Reviews, challenges, advances

AGRICULTURAL MICROBIOLOGY AND SYMBIOGENETICS: SYNTHESIS OF CLASSICAL IDEAS AND CONSTRUCTION OF HIGHLY PRODUCTIVE AGROCENOSES

(review)

N.A. PROVOROV[1] ✉, I.A. TIKHONOVICH[1, 2]

[1]*All-Russian Research Institute for Agricultural Microbiology,* 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail provorovnik@yandex.ru (✉ corresponding author);
[2]*Saint-Petersburg State University,* 7-9, Universitetskaya nab., Petersburg, 199034 Russia, e-mail arriam2008@yandex.ru
ORCID:
Provorov N.A. orcid.org/0000-0001-9091-9384   Tikhonovich I.A. orcid.org/0000-0001-8968-854X
The authors declare no conflict of interests

A b s t r a c t

Agricultural microbiology (AM) is presented as a discipline addressing the prokaryotic and eukaryotic microorganisms that influence operation of the major components of agrocenosis — plants, animals and soils. Development of AM is based on the synthesis of ideas and methods of microbiology, plant physiology, soil science and genetics. This synthesis is aimed to study the organization and evolution of biosystems in which symbiotic microorganisms perform adaptively important functions in cooperation with each other and with host organisms. Upon migration from environment into the endosymbiotic niches of plants and animals, microorganisms form with them multicomponent complexes — holobionts (E. Rosenberg, I. Zilber-Rosenberg, 2018). They possess own systems of heredity, symbiogenomes and hologenomes, which have become the subjects of a new discipline, symbiogenetics (I.A. Tikhonovich, N.A. Provorov, 2012). Microorganisms forming symbioses with plants perform the important adaptive functions — nutritional (N_2 fixation, absorption of soil nutrients, firstly phosphates), defensive (biocontrol of pathogens and phytophagans) and regulatory (synthesis of phytohormones that optimize plant development and improve their resistance to adverse environment) (I.A. Tikhonovich, N.A. Provorov, 2009). The broadly studied and practically important plant symbionts include: a) nodule bacteria or rhizobia (*Rhizobiales*) — N_2-fixing symbionts of legumes; b) arbuscular mycorrhizal fungi (*Glomeromycota*) — phosphate-mobilizing symbionts of a wide range (more than 80 % species) of plants (A. Berruti et al., 2016); c) rhizospheric and endophytic bacteria (e.g., *Azospirillum*, *Bacillus*, *Pseudomonas*) which stimulate the development of plants and determine their resistance to antagonists (pathogens, pests) and stresses (drought, salinity of soils, their contamination with xenobiotics or heavy metals) (M.A. Hassani et al., 2018). In animals, trophic symbionts determine the assimilation of plant biomass (intestinal or rumen microbiota), synthesis of essential amino acids and cofactors (intestinal and intracellular symbionts), and N_2 fixation (symbionts of some herbivorous animals) (E. Rinninella et al., 2019). The study of microbial effects on plants and animals makes it possible to create microbial preparations that improve the nutrition of hosts, their resistance to biotic and abiotic stresses, and increase the soil fertility. In crop production, preparations of N_2-fixing and growth-stimulating bacteria are widely used, which ensure a drastic reduction in application of environmentally hazardous nitrogen and phosphorus fertilizers. Preparations of microorganisms that are antagonists of phytopathogens — *Pseudomonas*, *Bacillus* (B.J. Lugtenberg et al., 2001; V.K. Chebotar et al., 2009), rodents — *Salmonella enteritidis*, *Serratia plymuthica* (A. Soenens, J. Imperial, 2019) or phytophagous insects — *Bacillus thuringiensis*, *Beauveria bassiana* (A.V. McGuire, T.D. Northfield, 2020) are used broadly for their biocontrol to significantly reduce the pesticide load on agrocenoses. By studying the integrative functions of agronomically valuable microorganisms, AM invests a significant contribution to the fundamental biological research, including the genetic and molecular interactions of prokaryotes and eukaryotes, evolution of cell and of its genome, and formation of supraorganismal genetic systems (I.A. Tikhonovich, N.A. Provorov, 2012). Based on these studies, methods of symbiotic engineering are being developed aimed at constructing the highly productive

biosystems, including the cereal and vegetable cultivars capable of symbiosis with rhizobia, as well as N$_2$-fixing plants.

Agricultural microbiology (ACM) emerged at the end of the 19th century as a research field applied to plant nutrition and the biocontrol of plant pests [as cited by 1, 2]. By the 1930s, ACM became a synthetic discipline using the methods of microbiology, plant physiology and soil science. In the 1940s, gene-for-gene systems were discovered that determined the relationship between phytopathogens and their hosts [3], as well as between rhizobia and legumes [4]. It was shown that the relationship between microorganisms and their hosts can be described in terms of gene interaction [5], i.e., the epistasis (parasitic systems) or complementarity (mutualistic symbioses) (Table 1). The deepest integration of partners, that is, the formation of combined signal-receptor complexes and biochemical pathways, is characteristic of mutualistic relationships [6]. The result of integrative evolution was the formation of holobionts, supraspecific complexes with their own systems of heredity (hologenomes), which provide plants and animals with various adaptive functions [7].

1. Types of gene interaction in free-living and symbiotic organisms

Type of interaction	Microorganisms	
	free-living (5)	symbiotic (3, 4)
Epistasis	The gene of one allelic pair suppresses the expression of the gene of another allelic pair	Gene-for-gene interactions in parasitic systems (host suppression of pathogens)
Complementarity	Involvement of multiple non-allelic genes in the development of a trait	Functional integration of genes in mutualistic systems (formation by partners of combined signaling and biochemical pathways)

The practical use of the ACM achievements began with the selection of effective strains of rhizobia for the inoculation of legumes and the coordinated selection of microorganisms and plants to create optimal combinations of their genotypes [8]. These works determined the development of a new field of biotechnology, symbiotic engineering, aimed at constructing eco-safe agrocenoses in which the adaptive functions of plants and animals are performed by their symbionts [9].

The ACM development in Russia was initiated by P.I. Kostychev and S.P. Kostychev, who found that bacteria and fungi are actively involved in plant nutrition and in the formation of fertile soils [10]. To date, microbial-plant symbioses divided into three types (trophic, protective and regulatory) is the most studied [6]. Their emergence has a long history associated with the joint (coevolutionary) emergence of plants and mycorrhizal fungi on land [11]. Fungi acted as intermediaries between the most ancient plants that lacked roots (rhyniophytes, psilophytes) and the soil, and possibly as donors of symbiotic bacteria that passed from fungi to plants [12].

In natural ecosystems and agrocenoses, microorganisms carry out all the main stages of the cycle of substances associated with plant nutrition, the assimilation of plant food by animals, as well as the transformation of organic residues into humic substances. The performance of agronomically significant functions by microorganisms is determined by their circulation in the soil—plants—animals ecosystems. Most plant symbionts originated from soil bacteria and fungi, and many inhabitants of the digestive cavities of animals evolved on the basis of the microflora of host plants [8]. Moving into the internal environment of the hosts, microbial communities fall under their control and become even more integrated and functionally active. This integration is associated with deep transformations of

the metagenomes of microbial communities that occur under the influence of plants and animals, which indicates the possibility of managing agrocenosis as a single genetic system.

The purpose of this paper is to generalize and analyze modern ideas about the integrative and adaptive functions performed by microorganisms during the formation of terrestrial ecosystems. Based on the knowledge of the genetic organization of functionally integral and self-sufficient microbial-plant and microbial-animal complexes (holobionts), methods for managing agrocenoses by modifying their microbial components that determine the vital activity of agricultural organisms, as well as the formation of fertile soils, will be proposed.

Bioengineering of agricultural microorganisms. In Russia, work on obtaining agronomically valuable microorganisms began in the laboratory of agricultural bacteriology, founded at the end of the 19th century in St. Petersburg with the aim of selecting strains for the control of murine rodents [2]. The creation of domestic preparations of rhizobia (nitragins and their modern modifications) is associated with the works of V.P. Israeli et al. [13] and E.N. Mishustin [14]. In 1930, the All-Union (now All-Russian) Research Institute for Agricultural Microbiology became the center of these studies, where in the 1970s the development of genetic breeding methods for constructing effective microbial-plant symbioses began. An important step along this path was formulated by L.M. Dorosinsky [15] the main principle of rhizobia selection, which should be based on the complementarity of partner genotypes, which plays a much greater role in determining symbiotic efficiency (SE, the ability to increase plant productivity) than the adaptability of bacteria to local soil conditions.

Developing this approach, we assessed the genotypic contributions of partners to the development of legume-rhizobium symbiosis and showed that an increase in SE can be achieved by narrowing the specificity of the interaction between bacteria and plants [16]. According to the results of the analysis of variance of data on the interaction of different genotypes of partners, the highest productivity of legumes inoculated with rhizobia is achieved with the maximum contribution of nonadditive cultivar—strain interaction to the variation of SE parameters, which serves as a measure of the specificity of symbiosis [17].

Based on these data, a methodology was proposed for coordinated breeding of plants and bacteria aimed at creating optimal combinations of their genotypes [17]. It should be also taken into account that native microbial populations interacting with leguminous plants in the field are heterogeneous and contain many strains that are useless for hosts and even parasitic strains that compete with industrial strains of rhizobia for the formation of nodules [18]. A change in the composition of the microbial population that has penetrated into the plant in favor of mutualist strains is possible due to the directed selection of these strains by the host from the soil or the rearrangement of the composition of the endosymbiotic population in favor of active N_2-fixers. Thanks to these mechanisms, competitive interactions of several types are carried out in the microbial-plant system.

The first type includes the competition of soil strains of rhizobia for the formation of nodules in leguminous plants. Its study made it possible to identify a number of *cmp* genes in bacteria that control nodulation competitiveness, which usually does not correlate with the activity of N_2 fixation [18]. Therefore, industrial strains of rhizobia introduced into the agrocenosis often cannot compete with inefficient, but virulent (aggressive) local strains. In a number of works, the preferential selection of certain genotypes of rhizobia from the soil by leguminous plants was noted [19], which usually does not provide targeted extraction from populations of strains with high nitrogenase activity [20].

The second type includes the competition of groups of bacteria located in

different nodules of a leguminous plant for the products of photosynthesis supplied to them. This competition is based on a positive feedback between N_2 and CO_2 fixation processes, which provides a preferential supply of carbon to microbial genotypes that have formed nodules actively fixing N_2 [21, 22]. Selection for mutualist strains is of a group nature and is effective under the condition of clonality of the endosymbiotic population of rhizobia, which is determined by infection of plants with individual bacterial cells through root hairs [6].

Finally, competition of the third type occurs between different holobiont plants for survival under conditions of soil nitrogen deficiency [23]. Its success depends on how efficiently individual legume genotypes select active N_2-fixers from the soil population of rhizobia and distribute photosynthesis products in favor of those microbial genotypes that formed actively N_2-fixing nodules.

The methodology of coordinated selection of symbiosis partners, based on the analysis of their natural variability, was used in the development of genetic methods for creating symbiotically active microbial strains. At the first stage of the work, it was shown that in alfalfa rhizobia (*Sinorhizobium meliloti*) hybridization (transformation, transduction, conjugation) is a more efficient source of active N_2-fixers than mutagenesis [24]. The selection of strains for an increase in nitrogenase activity turned out to be more effective than for an increase in SE, which indicates a different genetic control of these traits.

An important step in the creation of effective strains of rhizobia was the molecular labeling of symbiotically specialized (*sym*) genes, most of which are not active outside the host. Usually, this labeling is carried out by means of transposon (Tn5) mutagenesis [25], which makes it possible to identify two groups of symbiosis regulators, positive and negative (Table 2). Upon inactivation of the former, the symbiotic activity of bacteria decreases or is lost; upon inactivation of the latter, it increases (26).

2. Rhizobia genes for positive and negative regulators of symbiosis

Features	Symbiotic regulators	
	positive (27)	negative (28)
Functions	Synthesis of nitrogenase (*nif*) and ienergy supply (*fix, dct*)	Synthesis of reserve nutrients (*pha*C, *phb*A, *glg*A) and extracellular polysaccharides (*egl*C, *rkp*C), energy saving (red)
Localization in the genome	In extrachromosomal clusters	Dispersed
Expression outside symbiosis	Only shown for some genes (*dct*)	Common to most genes
Symbiotic efficiency:		
for gene inactivation	Lost or drastically reduced	Increased
for gene amplification	Increased	No data

To obtain agronomically valuable strains of rhizobia, amplification of positive symbiosis regulators is also used. For example, when additional copies of *dct*-genes, which determine the transport of dicarboxylic acids, are introduced into alfalfa rhizobia, the N_2-fixing (C_2H_2-reductase) activity of bacteria increases by 60-100%, but the mass of plants, the main SE indicator, increases only by 15-20% [27]. A balanced increase in nitrogenase activity and SE is ensured by the inactivation of negative regulators of N_2 fixation (for example, genes that determine the conversion of carbohydrates obtained from plants into reserve nutrients), which, in combination with amplification of its positive regulators, can be used to construct effective strains of rhizobia [28].

Construction of holobionts. The main result of the colonization of the internal environment of eukaryotic hosts by microorganisms is the formation of new biological units — holobionts. Using the legume-rhizobium symbiosis as an example, one can see that the integrity of the holobiont is determined by the partners' positive and negative feedbacks (Table 3). Negative feedback which acts at the early stages of interaction, increases the stability of the system, since, thanks

to it, the host strictly controls the size of the endosymbiotic population of rhizobia, limiting the number of nodules and the number of bacteria in each of them [29].

3. Feedback of partners in the system of legume-rhizobium symbiosis

Compared properties	Negative connections	Positive connections
Symbiosis stages	Early	Late
Interaction of partner genes	Epistasis (gene-to-gene)	Complementarity
Defined trait of symbiosis	Stability	Efficiency
Rhizobium genes	*nod*	*nif/fix*, *dct*
Plant genes	*NFR*	*GS/GOGAT/AAT*, *PEPC*
Controlled symbiotic processes	Host recognition and infection by bacteria, development of nodules	Formation of combined pathways of nitrogen-carbon metabolism

N o t e. Rhizobia genes control synthesis of lipo-chito-oligosaccharide Nod-factors (*nod*), the synthesis and regulation of nitrogenase activity (*nif/fix*), and the transport of plant-derived dicarboxylic acids (*dct*) [27]. Plant genes control the reception of Nod factors (*NFR*) [6], the assimilation of N_2 fixation products (*GS/GOGAT/AAT*), and dark CO_2 fixation (*PEPC*) [21, 22].

The positive feedback realized at the late stages of symbiosis plays an important role in the determination of SE, since the receipt of fixed nitrogen by the plant stimulates the supply of photosynthesis products to the nodules, which are used to ensure the nitrogenase reaction and bacterial reproduction [30]. Taken together, these connections determine the stability and integrity of the symbiosis, which, as shown by the results of mathematical and experimental modeling, are closely related to its adaptive functions. Indeed, the highest SE is achieved when plants and bacteria respond in a coordinated manner to external factors that affect the vital activity of partners [31].

The use of models of microbial-plant symbiosis showed that when moving from the external environment to the internal niches of plants or animals, soil microbiomes significantly change in composition and become more integrated, falling under the regulatory influence of the hosts [32]. Due to this regulation, symbionts coexist stably with the host, despite their diversity and rapid reproduction.

While in plants endosymbiotic microbiomes are formed on the basis of organisms that come mainly from the soil, in animals they are formed from organisms obtained from food. Holobionts formed by animals are characterized by deep integration necessary to perform symbiotic functions. For example, acquired immunity systems in vertebrates regulate the structure of microbiomes inhabiting the digestive organs (rumen, intestines), where symbionts degrade biopolymers obtained with food (primarily cellulose and pectin) and synthesize protein. The composition of these microbiomes is determined by the age of animals, their physiological state, and feeding regimen [33, 34] and is largely preserved during host reproduction [35]. The functional structure of the animal endosymbiotic microbiome associated with adaptive functions can be characterized using mathematical models based on fractal analysis. Their use for the analysis of the avian gut microbiome has shown [36] that gut microbiome integrity correlates with host productivity and may be a criterion for selecting bacterial strains used as feed probiotics.

The structural and functional integrity of holobionts is determined by the formation of superorganismal systems of heredity, the study of which is the subject of a new discipline, symbiogenetics [37, 38]. These systems were designated as symbiogenomes (only partner genes specialized for interaction participate) or hologenomes (all partner genes participate). Symbiogenomes arise as a result of the functional integration of partners, which is characteristic of facultative symbioses. As the mutual dependence of microsymbionts and hosts increases, the partners can move to structural integration, which is characteristic of obligate symbioses and leads to the formation of hologenomes [39].

A qualitatively new stage in increasing the integrity of holobionts is associated with the emergence of mechanisms for the regular (vertical) transfer of

micro-partners during host reproduction. It usually causes the genetic reduction of microorganisms, which are transformed into hereditary eukaryotic determinants that implement the strategy of pangenesis, i.e. the inheritance of acquired traits [40]. The deepest integration with the host cell is characteristic of its permanent organelles—mitochondria and plastids, many organelle genes were transferred to nuclear chromosomes [41], resulting in the formation of eukaryotic multicomponent genomes of mosaic origin.

The study of supraorganismal systems of heredity opens up the possibility of creating algorithms for their design, symbiotic engineering [9] as one of the most important areas of biotechnology. Its goal is to obtain new agronomically valuable biosystems, such as N_2-fixing plants. However, the direct way to solve this problem (introduction of *nif* genes into the plant genome) is not productive, since *nif* genes do not function in the eukaryotic cell [42]. The incorporation of *nif* genes into mitochondria or plastids which are evolutionarily associated with N_2-fixing bacteria is more realistic [43]. Deeply reduced cell organelles (hydrogenosomes, mitosomes, nonphotosynthetic plastids) which lack their own genomes and maintain anaerobic conditions necessary for nitrogenase activity can be considered as promising recipients of these genes [44].

Ecologically friedndly agricultural technologies. Microorganisms that carry out the main stages of metabolism between soil, plants and animals play a key role in the formation of natural ecosystems. One of the main tasks of agricultural microbiology is the study of the circulation of microorganisms in agrocenoses as a factor in their stability and productivity [8]. By interacting with microorganisms, plants and animals are able to develop with minimal agrochemical impact due to the symbionts fulfilling the functions of feeding the hosts, protecting them from pathogens and stresses, and increasing soil fertility [45-48].

An important condition for the development of environmentally friendly agricultural technologies is to increase the efficiency of symbiotrophic plant nutrition, including the assimilation of sparingly soluble phosphates by mycorrhizal fungi and rhizospheric bacteria [49]. It is of interest to develop combined preparations containing mineral fertilizers and microorganisms that allow plants to make the most of the nitrogen and phosphorus compounds introduced into the soil, reducing the accumulation of harmful products of their transformation [50].

Symbiotic microorganisms play an equally important role in the nutrition of animals, especially herbivores. Being in the digestive organs, microbes destroy entering biopolymers and synthesize protein and metabolites which are deficient in host plants [51]. To optimize the microflora in animal's body, probiotic preparations are actively used, primarily lactobacilli which increase the digestibility of feed and carry out biocontrol of pathogenic organisms [33, 34].

Structural-functional and genetic integration of prokaryotes and eukaryotes is one of the main areas of symbiotic engineering aimed at the formation of supraorganismal complexes for agricultural and environmental purposes [9]. Analysis of the mechanisms of interaction of microorganisms with plants and animals should be considered as a necessary condition for the development of algorithms for constructing effective agrocenoses. Conderning plants, it includes the improvement of natural (nodule, endophytic, epiphytic) symbioses, aimed at creating microbial preparations that replace environmentally hazardous agrochemicals. The construction of new biosystems, including transgenic plants capable of synthesizing metabolites used as edible vaccines, is also being discussed [52].

As a promising area of symbiotic engineering, one can consider the creation of fundamentally new photosynthetic systems for use in green energy. The development of approaches to solve this problem is associated with the study of

symbioses formed by pro- and eukaryotic phototrophic microorganisms with animals, protozoa, and fungi [53]. An example of the emergence of new systems of photosynthesis in nature are kleptoplasts, plastids extracted by animals from the plants they eat and maintained in cells by new hosts for a long time as effective sources of carbon (54).

Heterotrophic protozoan *Alveolata* groups, which apparently originated from organisms that previously had plastids, and contain numerous genes derived from cyanobacteria, are promising for the construction of new photosynthesis systems [55]. Obviously, such organisms are pre-adapted to the maintenance of photobionts and can be used to create new CO_2 fixation systems with a high biotechnological potential.

The creation of agricultural technologies of the future should be based on the study of the processes of evolution of natural biosystems, aimed at increasing their integrity, environmental sustainability and productivity. The appearance of these systems was the result of the co-evolution of symbionts and their hosts, during which partners formed unified genetic systems. As a result of human-directed evolution [56], the problems of plant transition to symbiotrophic nutrition can be solved in the near future, which will lead to partial, and under certain conditions, to complete replacement of mineral fertilizers with environmentally friendly microbial preparations. The introduction of endophytic microorganisms into plants, which make the hosts immune to infection by phytopathogens, also seems promising. The solution of these problems requires the integrated use of microbiology, symbiogenetics, and genetic engineering approaches, the combination of which is a priority task for agricultural biology.

Thus, the use of methods of agricultural microbiology and symbiogenetics is associated with the development of environmentally friendly agricultural technologies based on the use of symbiotic microorganisms that provide symbiotrophic nutrition for plants and animals and protection from parasites and pests. The solution of these problems will allow to partially, and in some cases completely abandon environmentally hazardous fertilizers and protective equipment. The design of ecologically safe agrosystems should be based on the formation of stable supraorganismal complexes (holobionts) which have their own systems of heredity, resulting from the genetic integration of plants and animals with beneficial microorganisms.

R E F E R E N C E S

1. Voznyakovskaya Yu.M. *Mikroflora rasteniy i urozhay* [Plant microflora and yield performance]. Moscow, 1969 (in Russ.).
2. Kandybin N.V. *Bakterial'nye sredstva bor'by s gryzunami i vrednymi nasekomymi: teoriya i praktika* [Bacterial means of control of rodents and harmful insects: theory and practice]. Moscow, 1989 (in Russ.).
3. Flor H.H. Genetics of pathogenicity in *Melampsora lini. J. Agric. Res.*, 1946, 73: 335-357.
4. Nutman P.S. Genetic factors concerned in the symbiosis of clover and nodule bacteria. *Nature*, 1946, 157: 463-465 (doi: 10.1038/157463a0).
5. Lobashev M.E. *Genetika* [Genetics]. Leningrad, 1967 (in Russ.).
6. Provorov N.A., Vorob'ev N.I. *Geneticheskie osnovy evolyutsii rastitel'no-mikrobnogo simbioza* [Genetic bases of the evolution of plant-microbial symbiosis]. St. Petersburg, 2012 (in Russ.).
7. Rosenberg E., Zilber-Rosenberg I. The hologenome concept of evolution after 10 years. *Microbiome*, 2018, 6: 78 (doi: 10.1186/s40168-018-0457-9).
8. Tikhonovich I.A., Provorov N.A. Agricultural microbiology as the basis of ecologically sustainable agriculture: fundamental and applied aspects. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 3: 3-9 (in Russ.).
9. Provorov N.A., Onishchuk O.P. Evolutionary-genetic bases for symbiotic engineering in plants — a mini review. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(3): 464-474 (doi: 10.15389/agrobiology.2018.3.464eng).

10. Kostychev S.P. *Fiziologiya rasteniy* [Plant physiology]. Moscow-Leningrad, 1937 (in Russ.).
11. Karatygin I.V. *Trudy Botanicheskogo instituta RAN*. Vyp. 9 [Proceedings of the Botanical Institute of the Russian Academy of Sciences]. St. Petersburg, 1993 (in Russ.).
12. Provorov N.A., Shtark O.Yu. *Mikologiya i fitopatologiya*, 2014, 48(3): 151-160 (in Russ.).
13. Izrail'skiy V.P., Runov E.V., Bernard V.V. *Kluben'kovye bakterii i nitragin* [Nodule bacteria and nitragin]. Moscow, 1933 (in Russ.).
14. Mishustin E.N. *Mikroorganizmy i produktivnost' zemledeliya* [Microorganisms and agricultural productivity]. Moscow, 1972 (in Russ.).
15. Dorosinskiy L.M. *Kluben'kovye bakterii i nitragin* [Nodule bacteria and nitragin]. Leningrad, 1970 (in Russ.).
16. Provorov N.A., Simarov B.V. *Genetika*, 1992, 28(6): 5-14 (in Russ.).
17. Provorov N.A., Tikhonovich I.A. Genetic resources for improving nitrogen fixation in legume-rhizobia symbiosis. *Genetic Resources and Crop Evolution*, 2003, 50(1): 89-99 (doi: 10.1023/A:1022957429160).
18. Onishchuk O.P., Vorob'ev N.I., Provorov N.A. *Prikladnaya biokhimiya i mikrobiologiya*, 2017, 53(2): 127-135 (in Russ.).
19. Carelli M., Gnocchi S., Fancelli S., Mengoni A., Paffetti D., Scotti C., Bazzicalupo M. Genetic diversity and dynamics of *Sinorhizobium meliloti* populations nodulating different alfalfa cultivars in Italian soils. *Applied and Environmental Microbiology*, 2000, 66(11): 4785-4789 (doi: 10.1128/AEM.66.11.4785-4789.2000).
20. Masson-Boivin C., Sachs J.L. Symbiotic nitrogen fixation by rhizobia-the roots of a success story. *Curr. Opin. Plant Biol.*, 2018, 44: 7-15 (doi: 10.1016/j.pbi.2017.12.001).
21. Barnes D.K., Heichel G.H., Vance C.P., Ellis W.R. A multiple-trait breeding program for improving the symbiosis for N_2 fixation between *Medicago sativa* L. and *Rhizobium meliloti*. *Plant and Soil*, 1984, 32(2): 303-314 (doi: 10.1007/BF02184269).
22. El Yahyaoui F., Küster H., Ben Amor B., Hohnjec N., Pühler A., Becker A., Gouzy J., Vernié T., Gough C., Niebel A., Godiard L., Gamas P. Expression profiling in *Medicago truncatula* identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. *Plant Physiology*, 2004, 136(2): 3159-3176 (doi: 10.1104/pp.104.043612).
23. Awika H.O., Mishra A.K., Gill H., DiPiazza J., Avila C.A., Joshi V. Selection of nitrogen responsive root architectural traits in spinach using machine learning and genetic correlations. *Sci. Rep.,* 2021, 11: 9536 (doi: 10.1038/s41598-021-87870-z).
24. Simarov B.V., Aronshtam A.A., Novikova N.I., Sharypova L.A., Bazhenova O.V., Provorov N.A. *Geneticheskie osnovy selektsii kluben'kovykh bakteriy* [Genetic basis for the selection of nodule bacteria]. Leningrad, 1990 (in Russ.).
25. Simon R., Priefer U., Pühler A. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. *Nat. Biotechnol.,* 1983, 1(3): 784-791 (doi: 10.1038/nbt1183-784).
26. Sharypova L.A., Onishchuk O.P., Chesnokova O.N., Fomina-Eshenko J.G., Simarov B.V. Isolation and characterization of *Rhizobium meliloti* Tn5 mutants showing enhanced symbiotic effectiveness. *Microbiology*, 1994, 140(3)): 463-470 (doi: 10.1099/00221287-140-3-463).
27. Onishchuk O.P., Vorob'ev N.I., Provorov N.A., Simarov B.V. *Ekologicheskaya genetika*, 2009, 7(2): 3-10 (in Russ.).
28. Provorov N.A., Onishchuk O.P., Yurgel' S.N., Kurchak O.N., Chizhevskaya E.P., Vorob'ev N.I., Zatovskaya T.V., Simarov B.V. *Genetika*, 2014, 50(11): 1273-1285 (in Russ.).
29. Ferguson B.J., Indrasumunar A., Hayshi S., Lin H.M., Lin Y.H., Reid D.E. Gresshoff P.M. Molecular analysis of legume nodule development and autoregulation. *Journal of Integrative Plant Biology*, 2010, 52(1): 61-76 (doi: 10.1111/j.1744-7909.2010.00899.x).
30. Denison R.F., Kiers E.T. Lifestyle alternatives for rhizobia: mutualism, parasitism and foregoing symbiosis. *FEMS Microbiology Letters*, 2004, 237(2): 187-193 (doi: 10.1111/j.1574-6968.2004.tb09695.x).
31. Vorob'ev N.I., Provorov N.A. *Ekologicheskaya genetika*, 2013, 11(4): 73-85 (doi: 10.17816/ecogen11473-85) (in Russ.).
32. Hassani M.A., Durán P., Hacquard S. Microbial interactions within the plant holobiont. *Microbiome*, 2018. 6: 58 (doi: 10.1186/s40168-018-0445-0).
33. Laptev G.Yu., Yyldyrym E.A., Dunyashev T.P., Il'ina L.A., Tyurina D.G., Filippova V.A., Brazhnik E.A., Tarlavin N.V., Dubrovin A.V., Novikova N.I. Peculiarities of taxonomic and functional characteristics of rumen microbiota of dairy cows suffered from ketosis. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(2): 356-373 (doi: 10.15389/agrobiology.2021.2.356eng).
34. Laptev G.Yu., Yyldyrym E.A., Dunyashev T.P., Il'ina L.A., Tyurina D.G., Filippova V.A., Brazhnik E.A., Tarlavin N.V., Dubrovin A.V., Novikova N.I., Bol'shakov V.N., Ponomareva E.S. Biodiversity and predicted metabolic functions of the rumen microbiota depending on feeding habits at different stages of the physiological cycle of dairy cows. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(4): 619-640 (doi: 10.15389/agrobiology.2021.4.619eng).
35. Li W., Tapiainen T., Brinkac L., Lorenzi H.A., Moncera K., Tejesvi M.V., Salo J., Nelson K.E.

Vertical transmission of gut microbiome and antimicrobial resistance genes in infants exposed to antibiotics at birth. *The Journal of Infectious Diseases*, 224(7): 1236-1246 (doi: 10.1093/infdis/jiaa155).

36. Vorob'ev N.I., Egorov I.A., Kochish I.I., Nikonov I.N., Lenkova T.N. Fractal analysis of frequency-taxonomic profile of broiler's gut microbiota for studying the influence of probiotics on bird development. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(2): 400-410 (doi: 10.15389/agrobiology.2021.2.400eng).

37. Tikhonovich I.A., Provorov N.A. From plant-microbe interactions to Symbiogenetics: a universal paradigm for the inter-species genetic integration. *Annals of Applied Biology*, 2009, 154(3): 341-350 (doi: 10.1111/j.1744-7348.2008.00306.x).

38. Tikhonovich I.A., Provorov N.A. *Genetika*, 2012, 48(4): 437-450 (in Russ.).

39. Provorov N.A., Tikhonovich I.A., Vorob'ev N.I. *Simbioz i simbiogenez* [Symbiosis and symbiogenesis]. St. Petersburg, 2018 (in Russ.).

40. Provorov N.A. Genetic individuality and inter-species altruism: modelling of symbiogenesis using different types of symbiotic bacteria. *Biologocal Communications*, 2021, 66(1): 65-71 (doi: 10.21638/spbu03.2021.108).

41. Smith D.R., Keeling P.J. Mitochondrial and plastid genome architecture: reoccurring themes, but significant differences at the extremes. *Proc. Natl. Acad. Sci. USA*, 2015, 112(33): 10177-10184.

42. Curatti L., Rubio L.M. Challenges to develop nitrogen-fixing cereals by direct *nif*-gene transfer. *Plant Sci.*, 2014, 225: 130-137 (doi: 10.1016/j.plantsci.2014.06.003).

43. Burén S., Pratt K., Jiang X., Guo Y., Jimenez-Vicente E., Echavarri-Erasun C., Dean D.R., Saaem I., Gordon D.B., Voigt C.A., Rubio L.M. Biosynthesis of the nitrogenase active-site cofactor precursor NifB-co in *Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences*, 2019, 116(50): 25078-25086 (doi: 10.1073/pnas.1904903116).

44. Tovar J., León-Avila G., Sánchez L.B., Sutak R., Tachezy J., van der Giezen M., Hernández M., Müller M., Lucocq J.M. Mitochondrial remnant organelles of *Giardia function* in iron-sulphur protein maturation. *Nature*, 2003, 426(6963): 172-176 (doi: 10.1038/nature01945).

45. Lugtenberg B.J.J., Dekkers L., Bloemberg G. Molecular determinants of rhizosphere colonization by *Pseudomonas. Annual Review of Phytopathology*, 39: 461-490 (doi: 10.1146/annurev.phyto.39.1.461).

46. Chebotar V.K., Makarova N.M., Shaposhnikov A.I., Kravchenko L.V. Antifungal and phytostimulating characteristics of *Bacillus subtilis* Ch-13 rhizospheric strain, producer of bioprepations. *Appl. Biochem. Microbiol.*, 2009, 4: 419-423 (doi: 10.1134/S0003683809040127).

47. Soenens A., Imperial J. Biocontrol capabilities of the genus *Serratia. Phytochemistry Reviews*, 2019, 19: 577-587 (doi: 10.1007/s11101-019-09657-5).

48. McGuire1 A.V., Northfield T.D. Tropical occurrence and agricultural importance of *Beauveria bassiana* and *Metarhizium anisopliae. Front. Sustain. Food Syst.*, 2020, 4: 6 (doi: 10.3389/fsufs.2020.00006).

49. Berruti A., Lumini E., Balestrini R., Bianciotto V. Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in Microbiology*, 2016, 6: 1559 (doi: 10.3389/fmicb.2015.01559).

50. Zavalin A., Chebotar V., Alferov A., Chernova L., Shcherbakova E., Chizhevskaya E. Nitrogen use by plants and nitrogen flows after application of standard and biomodified nitrogen fertilizers on barley. *Biological Communications*, 2021, 66(4): 4283-4289.

51. Rinninella E., Raoul P., Cintoni M., Franceschi F., Miggiano G.A.D., Gasbarrini A., Mele M.C. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet and diseases. *Microorganisms*, 2019, 7(1): 14 (doi: 10.3390/microorganisms7010014).

52. Savel'eva N.V., Burlakovskiy M.S., Emel'yanov V.V., Lutova L.A. *Ekologicheskaya genetika*, 2015, 13(2): 77-99 (in Russ.).

53. Green B.R. Chloroplast genomes of photosynthetic eukaryotes. *The Plant Journal*, 2011, 66(1): 34-44 (doi: 10.1111/j.1365-313X.2011.04541.x).

54. Serôdio J., Cruz S., Cartaxana P., Calado R. Photophysiology of kleptoplasts: photosynthetic use of light by chloroplasts living in animal cells. *Phil. Trans. R. Soc. B.*, 2014, 369(1640): 20130242 (doi: 10.1098/rstb.2013.0242).

55. Ševčíková T., Horák A., Klimeš V., Zbránková V., Demir-Hilton E., Sudek S., Jenkins J., Schmutz J., Přibyl P., Fousek J., Vlček C., Lang B.F., Oborník M., Worden A.Z., Eliáš M. Updating algal evolutionary relationships through plastid genome sequencing: did alveolate plastids emerge through endosymbiosis of an ochrophyte? *Sci. Rep.*, 2015, 5: 10134 (doi: 10.1038/srep10134).

56. Vavilov N.I. *Selektsiya kak nauka. Obshchaya selektsiya rasteniy* [Selection as a science. General plant breeding]. Moscow, 1934 (in Russ.).