	43,1 ± 1,1	8,5	24,5
	300	40,3 ± 1,7	5,6	16,4
LSD _{0,05}		4,6		

Table 1. Soybean seeds harvest with the application of various preparations forms of nodule bacteria with different homologous lectin concentrations (average of the 2 experiments) [80].

Lectin concentration, µg/ml	Seed yield, cwt/ha				Yield increment relative to control	
	I	II	III	Average of the 3 experiments	cwt/ha	%
0	36,2 ± 0,7	33,1 ± 1,8	22,5 ± 0,7	30,6 ± 1,1	–	–
100	45,1 ± 1,4	41,2 ± 0,8	25,8 ± 1,1	37,4 ± 1,1	6,8	22,2
300	41,9 ± 1,5	38,8 ± 2,0	27,3 ± 1,0	36,0 ± 1,5	5,4	17,6
LSD _{0,05}	4,2	5,1	2,5			

Table 2. Productivity of soybean inoculated with biological rhizobial preparation on the solid carrier (perlite) and modified with homologous lectin [80].

Note. I, II - 2005., III - 2006.

The economic efficiency of using bacterial preparations to inoculate the seeds of legume plants before sowing depends on the increase of yield, its cost and additional expenses. The calculation of economic efficiency of soybean production via a typical technological map was shown on a farm in the Kiev region (Table 3). By using bacterial preparations of soybean nodule bacteria (application of inoculation) the yield of the crop increased throughout Ukraine by an average of at least 12%, which makes it possible to increase the profitability of production to 27.8% (see Table 3). The use of bacterial preparations modified by homologous lectin in the conditions of our field trials led to an increase in soybean yield of no less than 9.8% in comparison with conventional inoculation (see Table. 1 and 2). A comparative analysis of various indicators of economic efficiency of soybean cultivation points to the feasibility of using bacterial preparations to inoculate seeds (see Table 3). The profitability of production at the same time increased by 11.7%. The maximum profitability (39.2%) was observed when using bacterial preparations modified by homologous lectin, indicating the benefits of their use. Table 4 also shows that the use of bacterial preparations reduced production costs and increased net income. Thus, the cultivation of soybeans in the conditions and fields of Ukraine can produce about 0.4 hryvnia net profit to every 1 hryvnia spent. Therefore different preparatory forms of nitrogen-fixing microorganisms effectively increase the productivity of plants and can be recommended for agricultural production. The use of bacterial agents leads to a slight increase in the cost of production while the economic effect of using nitrogen-fixing bacteria is achieved due to the additional crop yield, the savings made on fertilizers and the reduction of other production energy costs.

Parameter	Unit of measure	Control	Application of inoculation	Biopreparation with lectin
Seed yield	cwt/ha	20,0	22,4	24,6
Supplementary yield	cwt/ha	–	2,4	4,6
Cost of yield	UAH/ha	2400,0	2688,0	2952,0
Costs of biopreparation	UAH/ha	–	36,0	53,0
Net cost	UAH/cwt	103,34	93,88	86,17
Net profit	UAH/ha	333,13	585,13	832,13
Profitability	%	16,1	27,8	39,2

Table 3. Economic effect of bacterial preparations application while producing soybean seeds (the condition of 2006), the preparations being modified with homologous lectin [80].

Note. UAH - Ukrainian hryvna.

5. Conclusion

Given the uniqueness of the biochemical processes of nitrogen fixation by microorganisms and global importance of biological nitrogen fixation in the nitrogen cycle in the biosphere, this phenomenon requires further study and attention. Scientists still face the problem of finding new nitrogen-fixing communities, the creation of genetically engineered micro- and macro- symbionts, as well as studying specific mechanisms of the relationship between plants and microorganisms.

A comprehensive study of plant-bacterial symbioses and associations made possible the use of biological nitrogen fixation in practice and opened up prospects for the management of this process. Work is presently being done on the generation, testing and introduction of new strains of *Rhizobium* and associative nitrogen fixers for the cultivation of plants in our latitudes. Among the promising works in the creation of biological preparations based on symbiotic nitrogen fixers are those regarding the creation of agricultural consortiums with cyanobacteria, the use of bacterial and synthetic polysaccharides, and plant lectins. The biological preparations created based on beneficial microorganisms are an excellent alternative to mineral fertilizers. However, they have not yet been widely applied, which of course does not facilitate the resolution of a number of existing environmental and economic problems.

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