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## **THE CONCEPTION OF MICROBIAL PREPARATIONS DEVELOPMENT FOR A CROP PRODUCTION**

***Kakhramon Davranov<sup>1</sup>, Vyacheslav Shurigin<sup>2</sup>,  
Sitara Samadiy<sup>2</sup>, Bakhora Djalolova<sup>2</sup>***

*<sup>1</sup>Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan,  
4 Navoiy Str., Tashkent, 100128, Uzbekistan*

*<sup>2</sup>National University of Uzbekistan,  
4 University Str., Almazar district, Tashkent, 100174, Uzbekistan  
e-mail: slaventus87@inbox.ru*

*As a result of the analysis of scientific literature data and based on our experimental data, we present the conception of mutual relations between beneficial microorganisms and plants in the soil: a) for the purposeful management of processes in agro- and natural phytocenoses it is necessary to consider all components of the plant-microbial community (plant, arbuscular mycorrhizas, nodule bacteria and all microorganisms stimulating plants growth and development, both endophytic and epiphytic), as a uniform gen-metabolic net; b) it is necessary to consider, that the rate of microsymbionts genomes evolution exceeds the rate of plant evolution and consequently the plant should be considered as the most genetically stable in time, key component controlling the efficiency of plant-microbial systems functioning. According to the basic conception of intensive agriculture within several decades all over the world, the selection of new breeds of agricultural plants was conducted on a high agrotechnical level with the application of high doses of fertilizers and chemicals. The attention was paid only to the negative influence of a pathogenic microbiota on plants and according to this, the resistance of plants to such conditions. As a rare example, in the case of legumes farmers took into account the symbiotic nitrogen fixation that is mutualistic interactions of plants with nodule bacteria. It is necessary to notice, that the genetic systems of legumes controlled the interaction with nodule bacteria, arbuscular mycorrhiza and plant growth promoting bacteria (PGPB), have a series of common genes. It should be considered as a uniform polygene net and to be the object of selection on integral feature “efficiency of interaction with microorganisms beneficial to plants”, instead on separate signs characterizing the efficiency of formation of separate types of mutually advantageous plant-microbial symbioses; c) at the beginning of plant selection history, not having the effective technologies and knowledge about the structure of plant genetic system, monitoring mutual relations of plants with microorganisms, scientists breeders intuitively followed the laws dictated by nature-conducted selection against an undisturbed structure of plant beneficial microorganisms community on the integral feature of plant productivity. Today it is necessary to return to the initial practice of selection, but with the account of effective technologies and new knowledge of plant-microbial interactions and to make plant selection to increase the symbiotic potential of such interaction against its maximum genetic diversity. It is reasonable to conduct plant selection on such features as additional biomass, saved up by the formation of the mutualistic plant-microbial system and quality of received production. Due to such selection, the commercial breeds of agricultural plants maximum efficiently operating the interaction with microorganisms in agrophytocenosis will be received. As a result of applying this conception, the technologies for production of complex microbial preparations BIST, Pseudorizobin and Zamin were developed, patented and successfully tested in the Republic of Uzbekistan.*

*Keywords: microbial preparations, crop production, biodiversity, agrophytocenosis.*

The increase of agricultural plant's productivity and obtaining the pollution-free crop products using biological preparations based on plant growth promoting bacteria (PGPB) is important both for safety of the environment and for health

of humanity. However, the use of such biological preparations is limited due to lack of knowledge in the field of ecology and genetics of microorganisms and new plants breeds interacting with beneficial strains of microorganisms and also because of

the role of the agroecological factors defining the effective functioning of plant-bacteria associations.

It is known that the number of microbial populations is an important criterion of their activity and status in a biogeocenosis. Environmental conditions make an essential impact on the composition and activity of microbial communities, including N<sub>2</sub>-fixing, in the soil and plants rhizosphere [1]. It was established, that the specificity of various plant breeds [2] and species reaction on bacterization is caused by distinctions in plants' ability to conserve or lose associative connections with bacteria depending on their cultivation conditions. In turn, the introduced microbial population enters the active interaction with soil autochthonous microbiota and plants rhizosphere that renders a significant impact on the efficiency of inoculated plants [1, 3, 4]. However, due to the salinization of the fertile soil, plants educe the new ways of adaptation to various changes in environmental conditions such as abiotic and biotic. The ability of plants and primitive root systems to exist within the frames of mutually beneficial symbiotic relations with various groups of microorganisms generated during evolution caused the further evolution and strategy of the Kingdom Plantae terraneous mode of life [5].

Within two decades, in cooperation with various foreign institutes, in the frames of performance of scientific-technical grants of GNTP RUz and the international grants, we are engaged in working out of the new forms of microbial preparations of complex action to increase the efficiency and environmental safety of plant growing. The developed preparations were checked in laboratory, vegetative and field experiments in various agroclimatic and soil conditions of Uzbekistan.

The **purpose** of research was to study the crops interaction with beneficial microbiota functioning in the complex genetically and metabolically-integrated system (association), interacting with each other, and with a host plant. The second important aim was to work out the conception of use of complex microbial preparations promoting increase of content and biological diversity of PGPR for the purpose of compensation of lost microbial parts and chains in agrophytocenoses under increasing agrotechnological and chemical load on soil in a process of plant growing.

What does such a conception give?

- firstly, the increase of efficiency and productivity of agricultural plants and environmental safety of their production;

- secondly, an economically and ecologically defensible decrease of chemogenic load on agrolandscape;

- thirdly, the introduction of microorganisms' groups beneficial to plants, capable on a competitive or antagonistic basis to remove soil sickness by the active achievement of the alimentary ecological niches in the system of "soil-microbiota-plant" into soil microbiota composition.

- fourthly, the restoration of soil communities' structure, functionally important for agricultural plants;

- fifthly, the opposition to the resident, resistant to chemical load forms of phytopathogenic microorganisms (fungi, bacteria, viruses) with the use of natural mechanisms [6, 7];

- sixthly, biologically proved optimization of mineral nutrition elements consumption by agricultural plants;

- seventhly, the embodiment in life of the developed conception ideas allows refusing the application of agrochemicals and starting to use microbial preparations [2, 8].

### **Plant-microbial interactions**

It is necessary to notice, that the huge amount of experimental data about plant-microbial systems of legumes, such as clover (*Trifolium spp.* L), pea (*Pisum sativum* L), soybean (*Glycine max* L), bean (*Phaseolus vulgaris* L), chickpea (*Cicer arietinum* L), alfalfa (*Medicago truncatula* Gaertn), Japanese laywoman (*Lotus japonicus*), etc. with nodule bacteria is collected in the literature.

The conclusions received from these experiments also extrapolated on non-leguminous species of agricultural plants.

It is stated that microorganisms colonize land plants. The phenotype of the majority of land plants in nature is a product of extremely coordinated expression of plants and microorganisms genes. Actually, the plant phenotype in many respects is "an extended phenotype", depending on one or several microorganisms [9]. In other words, a plant and the microorganisms being in a state of organismic and consequently, genetic and metabolic integration, represent over-organismic system possessing adaptable properties, which the organisms integrated into the system separately, do not possess. Mutually advantageous (mutualistic) relations of microorganisms with plants primarily considered, as interesting, but specific events, which are characteristic only for some plant species or families. Similarly, the majority of microorganisms colonizing (infecting) plant,

considered as pathogenic, having an unfavorable influence on its development. However, the presence of microorganisms inside and on a surface of plants is a rule, instead of the exception. There are many of such examples: microorganisms, plants pathogens, mutualistic fungal endophytes, mycorrhizal fungi, nodule bacteria, and other nitrogen-fixing bacteria and beneficial for plant microorganisms of rhizosphere and phyllosphere, more of which grow inside the plant tissues and thus are endophytes [10].

Materials on the evolution of a plant-microbial system testify that plants were associated with microorganisms during all process of their evolution, representing plant-microbial evolutionary continuum [11]. Therefore, for example, mycorrhizal fungi form practically ubiquitous associations with plant roots. Fungi get access to soil resources and exchange them to plants photosynthates [12]. The second classical example of rather well studied mutualistic plant-microbial symbiosis is a symbiosis, which forms Fabaceae (leguminous) family plants and genetically various nodule bacteria grouped in order Rhizobiales in the specialized plant's organs – nitrogen-fixing nodules [13, 14, 15]. In nodules bacteria, differentiated in organelle-like symbiotic form – bacteroids which supply the plant with nitrogen, fixed from atmosphere, in exchange for plant photosynthates [14, 16].

For plant growth and development, the big role is played also by bacterial endophytes rendering various beneficial influences on plant metabolism, including nitrogen fixation without nodules formation [17, 18]. Together with rhizosphere bacteria, these bacteria form numerous functional groups of PGPB. Their stimulating effect is provided with various mechanisms:

- direct absorption of mineral nutrients ( $N_2$ -fixation, transformation of inorganic phosphates into a biologically available form, production of siderophores facilitating the absorption of metal ions, etc.);

- synthesis of hormone-like biologically active agents;

- activation of plants "resistance" genes;

- direct antibiosis against pathogen microorganisms [17–20].

The following genera of bacteria are related to PGPB: *Pseudomonas*, *Bradyrhizobium*, *Azorhizobium*, *Bacillus*, etc. Procedures of isolation of endophytic bacteria are normally oriented on certain functional groups or optimized for specific bacterial antibiotics. It is necessary to notice, that

biodiversity of endophytic bacteria in different plants and their functional role in vital activities of host plants still not well understood.

Today, the idea of production of a certain microbial preparation, capable in a revolutionary way to change the existing systems of plant growing, has outgrown in the belief supported with numerous experimental data and facts, received basically, at development of the theory and practice of "biological" agriculture, and also in the course of attentive studying of a "wild" nature phenomena. It is known, that in the modern conception of agriculture development, the special role belongs to legume cultures, which are the basic ingredients of a plant growing production technologies. It is known, that legumes are capable to form, at least, two types of mutually advantageous (mutualistic) endosymbioses. First of them – formation of arbuscular mycorrhizas with fungi, the second – formation of legume-rhizobial symbiosis with nodule bacteria of various phylogenetically far from each other taxons: *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium* and many other bacteria combined under the general name "rhizobia" [21]. Both these mutualistic relations are characterized by the formation of specialized symbiotic structures – compartments, a high degree of genetic and metabolic integration of partners, and efficiency of the functioning of such symbioses are substantially monitored by host plants.

Legume-rhizobial symbioses possess high specificity of interaction: certain species and strains of nodule bacteria form compatible pairs only with certain legumes genera or species. Thus, the specialized compartments are formed on plant roots – the nodules giving an environmental niche and conditions for atmospheric nitrogen fixation. Thanks to the formation of such symbiosis, legumes can grow in subtropics, free of bound nitrogen [22, 23].

The realization of the fixation process defines the significant role of legume-rhizobial symbioses in the nitrogen cycle in nature.

As well as for many other plants, for legumes an association formation with various plant beneficial microorganisms stimulating their growth and development is characteristic. Rhizospheric, phyllospheric and endophytic bacteria, such as *Agrobacterium*, *Azospirillum*, *Bacillus*, *Erwinia*, *Flavobacterium*, *Paenibacillus*, *Pseudomonas*, *Streptomyces* and many other though do not form specialized symbiotic structures and their interaction with plants is not species – or strain-specific, nevertheless, there is undoubtedly a

metabolic integration between partners, as it was noted above. There are various mechanisms explaining the positive influence of PGPB on plant growth and development. It is necessary to notice, that the plant plays an important role in such interaction [24–27].

Thus, mutually advantageous symbioses of legumes with plant beneficial microorganisms, give plants additional possibilities for survival in various conditions, and to microsymbiotes an environmental niche and products of photosynthesis. Such plant-microbial systems promote the maintenance of soil's natural fertility and biological diversity of ecosystems. Application of microbial preparations based on plant beneficial microorganisms allows to the reduction of expenses for agrochemical and to reduce chemogenic load on the environment that promotes principles of adaptable agriculture.

In agriculture practice, various variants of microbial preparations are used: monomicrobial, di-, three- and polymicrobial (or complex). The efficiency of monomicrobial biological preparations in most cases appears low in comparison with other types of biopreparations. It can be explained by high competition or horizontal transgenesis between representatives of indigenous microbiota and introduced organisms [24]. Besides, interactions of many modern breeds of cultivated plants with beneficial microorganisms are not so effective, because the selection of such breeds was made against high doses of fertilizers and plants chemical protection preparations, without an account of efficiency of interaction with beneficial microorganisms; as a result, there are casual losses of genes alleles valuable to plant-microbial interaction [11].

In spite of the fact that the plant has a long life cycle and more stable, than microorganisms genome structure, is the organizing and managing element of mutually advantageous plant-microbial systems, in modern selection work the increase of efficiency of plant-microbial interactions does not pay sufficient attention. Thanks to modern achievements in the field of a physical and chemical biology, especially molecular and cell biology, the significant progress in understanding of mechanisms of mutualistic plant-microbial systems interactions was reached. The fact that similar mechanisms having apparently, common evolutionary parentage participate in gen-metabolic integration of plants with beneficial microorganisms is especially significant. It is stated that the first stage of symbioses formation between plants and microorganisms is the process of diffusing signal

molecules exchange, before physical contact and providing the mutual detection and recognition of partners. According to the opinion of Hirsch et al. [28], in legume-rhizobial symbiosis, the role of the specific signal molecules secreted by plant belongs to flavonoids. These compounds in nodule bacteria cells induce synthesis of Nod-factors (Nodulation factor) – lipid chitooligosaccharide molecules, which structure are unique for each specific combination “bacterium/plant” [29]. In turn, the plant, thanks to the presence of specific receptor proteins distinguishes Nod-factors and after that activates programs of legume-rhizobial development [30].

Chemotaxis (directed locomotion of microorganisms in a gradient of substances – effectors concentration) also can be considered as an important component of the process of microorganisms’ “recognition” by the plant. Therefore, it was shown that mutant on motility and genes of chemotaxis strains of rhizosphere bacteria *Pseudomonas fluorescens* colonizes plant roots worse in comparison with wild strain and is not capable to colonize roots at joint inoculation with mutant and wild strains, so they have depressed competitiveness [31, 32].

The partners’ mutual recognition takes place and at the following stage of symbiosis development – during the establishment of immediate contact between microsymbiont and plant root. The mechanism of bacteria attachment to host-plant cells seems to be universal. Both PGPB and rhizobia, which work as PGPB at interaction with non-legumes, form biofilms on roots surface and synthesize exopolysaccharides, interreacting with plant lectins that can define the specificity of symbiosis [33, 34]. It is necessary to note, that some exopolysaccharides of rhizobia are necessary not only for attachment of bacteria to root hairs, but also for successful process of colonization, but for the question, whether exopolysaccharides play a role of signals for plants still remains without answer.

In the course of recognition at a stage of rhizosphere bacteria attachment to plant the important role is played also by some components of the cell surface, such as glycoproteins, agglutinins (lectins) or even simple proteins [35]. Recently it was revealed that rhizospheric nitrogen-fixing bacterium *Paenibacillus polymyxa*, on the surface of its cells contains agglutinating proteins, which at contact with a plant are redistributed and change their localization [36].



Thus, the mutual recognition of partners in which the important role play both diffusing signals, and immediate interaction of surface molecules of partners, is the general property of mutualistic symbioses, formed not only by legumes but plants in general, by working out of effective microbial preparations these processes should be considered.

The next important stage at the production of effective microbial preparations is the study of the colonization process and symbiotic compartments formation. There is a question, why? Because, plants play an active role in the penetration of microsymbionts into the root, and at the development of various symbioses similar mechanisms are involved [37]. Nodule bacteria pass into the root of the majority species of legumes through root hair cells – rhizoderma. It is important to notice, that root colonization, including root hairs and a cortex, occurs through the special tubular structure (an infectious filament) which is under construction a plant deep into root from cell to cell. Formation of an infectious filament is preceded by the formation of the cytoskeleton cylinder (preinfectious filament) in root cells defining the direction of an infectious filament growth [38].

Penetration of arbuscular mycorrhizas into the plant through rhizoderma cells and root cortexes also occurs with at assistance of a host cytoskeleton. The special structure, which is called “the apparatus previous to penetration”, is thus formed [39]. It is stated, that rearrangement of a cytoskeleton and infiltration of rhizobia and arbuscular mycorrhizas is accompanied by core migration and plant cell cytoplasmic membrane invagination [37, 39]. There are global restructurings of plant cells interacting with microorganisms in the world.

It is also shown, that in place of rhizobacteria penetration into plant roots, occurs also modification and the cellular wall becomes thin, related to hydrolysis of its components with the assistance of rhizobacteria specialized enzymes: endoglucanases and endopolygalacturonases [40]. It is necessary to notice, that in the modification of plant cell walls take part not only microbial enzymes but also some plant hydrolases, for example,  $\beta$ -glycosidases, proteinases, etc. [36]. Compant et al. [40] showed that bacteria in pour into root through rhizodermal and cortical cells and having overcome the endoderm get to root vascular system.

During the evolution of legume-rhizobial symbiosis, the process of endocytosis-like capture of nodule bacteria by plant cell cytoplasm is formed [41]. In this process bacteria detached from the

infectious filament and surrounds with peribacteroid membrane of plant and form symbiosomes [42]. Then, inside symbiosomes nodule bacteria undergo differentiation to organelle-like structures – bacteroids that are responsible for atmospheric nitrogen fixation. At the development of “archaic” legume-rhizobial symbioses, the nitrogen fixation occurs without endocytosis in fixation filaments [42, 43].

The symbiotic membrane (peribacterial) provides metabolic integration of partners at endosymbiosis. During the process of endosymbiosis the active zone (peribacterial) contains polysaccharides and enzymes, synthesized by both partners [37]. It forms between symbiotic membrane and microsymbiont cell. Such interaction based of interchange of metabolites reflects the evolutionary relationship of legume-rhizobial symbiosis.

Unlike arbuscular mycorrhizas, endophytic bacteria, while passing into a plant, do not form specific symbiotic compartments, and localized in intercellular space in an entry place, or distribute in vessels of all plant. Thus, the source of endophytic bacteria is rhizosphere microbiota. Many soil bacteria can penetrate the root hairs and place of lateral roots formation or damaged plant tissues. Naturally, getting in plants, they change some properties, adapting to new ecological conditions. In such conditions only the bacteria forming mutual relations with a plant, stay alive and functional.

In nodules, mycorrhizal roots and at interactions of plants with PGPB the activation of the processes similar to defence reactions of a plant on pathogens was observed [44, 45]. It is known that at mutualistic interactions these reactions of a plant are less expressed, than at a pathogenic, and, possibly, are under the control of regulatory symbiotic genes. As elicitors, invoking plant defence reactions at interactions with beneficial microorganisms, Nod-factor and specific surface polysaccharides of rhizobacteria, Myc-factor and/or components of a cell wall of arbuscular mycorrhizas and many other molecules, which are on a surface or secreted by PGPB can serve [46]. The activation of plant protective mechanisms as a result of inoculation with beneficial microorganisms can be one of the causes of increase of plant system resistance to pathogens.

The big role in regulation of symbiotic interactions with microorganisms is played also by hormonal system of plants. For example, cytokinin is one of the main positive regulators of legume-rhizobial symbiosis [47]. The ethylene which is the

universal signal molecule, starting plant defence reactions in reply to stressful conditions, reduces pathogenic infection, and also plays a role of a negative regulator in formation of legume-rhizobial symbiosis [48] and at interaction with PGPB [49].

Generalizing the above-mentioned facts, we came to the following conclusions:

1. Despite the significant distinctions in taxonomy, specificity, structural organization and mechanisms of the positive effect of mutualistic microsymbionts on plant growth, there are general biological bases of formation and functioning of mutually advantageous plant-microbial systems that testifies to evolutionary antiquity of such mutual relations. A comparison of legume-rhizobial symbiosis and arbuscular mycorrhizas development it is not difficult to notice, that the most expressed resemblance of these two processes is observed at recognition stages (Nod- and Myc-factors), colonization (infectious filament and apparatus previous to infiltration), formation of symbiotic compartments (infectious filament and intraroot mycelium, peribacteroid membrane and periarbuscular membrane), induction of local defense reactions, their intensity, and principles of system regulation. At the interaction of plants with PGPB some general mechanisms, such as attachment of nodule bacteria to root or activation of plant defense reactions are realized. It means that there are similar systems of the genetic control of various mutual relations or symbioses between microorganisms and plants.

2. The rhizosphere is the dynamical medium where microorganisms are subject to the influence of root exudates and inside- or interspecific interactions with each other and with plants. The arbuscular mycorrhiza modifies the environment, forming mycorrhizosphere. For example, stimulation of root exudates because of interaction with arbuscular mycorrhizas results in qualitative and quantitative changes of bacterial communities of the rhizosphere. Arbuscular mycorrhizas show synergism at interaction with PGPB (both indigenous and introduced), carrying out biocontrol, nitrogen fixation and phosphates mobilization at double and complex inoculation. Formation of arbuscular mycorrhizas enhances nodules formation by legumes and nitrogen fixation by rhizobia, in particular by means of stimulation of a host plant mineral nutrition.

3. Life activity of arbuscular mycorrhizas is associated with symbiotic bacteria, which can stimulate the growth of arbuscular mycorrhizas at the presymbiotic stage of development previous

to the establishment of immediate contact of a microsymbiont with plant root. At joint inoculation of plants with rhizobia and PGPB, it is observed the synergetic effect in particular related to the synthesis of indolyl-3-acetic acid by PGPB, stimulating nodules formation.

Thus, the potential of microbial synergism with regard to general evolutionary sources of various mutualistic plant-microbial systems allows talking about the perspective of biotechnology of multicomponent symbioses creation raising productivity and quality of agricultural legumes and non-legumes that is about the perspective of working out and application of complex microbial preparations based on plant beneficial microorganisms. Thus, the physiological and genetic adaptations of microorganisms to local environmental conditions are very important. Hence, by working out such biotechnology it is expedient to use a set of microbial isolates, adapted for concrete environmental conditions [2].

As it was noted, there is a system work on the creation of effective microbial preparations for plants that are carried out in NUU. In spite of the fact that effective technologies were developed and a number of microbial preparations were produced, the ecological estimation of their efficiency until now is not given. The performance of such work will give a chance to receive enough high yields of cotton and cereals in conditions of agrocenosis of Uzbekistan [50–54].

It is experimentally proved that as criteria for an assessment of efficiency of chickpea interaction with microorganisms beneficial to this plant (*Rhizobium+Pseudomonas*) it is possible to use an index of augmentation of its biomass and seed productivity by inoculation with a complex of beneficial bacteria isolated by us (Table 1) [53]. Bacterial strains were isolated by the authors of the article and stored in the Collection of microorganisms at the Department of Microbiology and Biotechnology of the National University of Uzbekistan, as well as in the Collection of industrially important microorganisms of the Institute of Microbiology of AS RUz.

These data give the chance to conclude that to increase the potential of interaction with beneficial microorganisms, it is necessary to carry out the selection work among various plant breeds. The selection programs directed on the increase of legumes symbioses efficiency is necessary to conduct with a glance of knowledge about processes of mutualistic symbioses development. In particular, the sequences of plant genes defining the

**Table 1**

**The effect of Jakhongir breed chickpea seeds inoculation with bacteria of *Rhizobium* and *Pseudomonas* genera on plants growth and development**

Type of inoculant	Nodules number	Stem length (cm)	Root length (cm)	Fresh weight of stem with leaves (g)	Root fresh weight (g)	Dry weight of stem with leaves (g)	Root dry weight (g)	Pods number	Yield (c/ha)
Control	22±2.91	48±2.91	25±2.75	95.75±2.14	6.56±0.67	21.03±0.74	1.77±0.18	36±3.53	17.84±1.21
<i>Rhizobium</i> sp.4	67±4.99	58±5.25*	28±2.91	112.89±5.75	8.57±1.40	21.47±1.52	1.96±0.38	41±3.77	20.45±1.37
<i>Rhizobium</i> sp.6	38±2.91	55±4.37	26±2.75	113.83±5.80	8.04±1.57	21.47±1.61	1.94±0.28	39±3.53	19.61±1.31
<i>Rhizobium</i> sp.9	43±4.22	56±3.53	31±3.20	98.9±3.95	7.09±0.90	20.64±1.57	1.77±0.14	39±2.91	19.56±1.30
<i>Rhizobium</i> sp. IC53	32±3.20	58±4.37	27±2.91	108.81±5.05	8.76±0.99	20.11±1.54	2.02±0.25	40±2.91	20.13±1.33
<i>Rhizobium</i> sp.4 + <i>Pseudomonas</i> sp.66	97±5.62*	62±4.99	35±3.77	123.67±6.55*	9.64±1.87*	21.63±2.32*	2.41±0.41*	45±5.62*	22.21±1.42*
<i>Rhizobium</i> sp.6 + <i>Pseudomonas</i> sp.66	54±3.53	59±4.37	32±3.77	118.17±5.29	9.25±1.51	21.53±1.49	2.36±0.24	43±4.99	21.57±1.36
<i>Rhizobium</i> sp.9 + <i>Pseudomonas</i> sp.66	63±4.99	60±4.37	34±3.77	109.9±4.75	9.38±0.90	20.13±1.44	2.38±0.21	40±3.53	20.14±1.34
<i>Rhizobium</i> sp.IC53 + <i>Pseudomonas</i> sp.6	41±3.77	61±4.37	35±2.91	121.38±5.28	10.65±1.32	21.49±1.52	2.63±0.25	44±4.99*	21.85±1.38*

Legend: \* – statistically significant at p≤0.05

stability and efficiency of symbioses can be used as DNA-markers facilitating selection of legumes breeds with the high performance of interaction with beneficial microorganisms.

The interaction of microbial communities with a host plant is a complex process defined by genetic integration of partners and mentions the whole complex of the vital factors, such as feeding, growth and development regulation, resistance to both biotic and abiotic factors and settling of new territories.

The mechanisms of plants-microorganisms interactions can be “straight lines” (coordinated feeding, synthesis of biologically active and hormone-like substances) or direct microbial antibiosis (inhibition of plant-pathogen microorganisms growth), and also mediated (regulation of “resistant genes” activity of a host plant or through interaction on higher trophic levels).

Microorganisms, associated with the plant, are heterotrophs and demand the expenses from a host plant whereas the “benefit” of a plant from interaction with microorganism metabolically integrated with it depends on many factors including the interaction of the plant-microbial system with the environment.

The concept of “plant without microorganisms” is not correct. It has no right to exist because such important phenomena as phenotypic and epigenetic plasticity of plants, phenotypic “norm” of plants in ecosystems and plant evolution cannot be carried out without mutual relations of plants with microorganisms. It is offered to introduce into practice the molecular-genetic methods using diagnostic fragments of transcribed genes for an assessment of microorganisms’ true diversity, associated with wild plants and not bound to their cultivation on artificial nutrient mediums. First of all, it concerns endophytic microorganisms as they are presented as high evolutionary-specialized forms to exist in the endosphere, capable to grow on artificial nutrient mediums and incapable (not cultivated). For the formulation of the hypotheses, explaining the role of endophytic microorganisms found in a plant the specters of microorganisms should be correlated with species diversity of host plants and diversity of environmental conditions. It is necessary to compare various combinations of “host plant – endophytic microorganism” in different typical environmental conditions and to study genetic, epigenetic and physiological mechanisms of evolutionary plant-microbial continuum transferring on a continuous scale “mutualism – parasitism”.

It is well known, that the bigger the microbial diversity of soils, the higher their fertility. It is a basis for the creation and application of complex plant beneficial microorganisms to increase soil fertility. Here is a question: “whether modern breeds are ready to operate with evolutionary verified and biologically defensible strategy of terrestrial plants, functions division and resources redistribution in the frames of genetically and the metabolically-integrated plant-microbial system?” It is necessary to notice, that many modern breeds of cultivated plants inefficiently interact with beneficial microorganisms, only because their selection was carried out against high doses of fertilizers and other chemical combinations used for plants protection, and the most important thing it is without the account of signs of interaction with plant beneficial microorganisms. At such plants, the alleles of the gene valuable to interaction with beneficial microorganisms can be lost [11].

#### **The conception of mutual relations between beneficial microorganisms and plants in a soil**

As a result of the above-stated scientific data analysis and on the basis of our experimental data the conception of mutual relations between beneficial microorganisms and plants in the soil can be presented:

a) for the purposeful management of processes in agro- and natural phytocenoses it is necessary to consider all components of the plant-microbial community (plant, arbuscular mycorrhizas, nodule bacteria and all microorganisms stimulating plants growth and development, both endophytic and epiphytic), as a uniform gen-metabolic net.

b) it is necessary to consider, that the rate of microsymbionts genomes evolution exceeds the rate of plant evolution and consequently the plant should be considered as most genetically stable in time, key component controlling the efficiency of plant-microbial systems functioning.

According to the basic conception of intensive agriculture within several decades all over the world, the selection of new breeds of agricultural plants was conducted on a high agrotechnical level with the application of high doses of fertilizers and chemicals. Attention was paid only to the negative influence of a pathogenic microbiota on plants and according to this, the resistance of plants to such conditions [50, 51].

As a rare example, in the case of legumes farmers took into account the symbiotic nitrogen fixation that is mutualistic interactions of plants with nodule bacteria. It is necessary to notice, that the genetic



systems of legumes controlled the interaction with nodule bacteria, arbuscular mycorrhiza and PGPB, have a series of common genes and should consider as a uniform polygene net and to be the object of selection on integral feature “efficiency of interaction with microorganisms beneficial to plants”, instead on separate signs characterizing the efficiency of formation of separate types of mutually advantageous plant-microbial symbioses [11].

c) At the beginning of plant selection history, not having the effective technologies and knowledge about the structure of plant genetic system, monitoring mutual relations of plants with microorganisms, scientists breeders intuitively followed the laws dictated by nature – conducted selection against an undisturbed structure of plant beneficial microorganisms community on the integral feature of plant productivity. Today it is necessary to return to the initial practice of selection, but with the account of effective technologies and new knowledge of plant-microbial interactions and to make plant selection to increase the symbiotic potential of such interaction against its maximum genetic diversity. It is reasonable to conduct plant selection on such features as

additional biomass, saved up by the formation of the mutualistic plant-microbial system and quality of received production. Because of such selection, the commercial breeds of agricultural plants maximum efficiently operating the interaction with microorganisms in agrophytocenosis will be received [52].

As a result of applying this conception, the technologies for production of complex microbial preparations BIST, Pseudorizobin [2, 53] and Zamin [54] were developed, patented and successfully tested in the Republic of Uzbekistan.

BIST is a microbial preparation based on the bacteria *Pseudomonas putida* SKB-251 and *Bacillus subtilis* SKB-256 stored in the “Collection of industrially important microorganisms” of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. We selected a nutrient medium for the active growth of these two cultures during co-cultivation. Under field stressful conditions, when *B. subtilis* transforms to spores, *P. putida* grows normally, manifesting its biological functions. BIST was patented in the Republic of Uzbekistan (No. IAP 04299) and the Eurasian patent (No. 021467) was also obtained for it [55, 56].

**Table 2**

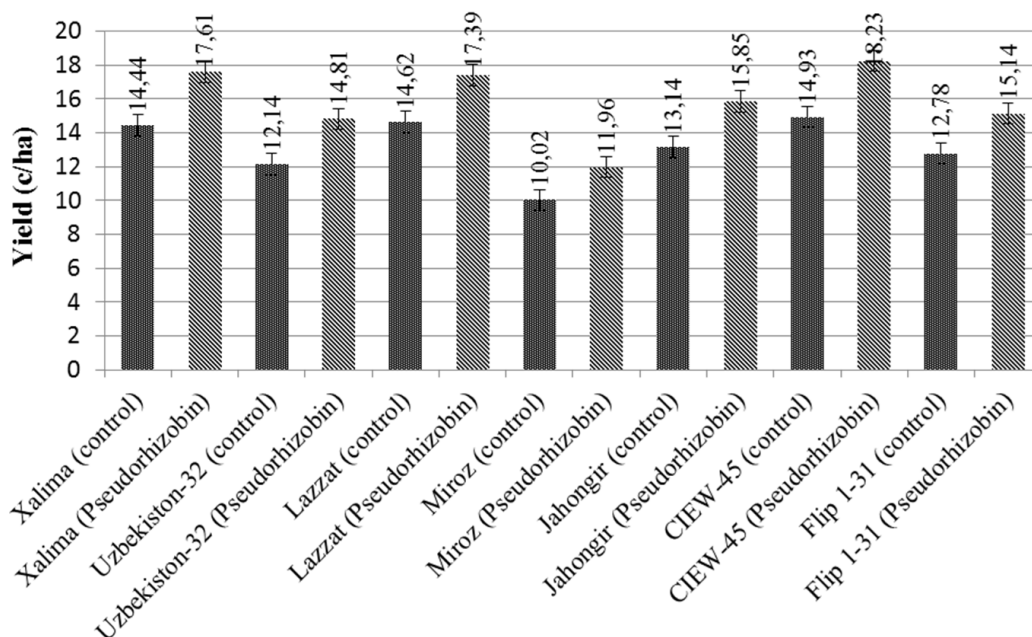
**The effect of microbial preparations BIST and Zamin on yield of different wheat breeds in a field conditions**

Wheat breeds	Place of origin	Yield (c/ha)			Types of wheat
		Without preparations	BIST	Zamin	
Sanjar-8	Uzbekistan	27.4–30.5	31.5–35.6	32.3–35.5	Facultative two-handed
Krasnodarskaya 99	Russia	42.7–45.4	48,5–51.8	50.5–53.3	Winter
Qayroqtosh	Uzbekistan	22.5–25.3	25.5–28.2	25.7–29.4	Dry

**Table 3**

**The effect of microbial preparations BIST and Zamin on yield of different cotton plant breeds in a field conditions**

Cotton plant breeds	Place of origin	Yield (c/ha)			Fiber quality
		Without preparations	BIST	Zamin	
AN-Bayavut 2	Scientific Production Association “Biolog”, Uzbekistan	27.2–30.5	30.7–34.7	31.6–35.6	Type 5
C-6524	Institute for Cotton Breeding, Uzbekistan	28.8–31.6	32.9–36.2	33.8–37.1	Type 4-5
Sulton	Institute for Cotton Breeding, Uzbekistan	35.2–38.1	39.3–43.2	41.1–49.0	Type-4
Kelajak	Institute of Genetics of the AS RUz, Uzbekistan	37.4–40.3	42.8–46.5	43,5–46.8	Type-4
Chorloq-4	Center of Genomics and bioinformatics of AS RUz	37.8–39.5	43.2–45.5	44.1–45.9	Type-2



**Fig. 1. The influence of microbial preparation Pseudorhizobin on yield of different chickpea breeds in saline soil ( $\text{SO}_4^{2-} + \text{Cl}$ )**

Zamin is a microbial preparation based on bacteria *Bacillus subtilis* SKB-309, *Bacillus megatherium* SKB-10 and *Pseudomonas stutzeri* SKB-308 for pre-sowing treatment of grain seeds and industrial crops. This microbial preparation increases seed germination rate, growth and yield of crops; stimulates the growth of the root system; suppresses phytopathogenic microbiota of seeds and soil; increases the immunity of plants to diseases and pests. Zamin was patented in the Republic of Uzbekistan (No. IAP 05254) [57].

The microbial preparation Pseudorhizobin is intended for pre-sowing treatment of chickpea seeds and growing plants in saline soils. The basis of the preparation are salt-tolerant bacterial strains *Mesorhizobium ciceri*-4 and *Pseudomonas chlororaphis*-66, which actively colonize the chickpea rhizosphere, and enter into direct symbiosis with it. Due to nitrogen fixation and the release of biologically active substances, bacteria help to increase yield and enhance plant immunity against phytopathogenic microorganisms, also sharply decrease the content of nitrates in the crop. Bacterial strains are stored in the collection of Microbiology and biotechnology department of the National University of Uzbekistan. Pseudorhizobin was patented in the Republic of Uzbekistan (No. IAP 05272) [58].

BIST and Zamin were tested on different cultivars of cotton and wheat (Tables 2 and 3), and Pseudorhizobin was tested for growing chickpea in laboratory, greenhouse and field conditions (Fig. 1).

As can be seen from the tables 2 and 3 and figure 1 the microbial preparations BIST, Zamin and Pseudorhizobin significantly increased the yield of various breeds of tested crops.

Thus, the innovative technology based on mutual relations of plants with microorganisms already gives practical results.

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## КОНЦЕПЦІЯ СТВОРЕННЯ МІКРОБНИХ ПРЕПАРАТІВ ДЛЯ РОСЛИННИЦТВА

Кахрамон Давранов<sup>1</sup>, В'ячеслав Шуригін<sup>2</sup>,  
Ситора Самадий<sup>2</sup>, Бахора Джалолова<sup>2</sup>

<sup>1</sup>Інститут Мікробіології Академії Наук  
Республіки Узбекистан,  
вул. Навої, 4, Ташкент, 100128, Узбекистан  
<sup>2</sup>Національний університет Узбекистану,  
вул. Університетська, 4, Алмазарський район,  
Ташкент, 100174, Узбекистан

### Резюме

В результаті аналізу наукових літературних даних і на основі наших експериментальних даних представляємо концепцію взаємин корисних

мікроорганізмів і рослин у ґрунті: а) для цілеспрямованого управління процесами в агро- і природних фітоценозах необхідно враховувати всі компоненти рослинно-мікробної спільноти (рослини, арбускулярні мікоризи, бульбочкові бактерії і всі мікроорганізми, що стимулюють ріст і розвиток рослин, як ендofітні, так і епіфітні) як єдину генетичну мережу; б) необхідно враховувати, що швидкість еволюції геномів мікросімбіонтів перевищує швидкість еволюції рослин, і, отже, рослину слід вважати найбільш генетично стабільною у часі, ключовим компонентом, який контролює ефективність функціонування рослинно-мікробних систем. Згідно базової концепції інтенсивного землеробства протягом декількох десятиліть в усьому світі відбір нових сортів сільськогосподарських рослин проводився на високому агротехнічному фоні із застосуванням великих доз добрив і хімікатів. Увагу звертали тільки на негативний вплив патогенної мікробіоти на рослини і, відповідно, на стійкість рослин до таких умов. Як рідкісний приклад для бобових рослин фермери взяли до уваги симбіотичну фіксацію азоту, тобто взаємодію рослин з бульбочковими бактеріями. Слід зазначити, що генетичні системи бобових, що контролюють взаємодію з бульбочковими бактеріями, арбускулярною мікоризою і бактеріями, що сприяють росту рослин (PGPB-Plant Growth Promoting Bacteria) мають ряд загальних генів і повинні розглядатися як єдина полігенна мережа. Вона повинна бути об'єктом відбору за інтегральною характеристикою «ефективність» взаємодії з мікроорганізмами, корисними для

рослин, а не за окремими ознаками, що характеризують ефективність формування окремих видів взаємовигідних рослинно-мікробних симбіозів; в) на початку історії селекції рослин, не маючи ефективних технологій і знань про структуру генетичної системи рослин, відстежуючи взаємини рослин з мікроорганізмами, вчені-селекціонери інтуїтивно додержувались законів, продиктованих природою, і проводили селекцію за невід'ємною ознакою продуктивності рослин за умови непорушеної структури угруповання корисних для рослин мікроорганізмів. Сьогодні необхідно повернутися до первісної практики селекції, але з урахуванням ефективних технологій і нових знань про рослинно-мікробні взаємовідносини, а також проводити селекцію рослин для збільшення симбіотичного потенціалу такої взаємодії по відношенню до його максимальної генетичної різноманітності. Доцільно проводити селекцію рослин за такими ознаками, як додаткова біомаса, накопичена за рахунок формування мутуалістичної рослинно-мікробної системи, і якість одержуваної продукції. Завдяки такому відбору буде отримано комерційні сорти сільськогосподарських рослин, що максимально ефективно взаємодіють з мікроорганізмами агрофітоценозів. В результаті застосування даної концепції було розроблено технології виробництва комплексних препаратів BIST, Pseudorhizobin і Zamin, які запатентовано і успішно апробовано в Республіці Узбекистан.

*Ключові слова:* мікробні препарати, рослинництво, біорізноманіття, агрофітоценоз.

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