AGRICULTURAL BIOLOGY,
2019, V. 54, № 5, pp. 847-862
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN2412-0324 (Englished.Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

## Reviews, challenges

*UDC 633.31:631.461.52:577.21*

doi: 10.15389/agrobiology.2019.5.847eng
doi: 10.15389/agrobiology.2019.5.847rus

# ROOT NODULE BACTERIA: PERSPECTIVES OF MONITORING SYMBIOTIC PROPERTIES BY APPLYING GENETIC MARKERS

(review)

**M.L. ROUMIANTSEVA**

*All-Russian Research Institute for Agricultural Microbiology,* 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail mroumiantseva@yandex.ru
ORCID:
Roumiantseva M.L. orcid.org/0000-0001-5582-6473
The author declares no conflict of interests
Acknowledgements:
Supported financially by the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 14.607.21.0178, RFMEFI60717X0178)
*Received July 31, 2019*

Abstract

Alfalfa and soybeans are widely cultivated economically valuable fodder and leguminous crops the yield of which directly depends on bacterial microsymbionts. The legume seeds inoculation by nodule bacteria (rhizobia) is significantly increased the productivity of the plant-microbial system both in typical and in adverse growing conditions, for example, on degraded soils, including those subjected to salinization, waterlogging, aridity, etc. That is why obtaining new strains capable of forming highly productive and stress-tolerant symbiotic systems with leguminous plants is highly requested in agriculture. Modern technologies for the production of highly productive and environmentally friendly varieties of legumes require the use of a biogeocenotic approach which primary takes into account the symbiotrophic indicators (Z.S. Shamsutdinov, 2014). The formation of highly productive plant-microbial systems is based on the principle of complementarity of the interaction of macro- and microsymbiont genomes (I.A. Tikhonovich et al., 2015), that is ensured their successful introduction into agrocenoses, which differ in agroclimatic and soil conditions. Virulence, competitiveness, host specificity and effectiveness of nitrogen-fixing activity, which root nodule bacteria exhibit in relation to a certain species and sometimes to the variety of legume host plant, are among the symbiotically significant and genetically determined properties of rhizobia. All of the above symbiotically significant characteristics are determined by numerous groups of rhizobia genes. The review presents an analysis of data on the genes of soybean and alfalfa microsymbionts, the participation of which in the control of symbiotic activity and stress tolerance has been experimentally proven. Nodule bacteria of the species *Sinorhizobium meliloti*, *S. fredii* and *Bradyrhizobium japonicum*, contrastingly differing in the genetic and morphophysiological characteristics are the most studied. Analysis of recently published data on the main groups of symbiotically significant genes (i.e. *nod* genes involved in the synthesis and decoration of the Nod factor signal molecule initiating the nodulation process during plant-microbial interaction, the *nif*, *fix*, and *eff* groups of genes responsible for the nitrogen fixation and symbiotic effectiveness) indicates a continuing high degree of incompleteness and fragmentation data for both fast- and slow-growing rhizobia species. At the same time, according to published data, allelic polymorphism for these genes is a factor that plays an important role in varying signaling, host specificity, and symbiotic effectivity in both fast and slow-growing species of nodule bacteria. It is concluded that a coupled analysis of sequences of genes from functionally different groups relating to the formation of highly effective stress-tolerant symbioses, which are represented by *sym* genes (symbiosis), *srg* (stress related genes; genes of resistance to stress factors), QS (quorum sensing genes), or *sym-srg*-QS genes, is promising for the search and creation of molecular markers associated with the symbiotic and adaptive properties of nodule bacteria and it is promising for monitoring them under laboratory conditions and in microbiome of agrocenosis.

Keywords: nodule bacteria, *Sinorhizobium meliloti*, *S. fredii*, *Bradyrhizobium japonicum*, alfalfa, soybean, genes of symbiotic activity, effectiveness, resistance to abiotic stresses

Alfalfa and soybeans are the most widely cultivated economically valuable crops worldwide, therefore, their bacterial microsymbionts are in the spot-

light. Modern technologies for breeding highly productive and environmentally friendly varieties of legumes suggest the use of a biogeocenotic approach based on the symbiotic selection method [1]. This technology involving genetically selected nodule bacteria (rhizobium) considerably shortens the time to create new alfalfa varieties.

The method is based on a complementarity interaction of macro- and microsymbiont genomes [2, 3], which predetermines suitability of a plant-microbial system for the environmental conditions and agrocenoses. Virulence, competitiveness, capability to nodulate of a certain legume host and nitrogen-fixing activity are symbiotically significant and genetically determining properties of nodule bacteria. Seed treatment with rhizobia strains provides an increase in symbiotic productivity of legume plants both in typical and in adverse growing conditions, for example, on degraded soils, including salinization, waterlogging, aridity, etc. [4-6]. That is why obtaining new strains capable of forming highly productive and stress-tolerant symbiotic systems with leguminous plants are extremely relevant [7, 8).

Traditionally, obtaining effective nodule bacteria necessitates long-term plots experiments and field trials. In the result strains providing significant positive biometric (height, development of root system, etc.), biochemical (nitrogen content) and symbiotrophic (an increase in plant green biomass or dry matter) changes in plants [1, 4, 5, 9, 10] were selected. These are deemed qualitative and quantitative indicator parameters of symbiotic activity and effectiveness of root nodule bacteria strains. However, an improved plant green mass yield or dry matter production resulting from inoculation by selected strains may eventually decline [11], wherefore such strains are subjected to a supporting selection with isolation of new clones and the analysis of their symbiotic properties in multiple micro-vegetation and then vegetation experiments [12, 13]. Therefore, the development of molecular genetic approaches to search, select and monitore strains with high symbiotic properties seems extremely urgent.

In the modern view, the cause of decrease or loss of symbiotic activity and effectiveness in rhizobia strains may be the instability of their symbiotic genome [14]. The latter means the complex of *sym* genes (structural and regulatory) responsible for various stages of plant-microbial interaction which formed due to the coevolution of nodule bacteria with legume hosts. As per published data, the symbiotic genome of slow-growing and fast-growing nodule bacteria (i.e. *Bradyrhizobium* and *Sinorhizobium* genera, respectively) comprises at least five hundred *sym* genes [15]. Of the genes related to virulence and, thence, nodule formation, a group of common *nod* genes stands out. These genes determine synthesis of specific signal molecules, the Nod-factors, involved in plant-microbe interaction and are found in virtually all known rhizobia species [16]. Genes determining symbiotic activity are normally located on one or several plasmids or in a genomic island located on a chromosome. Such type of location could be a reason for potential loss of individual *sym* genes as well as their clusters, especially under the influence of various abiotic stress factors [17, 18]. Therefore, genotyping of strains for genes determining formation and functioning symbiosis and involved in stress tolerance of bacteria appears imperative.

This review focuses on genes which are experimentally proven to be involved in control of symbiotic activity and stress tolerance of root nodule bacteria of *Sinorhizobium* and *Bradyrhizobium* genera which are contrastingly different genetically and phenotypically, but most well studied. Genes related to symbiosis, but common for fast- and slow-growing rhizobia species may be promising in searching for candidate genes and molecular markers to facilitate analysis of genome stability and inheritance of symbiotic traits in nodule bacteria.

Alfalfa and soybean symbionts. Slow-growing *B. japonicum* and *B. elkanii* rhizobia form highly effictive symbiosis with *Glycine max* (L.) Merr. soybean cultivar in neutral or sub acid soils [19-21]. In alkaline soil, some soybean cultivars form symbiosis with fast-growing *S. fredii*, the typical symbionts of *G. soja* a wild soybean relative widely used in genetic improvement of *G. max* for many economically valuable traits. There is an opinion that use of fast-growing *S. fredii* bacteria in inoculation of soybean cultivars may be of environmental and practical importance due to an increase in agricultural production [22]. Symbionts of perennial tetraploid alfalfa (*Medicago varia*) are fast-growing *S. meliloti* rhizobia, however, in natural conditions, strains of closely related *S. medicae* species mainly forming effective symbiosis with annual diploid alfalfa cultivars, can also be a symbionts of *M. varia*.

Thus, both soybeans and alfalfa form an effective symbiosis with certain species of nodule bacteria, but also could form an ineffective one with bacteria of closely related and quite often with unrelated rhizobia species, which naturally results in considerable yield loss. Hence, the species attribution of nodule bacteria strains is essential for evaluation of their potential symbiotic effectiveness. The method of nucleotide sequence analysis of 16S rRNA gene proved to be the best as it enables characterization of strains not only at the species level but also identification of rhizobia genospecies [23, 24]. Analysis of ribosomal operon *rrn-rrl* intergenic sequence (ITS) or its part, e.g. *hin* region, is also frequently used [25-27]. The analysis of these sequences is useful not only for identification of strains species allocation but also for strain-specific characterization at the level of chromosomal markers [28], which, however, does not guarantee inheritance by strain(s) of genetic determinants of stress tolerance and symbiotic activity.

Genes determining symbiotic activity are located differently in genomes of bacteria of *Bradyrhizobium* and *Sinorhizobium* genera. In fast-growing *S. meliloti* and *S. fredii* species, *sym* genes are on one to two high-molecular megaplasmids exceeding 0.8-1.0 million bps in size but may also be found on chromosome (≥ 3.6 million bps) and on cryptic plasmids (30 to 600 kbps), the number of which in different strains varies from 0 to 5 [29]. In slow-growing *Bradyrhizobium*, *sym* genes are detected in so-called symbiotic island located on a chromosome which size varies from 643 to 998 kbps [30, 31]. Symbiotic islands have mosaic structure in which sequences determining symbiotic activity alternate with sequences that do not affect symbiotic properties or are senseless [30, 31]. Symbiotic islands are related to the type of genomic islands, which is also found in fast-growing nodule bacteria. However, in the latter, the genes relating to symbiotic activity or rhizobia fitness are sporadic and much less frequent [32]. Genomic islands of nodule bacteria are able to substantially affect the functional activity of genes located on a chromosome and/or on plasmids and may be involved in horizontal gene transfer [32-34]. Thence, detection of genomic islands in genomes of highly effective nodule bacteria is essential and is directly related to the selection of genetically stable strains for manufacturing biologicals.

*Nod* gene group. This group determines/regulates the synthesis of signal molecules (or Nod-factors) required to initiate nodule formation on the roots of the host plant, is best studied in rhizobia species considered in the review.

By the example of *B. japonicum* USDA110 strain it is shown that slow-growing bradyrhizobia contain two operons, *nodYABCSUIJnolMNOnodZ* and *nolYZ*, responsible for synthesis of core part of signal molecule, while fast-growing symbionts of soybean *S. fredii* (the strains HH103 and USDA257) have one operon united *nodABCIJnolOnoeI* genes [16, 35]. In case of alfalfa symbionts, *nod* genes are arranged into five operons, *nodABCIJ*, *nodFEGPQ*, *nodH*, *nodMnolFGnodN* and *nodLnoeAB* (the strain *S. meliloti* Rm1021) [36]. It should be

noted that the location and functional role of *nod* genes known now is a result from studying of a single strains including the above mentioned. Disruption of one of so-called common *nod* genes in the result of mutation or targeted alteration results in significant changes in nodulation and, normally, in decrease in or loss of symbiotic activity [37]. Activity of common *nod* genes of rhizobia is regulated by the product of *nodD1* gene which in *S. meliloti* plays a more significant role than its orthologs *nodD2* and *nodD3* [38]. The inducers of *nodD1* are flavonoids present in root exudates of the host plant, but inducers can also be betaines (osmoprotectors) found in alfalfa root exudates too. The homology of *nodD1* genes of *S. meliloti* Rm2011 strain and reference strains *B. japonicum* and *B. elkanii* does not exceed 75% (at amino-acid level), while homology between the above genes of *B. japonicum* and *B. elkanii* is 92%. In *B. japonicum* and *B. elkanii nodD1* genes are activated by different flavonoids, drawing to a suggestion that analysis of allelic polymorphism of *nodD1* genes in native bradyrhizobia strains may be benefit to identify strains differ in host specificity [39]. An important role in regulation of *nod* genes of bradyrhizobia is played by *nodD2* gene, which, in its turn, is controlled by *nolA* gene [40].

On the example of *B. japonicum* USDA110 strain it was shown the involvement of *nolA* gene in control of host specificity, since a corresponding mutants formed nodules on soybean plant roots much later than the parent strain, and were not capable to form nodules on the roots of *Vigna unguiculata* [41]. A two-component system of regulatory genes *nodVW* activated by flavonoid genistein are also related to host specificity of *B. japonicum* [39]. Activity of these genes is required for nodulation of siratro (*Macroptilium atropurpureum*) and vigna roots by bradyrhizobia, but not soybeans [39]. In addition to regulators reviewed above, the secretory protection system TTSS (Type III Secretion System) that is under control of *nodD1* in *B. japonicum* and *S. fredii* is also involved in interaction of rhizobia with certain plant hosts [42-44]. High activity of *tts* genes was noted at early stages of nodule formation, while the *tts* gene mutants were unable to form nodules on the roots of a number of plant hosts [42-44].

Particular role play *nod* genes involved in structural modification (or so-called decoration) of the core of Nod-factor, which makes the signal factor molecule species-specific. For example, the presence of sulfate group on reducible end of *S. meliloti* Nod-factor molecule is required to form symbiosis with alfalfa, which had been proven in studying respective mutants [45]. The Nod-factor sulfating is carried out by *nodH* gene product in connection with products of two other genes, *nodP* and *nodQ*. However, native strains capable to form symbiosis with alfalfa in acidic soils possess signal molecules which lack sulfate group [45]. In *S. fredii*, signal molecules are sulfate-terminated too, while decoration of Nod-factor includes fucosylation and methylation of fucosylated residue on reducible end of signal molecule for which the activity of *noeJ* gene and *nolK-noeL-nodZ-noeK* gene cluster are required [46]. Similar cluster is also present in *B. japonicum* but it includes also another two genes, *nolL* and *noeE*, involved in methylation of signal molecule.

Signal molecules of fast- and slow-growing rhizobia symbionts of soybeans have some structural differences. In bradyrhizobia they are acetylated while in sinorhizobia non-carbamylated at non-reducible end of the molecule [47]. Carbamylation is controlled by *nolO* and *nodU* genes. It was found that all of the studied so far *S. fredii* strains (HH103, 042B, USDA192, USDA193 and USDA257) had an insertion and a stop codon in *nolO* gene, and only in the first two mentioned strains structural modifications in *nodU* gene were found as well [47]. Obviously, the genes involved in signal molecule decoration, such as *nolO*

and *nodH*, may be of interest as candidate genes for searching marker sequences required to facilitate express search for fast-growing symbionts of soybeans and alfalfa, respectively.

It should be noted that the relationship of structural polymorphism of *nod* genes with the structure of signal molecules and their indirect impact on symbiotic potential of root nodule bacteria remains very poorly understood. Investigations carried out in our laboratory revealed high level of the structural polymorphism of common *nod* genes and species-specific *nodH* gene in geographically isolated native populations of *S. meliloti* as compared to reference strain Rm1021 [48]. It was shown that native *S. meliloti* strains harbouring divergent alleles (in comparison with the reference strain) formed symbiosis mainly with annual alfalfa species under typical growth conditions, while under model salinity conditions tested strains were differed in ability to form effective symbiosis with different alfalfa species [49]. For different species of rhizobia, a positive correlation between *nodA* gene structure and the type of synthesized Nod factors was shown according *in silico* analysis (50). Based on the data obtained, the authors proposed to use the analysis of the *nodA* gene to search symbiotically active rhizobia strains with a certain host specificity in various ecological niches (50). Thus, the analysis of the literature data on the structural organization of *nod* genes involved in synthesis and decoration of Nod-factor allows us to consider allele polymorphism of *nod* genes as an important factor of signaling and host specificity variations in both fast- and slow-growing nodule bacteria species.

*Nif*, *fix* and *eff* gene groups. These nodule bacteria genes are responsible, respectively, for nitrogen fixing and symbiotic activity. The *nif* genes determine the synthesis of nitrogenase responsible for conversion of nitrogen into compounds available for plant metabolism. Of 20 *nif* genes described for *Klebsiella pneumonia*, two genes, *nifDK* and *nifH* encoding an enzyme complex, are mainly studied [39, 51]. The structure of *nif* genes is almost identical in taxonomically different microorganisms, while their arrangement and regulation differ. Analysis of these genes is often used to assess the potential nitrogen fixing ability in newly discovered microorganisms [52].

*Fix* and *eff* gene groups are characterized as genes determining so-called central intermediate metabolism [39, 41]. *B. japonicum* or *S. meliloti* genes highly expressed in bacteroids comprise about 15-16% of their number in saprophytic bacteria. The genes of *B. japonicum* or *S. meliloti* with high expression in bacteroids make up about 15-16% in relation to their number in saprophytic forms of bacteria. Total number of genes involved in regulation of symbiotic activity differs significantly in rhizobia species, also varies between the strains of the same species and depends on the experimental conditions [53]. Thus, the number of genes the activity of which changes (increases or decreases) as a result of symbiotic interaction is 982 or 1288 for *S. meliloti*, and 1234 or 2778 for *B. japonicum* [53]. If we compare sinorizobia and bradyrhizobia, then the portion of genes for which the expression is increased is 37% and 54%, correspondingly, while the functions of the vast majority of these genes remain unknown (53, 54). PubMed NCBI database (https://www.ncbi.nlm.nih.gov/pubmed/) contains over sixty papers that include the information about the genes of *Bradyrhizobium* and *Sinorhizobium* genera involved in symbiotic effectiveness which can be classified as *fix* and *eff* genes, however, the number of genes of each genus directly studied in this respect does not go above twenty. Up to this time, there is no systematization of data for these genes, apparently due to the fact that their activity is mediated and/or that their products are involved in different cellular processes. For instance, *fixNOQP* genes determine the synthesis of cbb3-cytochrome oxidase playing a key role in generation of reducing-oxidation potential (redox potential)

in bacteroids [55]. We have correlated the *fixB*, *fixC*, *fixU*, *fixX*, *fixO* and *fixQ* genes products of *B. japonicum* with the corresponding COG groups (cluster of orthologous groups) of widely-used protein product classification system [56]. It turned out that the products of these genes are predominantly belong to C group (metabolism associated with energy processes), while *fixK2* and *fixN* gene products belong to group T (signal transduction mechanisms) and P (transport of inorganic ions and metabolism), respectively. Moreover, among the genes affecting symbiotic activity of *B. japonicum* there are genes determining the synthesis of ferrodoxins (*fdxN*; COG group C), ACC-deaminase (*acdS*; group E), hydrolase (*blr6420*; group C) homologous to the *pobA* gene product (involved in hydroxybenzoate conversion), and transcription factor (*blr6378*; group K). Therefore, *B. japonicum* genes involved in control of symbiotic effectiveness mainly belong to COG groups associated with amino acid and energy metabolism.

Similar groups of *fix* and *eff* genes in *S. meliloti* are located on megaplasmids and on a chromosome. Genetic analysis of Tn5 mutants of streptomycin resistant CXM1-105 strain which, in turn, is a derivate of 425a strain widely used in manufacturing biologicals in 1980-1990s, revealed 12 new genes involved in control of symbiotic effectiveness [57-59]. Increase in dry mass yield of alfalfa plants inoculated with such transposants was ranged from 15 to 34%. Analysis of products of these genes showed that 42% are responsible for transport and metabolism of inorganic ions (COG group P), while the rest are involved in transport and metabolism of amino acids (COG group E) and carbohydrates (COG group G). Also the products of some genes are related to cellular processes and signaling (COG group M), and to storing and processing of information (COG group K). Therefore, *S. meliloti* genes involved in control of symbiotic effectiveness are numerous, and their products belong to a larger number of different COG groups than in *B. japonicum* species considered above.

Thus, genes involved in control of symbiotic effectiveness of plant-microbial systems of soybeans or alfalfa are numerous in both cases. At the same time, genes mentioned above are primarily involved in metabolic processes in *B. japonicum*, while in *S. meliloti* they are involved in various cellular processes. In addition, homologous genes in the reviewed rhizobia species may or may not be related to the regulation of symbiotic effectiveness. As was recently demonstrated, *rirA* gene in *S. fredii* HH103 is involved in iron metabolism but directly affects symbiotic effectiveness as well, while nothing like that was found in *S. meliloti* [60]. The described differences between the considered species in genes involved in symbiotic effectiveness control rather indicate utmost lack of our knowledge about this process. It should be noted that native strains may not harboured some of genes or carry different alleles of genes of interest in which functionally significant structural changes have occurred, as it was reported [47, 61]. Apparently, analysis of native polymorphism of alleles of the discovered above candidate genes is one of the approaches to studying the regulation of symbiotic effectiveness in rhizobia.

Group of genes involved in bacteria fitness control. Fitness means the ability of microorganisms to occupy various environmental niches, and the formation of mutualistic symbiosis is also viewed as a part of the latter. Sustainability under different biotic and abiotic factors of soil environment (temperature, humidity, medium pH, pollutants, osmotic stress, particularly, salinization) promotes an increase of the population density. At the same time, bacterial tolerance to drying (temperature, humidity, osmotic stress) is an important technical parameter for routine seed treatment [62-64]. Therefore, enhancement of strain tolerance to various abiotic factors is of practical significance. Aridity and salinity can be attributed to the most common model abiotic factors [64, 65].

Accumulation of various ions or compounds, including $K^+$, amino acids (e.g. glutamate), carbohydrates (including trehalose) or osmoprotectors (ectoin, glycine, betaine and choline) by bacteria increases their tolerance to high osmolarity. *S. meliloti* rhizobia may be attributed to moderate halotolerants as over 71% of native strains grow well at 0.6 M NaCl [65]. For now, bacteria of this species have revealed at least six groups of genes involved in responses to different types of stresses, which are further designated as *srg* (**s**tress **r**elated **g**enes) genes.

Resistance to salinization is predetermined by the activity of *bet*, *pro*, *tre* groups of genes, and to low pH by *act*, *ots* and *hpr* groups of genes. Interestingly, the products of these genes relating to various metabolic processes also affect symbiotic activity. For instance, *S. meliloti* betaine synthesis genes (*bet* genes) are involved in carbon and nitrogen pathways [65]. Microvegetation tests at normal conditions and under salinization revealed that native strains of *S. meliloti*, in which certain alleles of the *bet* gene were detected, have a salt tolerant phenotype (tolerant to high salt concentrations) and significantly more often form symbiosis with increased effectivity [47, 65].

*B. japonicum*, on the contrary, grow poorly even at 0.05 M NaCl, thus, bradyrhizobia have a salt-sensitive phenotype. As it was recently demonstrated, *B. japonicum* with higher catalase activity was able to grow in 0.15 M NaCl media [66]. According to trascriptomic analysis, 441 genes of *B. japonicum* changed activity at 0.05 M NaCl [53]. For 13 genes, the highest increase in activity was detected, and these genes (except *rpoH2*) were characterized as genus-specific since in *S. meliloti* they were not associated with salt tolerance [53]. Apparently, bradyrhizobia sensitivity to osmotic stress probably was caused by lack of transport systems like BCCT (betaine, carnitine, choline) which are typical, for *S. meliloti* [67]. Experimentally was shown that *B. japonicum* strains capable to utilize trehalose formed highly effective symbiosis [67].

It has been suggested that the selection of bradyrizobia strains by ecfG, nepR and phyR genes which products are involved in the cascade regulation of bacterial tolerance to general stresses, as well as by genes of bll/r1465-69 locus involved in the response to temperature shock and UV-light tolerance, can be promising for identification of stress-tolerant strains (68, 69), and these candidate genes can be used to create appropriate marker sequences.

It should be concluded that *srg* genes involved in development of stress-tolerance are present in the genomes of not only fast-growing and salt tolerant sinorhizobia, but in slow-growing salt sensitive bradyrhizobia. Activity of these genes in bacteria of both genera is associated with the central metabolism and directly affects the symbiotic effectiveness and nitrogen fixation. All of the above proves that evaluation of genes involved in resistance to various stress factors is promising in practical aspect.

Tolerance of soil bacteria to adverse environmental factors also depends on the ability to synthesize polysaccharides. These are various macromolecules whether bound or not bound to cellular wall having a wide range of biological functions, from signaling to protection. Polysaccharides provide bacteria protection from drying, temperature changes and from bacteriophages, as well as from specific and non-specific host plant immunity responce, etc. These compounds may have adhesive properties that enable bacteria to colonize various surfaces by forming microcolonies and/or biofilms. Thus, polysaccharides ensure rhizobia fitness enabling them ability to occupy almost any ecological niche, including colonizing legume roots, and are crucial for overcoming of abiotic stresses by bacteria [70-72].

*S. meliloti* synthesize various polysaccharides, i.e. capsular polysaccha-

rides, exopolysaccharides such as EPSI (succinoglucan) and EPSII (galactoglucan), lipopolysaccharides and cyclic glucans [36, 73-75]. Genes determining polysaccharide synthesis (*exo-exp-lps*) are located primarily on the second megaplasmid which does not contain *nod-nif-fix* genes. It has been shown that mutations in some polysaccharide synthesis genes result in disruption of plant-microbe interaction event at early stages, wherefore nodules do not form [58, 67, 74, 76]. With that, mutations in some genes (e.g. *exoB*, *exoY* or *lpsL*) can, on the contrary, enhance nodule formation [77]. In fast-growing soybean rhizobia *S. fredii*, a cluster of genes similar to that of *S. meliloti* which determines the polysaccharide synthesis and is also located on megaplasmid was found [16]. However, *S. fredii* strains do not synthesize EPSII and do not bind soybean lectin. Moreover, bacteria of this species are more sensitive to salinization, less motile and adhere better to non-biotic surfaces than *S. meliloti* bacteria.

Bradyrhizobia polysaccharides bind soy lectin and accumulate polysaccharides in peribacteroid space. These polysaccharides may play an important role in survival of bacteria which were not transformed into bacteroids and, after destroying nodules, release into environment. In *B. japonicum* the *lps* locus including *rfaD*, *rfaF*, *lpcC* and *galE* genes (their products are heptose epimerase, heptosyltransferase, mannosyltransferase and glucose epimerase, respectively) is directly involved in reception and nodulation [78]. Of vast interest is *rfaL* (or *waaL*) gene the product of which is a key enzyme in biosynthesis of cell wall in bradyrhizobia. The product of this gene participates in providing bacterial tolerance to various stresses and escaping from plant defense mechanisms under inoculation was shown [78].

Important that rhizobia could utilize inositol and its derivatives which are widespread in environmental systems as a source of carbon. Inositol is a part of plant cell wall, it is a main form of storage of phosphorus in seeds, and also can be a signal molecule [79]. Inositol derivatives attract attention due to their therapeutic effect in diabetes and Alzheimer's disease. Extended clusters of *iol* genes are discovered in sino- and bradyrhizobia, but the best studied gene is *idhA* in *S. fredii*. The activity of this gene is interconnected with formation of nitrogen fixing nodules, while similar gene of *S. meliloti* is related to competitiveness [79].

Among the genes associated with bradyrhizobia fitness, a special focus belongs to *nosZ*. The *nosZ* gene mutants of *B. japonicum* have higher $N_2O$-reductase activity ($N_2O$ reduction to $N_2$) and remain capable to form highly effective nitrogen fixing symbiosis with soybeans [80, 81]. Use of biofertilizers for soybean crops based on native strains harbouring modified *nos*Z gene, in the authors' opinion, may contribute to reduction in nitrogen oxide (greenhouse gas) emissions [82]. There is also an opinion that allelic polymorphism of *hup* genes (responsible for hydrogen accumulation), *nap* genes (encoding periplasmic nitrate reductase), and *nos* genes (encoding nitrite reductase) can be used to search native effective soybean symbionts [7, 81, 82].

Of direct relation to bacteria fitness are also so-called quorum-sensing (QS) systems. They are function as a global regulation factors detected in almost all known bacteria species and can be involved in the coordinating interaction between prokaryotes and eukaryotes. During growing bacterial cell population upon to certain density the synchronous synthesis, accumulation and secretion of chemical signals, the acylated homoserine lactones (AHLs), occurs [83]. Several QS systems are found in *S. meliloti*, one of which is *sin*R/*sin*I responsible for synthesis of homoserinelactones which regulate EPSII synthesis. Strains with mutated *sin* gene form nitrogen fixing nodules on *M. sativa* roots in considerably less amount and much later. QS system in *S. fredii* plays important role in devel-

oping biofilm on *Glycine max* roots. Apparently, similar system is also present in *B. japonicum* but it differs substantially from those described above and those found in other rhizobia species. It has been demonstrated that with high density of bradyrhizobia cells the virulence, on the contrary, goes down due to production of bradyoxetin, a compound structurally similar to some antibiotics and siderophores [70]. For bradyrhizobia, the inhibition of *nod* genes activity at high cell density and presence of flavonoids were established; the process also involves *nodD2* and *nolA* genes [84] discussed above. Data currently accumulated evidence that QS systems are extremely diverse and have different regulatory mechanisms, but the investigation of their role in plant-microbe interactions is actually in its early stage.

Summarizing the above, it should primarily be noted that information on main groups of symbiotically significant genes of nodule bacteria are still limited, fragmented and hardly comparable not only for different genera and species, but even for the strains of the same species. The available data confirm the fact that for each type of nodule bacteria that differ in genetic and phenotypic characteristics, it is necessary to analyze species-specific and strain-specific genes, for example, the 16S rRNA gene and genes which activity is associated with the formation of symbiosis and the ability to withstand to abiotic stresses (sym, srg and QS genes). Confirmation of the stable inheritance of sym-srg-QS genes alleles related to the formation of highly effective and stress tolerant plant-microbe symbiotic systems will increase the likelihood of the strain retaining symbiotically significant properties during its lab storage, and such approach can also be used for monitoring of the strain in microbiome of agrocenosis. Conversely, the identification of structural modifications in sequences of one or several candidate genes, for example, according to PCR data (in particular RFLP), will indicate at a possible change or loss of the corresponding phenotypic properties by the strain. A joint analysis of sequences of genes of interest belonged to functionally different groups of genes involved in the formation of highly effective stress-tolerant symbioses can be used to develop genetic markers using the SNP technology adapted for haploid genomes (85). The introduction of such markers in modern molecular genetic studies on symbiogenomics will promote targeted design of highly productive nitrogen-fixing plant-microbial systems with extensive adaptive potential for the development of organic agriculture in geographically different Russian regions.

Thus, evaluation of joint inheritance of alleles of genes of root nodule bacteria responsible for the formation of highly effective and stress tolerant symbiotic systems with host legumes will enable express testing of symbiotically significant and adaptive properties of rhizobia even during lab storage. This also makes it possible to develop protocols providing strain viability and genetic stability in biologicals and in microbiomes of agrocenoses.

## REFERENCES


1. Shamsutdinov Z.Sh. Forage crops selection: progress and challenges. *Agricultural Biology* [*Sel'skokhozyaistvennaya biologiya*], 2014, 6: 36-45 (doi: 10.15389/agrobiology.2014.6.36eng).
2. Tikhonovich I.A., Andronov E.E., Borisov A.Yu., Dolgikh E.A., Zhernakov A.I., Zhukov V.A., Provorov N.A., Roumiantseva M.L., Simarov B.V. *Genetika*, 2015, 51(9): 973-990 (doi: 10.7868/S001667581509012X) (in Russ.).
3. Werner G.D.A., Cornwell W.K., Cornelissen J.H.C., Kiers E.T. Evolutionary signals of symbiotic persistence in the legume—rhizobia mutualism. *PNAS*, 2015, 112(33): 10262-10269 (doi: 10.1073/pnas.1424030112).
4. Roumiantseva M.L., Stepanova G.V., Muntyan V.S., Onishchuk O.P., Kozhemyakov A.P., Simarov B.V. V sbornike: *Aktual'nye napravleniya selektsiii I spol'zovaniya lyutserny v kormoproizvodstve*. Moscow, 2014: 133-142 (in Russ.).
5. Dragomir N., Pe I., Dragomir C.P.A., Toth S., Ravdan S. Enhancement of the capacity of

biological nitrogen fixation in alfalfa by bacterial inoculation with *Sinorhizobium meliloti* strains. *Romanian Journal of Grassland and Forage Crops*, 2010, 1: 17-23.
6. Mabrouk Y., Belhadj O. Enhancing the biological nitrogen fixation of leguminous crops grown under stressed environments. (Review). *African Journal of Biotechnology*, 2012, 11(48): 10809-10815 (doi: 10.5897/AJB10.2170).
7. O'Callaghan M. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology*, 2016, 100(13): 5729-5746 (doi: 10.1007/s00253-016-7590-9).
8. Checcucci A., Dicenzo G.C., Bazzicalupo M., Mengoni A. Trade, diplomacy, and warfare: the quest for elite rhizobia inoculant strains. *Frontiers in Microbiology*, 2017, 8: 2207 (doi: 10.3389/fmicb.2017.02207).
9. Meghvansi M.K., Prasad K., Mahna S.K. Symbiotic potential, competitiveness and compatibility of indigenous *Bradyrhizobium japonicum* isolates to three soybean genotypes of two distinct agro-climatic regions of Rajasthan, India. *Saudi Journal of Biological Sciences*, 2010, 17(4): 303-310 (doi: 10.1016/j.sjbs.2010.06.002).
10. Umarov B.R. *Universum: Khimiya I biologiya: elektronnyi nauchny izhurnal*, 2019, 4(58): 1. Available http://7universum.com/ru/nature/archive/item/7083.No date.
11. Gibson A.H., Demezas D.H., Gault R.R., Bhuvaneswari T.V., Brockwell J. Genetic stability in rhizobia in the field. *Plant and Soil*, 1990, 129(1): 37-44 (doi: 10.1007/BF00011689). (in Russ.).
12. Aronshtam A.A., Bazhenova O.V., Zaretskaya A.N., Kuchko V.V., Novikova N.I., Provorov N.A., Fedorov S.N., Fokina I.G., Chernova T.A. *Geneticheskie metody selektsii kluben'kovykh bakterii (metodicheskie rekomendatsii)* [Genetic methods for breeding nodule bacteria (guidelines)]. Leningrad, 1984 (in Russ.).
13. Denton M., Yates R., Seymour N., Herridge D. Northern, southern and western regions. Harvesting the benefits of inoculating legumes. In: *Rhizobial inoculants FACT SHEET*. Grains Research and Developments Corporation, January 2013. Available http://www.coretext.com.au. Accessed 26.09.2019.
14. Sachs J.L., Ehinger M.O., Simms E.L. Origins of cheating and loss of symbiosis in wild *Bradyrhizobium*. *Journal of Evolutionary Biology*, 2010, 23: 1075-1089 (doi: 10.1111/j.1420-9101.2010.01980).
15. Tian C.F., Zhou Y.J., Zhang Y.M., Li Q.Q., Zhang Y.Z., Li D.F., Wang S., Wang J., Gilbert L.B., Li Y.R., Chen W.X. Comparative genomics of rhizobia nodulating soybean suggests extensive recruitment of lineage-specific genes in adaptations. *PNAS*, 2012, 109(22): 8629-8634 (doi: 10.1073/pnas.1120436109).
16. Lopez-Baena F.J., RuIz-Sainz J.E., Rodriguez-Carvajal M.A., Vinardell J.M. Bacterial molecular signals in the *Sinorhizobium fredii*-soybean symbiosis. *International Journal of Molecular Sciences*, 2016, 17(5): 755 (doi:10.3390/ijms17050755).
17. Sachs J.L., Russell J.E., Hollowell A.C. Evolutionary instability of symbiotic function in *Bradyrhizobium japonicum*. *PLoS ONE*, 2011, 6(11): e26370 (doi: 10.1371/journal.pone.0026370).
18. Batista J.S., Hungria M., Barcellos F.G., Ferreira M.C., Mendes I.C. Variability in *Bradyrhizobium japonicum* and *B. elkanii* seven years after introduction of both the exotic microsymbiont and the soybean host in a cerrados soil. *Microbial Ecology*, 2007, 53(2): 270-284 (doi: 10.1007/s00248-006-9149-2).
19. Kaneko T., Nakamura Y., Sato S., Minamisawa K., Uchiumi T., Sasamoto S., Watanabe A., Idesawa K., Iriguchi M., Kawashima K., Kohara M., Matsumoto M., Shimpo S., Tsuruoka H., Wada T., Yamada M., Tabata S. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. *DNA Research*, 2002, 9(6): 189-197 (doi: 10.1093/dnares/9.6.225).
20. Melchiorre M., de Luca M., Gonzalez A.G., Suarez P., Lopez C., Lascano R., Racca R.W. Evaluation of bradyrhizobia strains isolated from field-grown soybean plants in Argentina as improved inoculants. *Biology and Fertility of Soils*, 2011, 47(1): 81-89 (doi: 10.1007/s00374-010-0503-7).
21. Gyogluu C., Jaiswal S.K., Kyei-Boahen S., Dakora F.D. Identification and distribution of microsymbionts associated with soybean nodulation in Mozambican soils. *Systematic and Applied Microbiology*, 2018, 41(5): 506-515 (doi: 10.1016/j.syapm.2018.05.003).
22. Chueire L.M.O., Hungaria M. $N_2$-fixation ability of Brazilian soybean cultivars with *Sinorhizobium fredii* and *Sinorhizobium xinjiangensis*. *Plant and Soil*, 1997, 196(1): 1-5 (doi: 10.1023/A:1004222218007).
23. Hoque M.S., Broadhurst L.M., Thrall P.H. Genetic characterization of root-nodule bacteria associated with *Acacia salicina* and *A. stenophylla* (*Mimosaceae*) across south-eastern Australia. *International Journal of Systematic and Evolutionary Microbiology*, 2011, 61(2): 299-309 (doi: 10.1099/ijs.0.021014-0).
24. Marinkovic J.B., Bjelic D.D., Tintor B.B., Ignjatov M.V., Nikolic Z.T., Dukic V.H., Balesevic-Tubic S.N. Molecular identification of *Bradyrhizobium japonicum* strains isolated from root nodules of soybean (*Glycine max* L.). *Zbornik Matice Srpskeza Prirod ne Nauke*, 2017, 132: 49-56

(doi: 10.2298/ZMSPN1732049M).
25. Roumiantseva M.L., Muntyan V.S., Mengoni A., Simarov B.V. *Genetika*, 2014, 50(4): 400-412 (doi: 10.7868/S0016675814040109) (in Russ.).
26. Tan Z., Hurek T., Vinuesa P., Muller P., Ladha J.K., Reinhold-Hurek B. Specific detection of *Bradyrhizobium* and *Rhizobium* strains colonizing rice (*Oryza sativa*) roots by 16S-23S ribosomal DNA intergenic spacer-targeted PCR. *Applied and Environmental Microbiology*, 2001, 67(8): 3655-3664 (doi: 10.1128/AEM.67.8.3655-3664.2001).
27. Zotov V.S., Punina N.V., Khapchaeva S.A., Didovich S.V., Mel'nichuk T.N., Topunov A.F. *Ekologicheskaya genetika*, 2012, 1(2): 50-63 (in Russ.).
28. Cherni A.E., Perret X. Deletion of rRNA operons of *Sinorhizobium fredii* strain NGR234 and impact on symbiosis with legumes. *Frontiers in Microbiology*, 2019, 10: 154 (doi: 10.3389/fmicb.2019.00154).
29. Guo H.J., Wang E.T., Zhang X.X., LiQ.Q., Zhang Y.M., Tian C.F., Chena W.X. Replicon-dependent differentiation of symbiosis-related genes in *Sinorhizobium* strains nodulating *Glycine max*. *Applied and Environmental Microbiology*, 2013, 80(4): 1245-1255 (doi: 10.1128/AEM.03037-13).
30. Kanehara K., Minamisawa K. Complete genome sequence of *Bradyrhizobium japonicum* J5, isolated from a soybean nodule in Hokkaido, Japan. *Genome Announcements*, 2017, 5(6): e01619-16 (doi: 10.1128/genomeA.01619-16).
31. Itakura M., Saeki K., Omor I. H., Yokoyama T., Kaneko T., Tabata S., Ohwada T., Tajima S., Uchiumi T., Honnma K., Fujita K., Iwata H., Saeki Y., Hara Y., Ikeda S., Eda S., Mitsui H., Minamisawa K. Genomic comparison of *Bradyrhizobium japonicum* strains with different symbiotic nitrogen-fixing capabilities and other *Bradyrhizobiaceae* members. *The ISME Journal*, 2009, 3(3): 326-339 (doi: 10.1038/ismej.2008.88).
32. Muntyan V.S., Cherkasova M.E., Andronov E.E., Simarov B.V., Roumiantseva M.L. *Genetika*, 2016, 52(10): 1126-1133 (doi: 10.7868/S0016675816080105) (in Russ.).
33. Sullivan J.T., Patrick H.N., Lowther W.L., Scott D.B., Ronson C.W. Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *PNAS*, 1995, 92(19): 8985-8989 (doi: 10.1073/pnas.92.19.8985).
34. Barcellos F.G., Menna P., da Silva Batista J.S., Hungria M. Evidence of horizontal transfer of symbiotic genes from a *Bradyrhizobium japonicum* inoculant strain to indigenous diazotrophs *Sinorhizobium* (*Ensifer*) *fredii* and *Bradyrhizobium elkanii* in a Brazilian Savannah soil. *Applied and Environmental Microbiology*, 2007, 73(8): 2635-2643 (doi: 10.1128/AEM.01823-06).
35. Kobayashi H., Broughton W.J. Fine-tuning of symbiotic genes in rhizobia: flavonoid signal transduction cascade. In: *Nitrogen-fixing leguminous symbioses. Nitrogen fixation: origins, applications, and research progress, vol. 7.* M.J. Dilworth, E.K. James, J.I. Sprent, W.E. Newton (eds.). Springer, Dordrecht, 2008: 117-152 (doi: 10.1007/978-1-4020-3548-7_5).
36. Barnett M.J., Long S.R. The *Sinorhizobium meliloti* Syr Mregulon: effects on global gene expression are mediated by *syr*A and *nod*D3. *Journal of Bacteriology*, 2015, 197:1792-1806 (doi:10.1128/JB.02626-14).
37. Roche P., Maillet F., Plazanet C., Debelle F., Ferro M., Truchet G., Prome J.-C., Denarie J. The common *nod*ABC genes of *Rhizobium meliloti* are host-range determinants. *PNAS*, 1996, 93(26): 15305-15310 (doi: 10.1073/pnas.93.26.15305).
38. Capela D., Carrere S., Batut J. Transcriptome-based identification of the *Sinorhizobium meliloti* NodD1 regulon. *Applied and Environmental Microbiology*, 2005, 71(8): 4910-4913 (doi: 10.1128/AEM.71.8.4910-4913.2005).
39. Shamseldin A. The role of different genes involved in symbiotic nitrogen fixation (review). *Global Journal of Biotechnology and Biochemistry*, 2013, 8(4): 84-94 (doi: 10.5829/idosi.gjbb.2013.8.4.82103).
40. Loh J., Stacey G. Nodulation gene regulation in *Bradyrhizobium japonicum*: a unique integration of global regulatory circuits. *Applied and Environmental Microbiology*, 2003, 69(1): 10-17 (doi: 10.1128/AEM.69.1.10-17.2003).
41. Pessi G., Ahrens C.H., Rehrauer H., Lindemann A., Hauser F., Fischer H-M., Hennecke H. Genome-wide transcript analysis of *Bradyrhizobium japonicum* bacteroids in soybean root nodules. *Molecular Plant-Microbe Interactions*, 2007, 20(11): 1353-1363 (doi: 10.1094/MPMI-20-11-1353).
42. Krause A., Doerfel A., Gottfert M. Mutational and transcriptional analysis of the type III secretion system of *Bradyrhizobium japonicum*, *Molecular Plant-Microbe Interactions*, 2002, 15(12): 1228-1235 (doi: 10.1094/MPMI.2002.15.12.1228).
43. Jimenez-Guerrero I., Perez-Montano F., Medina C., Ollero F.J., Lopez-Baena F.J. NopC is a rhizobium-specific type 3 secretion system effector secreted by *Sinorhizobium* (*Ensifer*) *fredii* HH103. *PLoS ONE*, 2015, 10(11): e0142866 (doi: 10.1371/journal.pone.0142866).
44. Zhao R., Liu X.L., Zhang Y.Z., Jiao J., Cui W.J., Zhang B., Wang X.L., Li M.L., Chen Y., Xiong Z.O., Chen W.X., Tian C.F. Adaptive evolution of rhizobial symbiotic compatibility mediated by co-evolved insertion sequences. *The ISME Journal*, 2018, 12(1): 101-111 (doi: 10.1038/ismej.2017.136).
45. Torres T.G., Del Papa M.F., Soria-Diaz M.E., Draghi W., Lozano M., Giusti M.L.,

Manyani H., Megias M., Serrano G.A., Puhler A., Niehaus K., Lagares A., Pistorio M. The nodulation of alfalfa by the acid-tolerant *Rhizobium* sp. strain LPU83 does not require sulfated forms of lipochitooligosaccharide nodulation signals. *Journal of Bacteriology*, 2011, 193(1): 30-39 (doi: 10.1128/JB.01009-10 2011).

46. Lopez-Baena F.J., Ruiz-Sainz J.E., Rodriguez-Carvajal M.A., Vinardell J.M. Bacterial molecular signals in the *Sinorhizobium fredii*-soybean symbiosis. *International Journal of Molecular Sciences*, 2016, 17(5): E755 (doi: 10.3390/ijms17050755).
47. Vinardell J.M., Acosta-Jurado S., Zehner S., Gottfert M., Becker A., Baena I., Blom J., Crespo-Rivas J.C., Goesmann A., Jaenicke S., Krol E., Mcintosh M., Margaret I., Perez-Montano F., Schneiker-Bekel S., Serrania J., Szczepanowski R., Buendia A.M., Lloret J., Bonilla I., Puhler A., Ruiz-Sainz J.-E., Weidner S. The *Sinorhizobium fredii* HH103 genome: a comparative analysis with *S. fredii* strains differing in their symbiotic behavior with soybean. *Molecular Plant-Microbe Interactions*, 2015, 28(7): 811-824 (doi: 10.1094/MPMI-12-14-0397-FI).
48. Roumiantseva M.L., Saksaganskaya A.S., Muntyan V.S., Cherkasova M.E., Simarov B.V. *Genetika*, 2018, 54(5): 524-534 (doi: 10.7868/S001667581805003X) (in Russ.).
49. Saksaganskaya A.S., Muntyan V.S., Rumyantseva M.L. *Materialy Mezhdunarodnoi nauchnoi konferentsii PLAMIC2018 «Rasteniya i mikroorganizmy: biotekhnologiya budushchego»* [Proc. Int. Conf. PLAMIC2018 «Plants and microorganisms: biotechnology of the future»]. Ufa, 2018: 222 (in Russ.).
50. Debelle F., Moulin L., Mangin B, Denarie J., Boivin C. Nod genes and Nod signals and the evolution of the *Rhizobium* legume symbiosis. *Acta Biochimica Polonica*, 2001, 48(2): 359-365.
51. KlippW. Historical perspective — development of *nif* genetics and regulation in *Klebsiella pneumonia*. In: *Genetics and regulation of nitrogen fixation in free-living bacteria*. W. Klipp, B. Masepohl, O.R. Gallon, W.E. Newton (eds.). KluwerAcademicPublishers, the Netherlands, 2005: 1-25 (doi:10.1007/1-4020-2179-8).
52. Baimiev An.Kh., Ivanova E.S., Gumenko R.S., Chubukova O.V., Baimiev Al.Kh. *Genetika*, 2015, 51(12): 1359-1367 (doi: 10.7868/S001667581511003X) (in Russ.).
53. Chang W.S., Franck W.L., Cytryn E., Jeong S., Joshi T., Emerich D.W., Sadowsky M.J., Xu D.,Stacey G. An oligonucleotide microarray resource for transcriptional profiling of *Bradyrhizobium japonicum*. *Molecular Plant-Microbe Interactions*, 2007, 20(10): 1298-1307 (doi:10.1094/MPMI-20-10-1298).
54. Becker A., Berges H., Krol E., Bruand C., Ruberg S., Capela D., Lauber E., Meilhoc E., Ampe F., de Bruijn F.J., Fourment J., Francez-Charlot A., Kahn D., Kuster H., Liebe C., Puhler A., Weidner S., Batut J. Global changes in gene expression in *Sinorhizobium meliloti* 1021 under microoxic and symbiotic conditions. *Molecular Plant-Microbe Interactions*, 2004, 17(3): 292-303 (doi: 10.1094/MPMI.2004.17.3.292).
55. Bueno E., Richardson D.J., Bedmar E.J., Delgado M.J. Expression of *Bradyrhizobium japonicum cbb3* terminal oxidase under denitrifying conditions is subjected to redox control. *FEMS Microbiology Letters*, 2009, 298(1): 20-28 (doi:10.1111/j.1574-6968.2009.01711.x).
56. Galperin M.Y., Makarova K.S., Wolf Y.I., Koonin E.V. Expanded microbial genome coverage and improved protein family annotation in the COG database. *Nucleic Acids Research*, 2015, 43: D261-269 (doi: 10.1093/nar/gku1223).
57. Onishchuk O.P., Chizhevskaya E.P., Kurchak O.N., Andronov E.E., Simarov B.V. *Ekologicheskaya genetika*, 2014, 12(1): 39-47 (in Russ.).
58. Onishchuk O.P., Sharypova L.A., Kurchak O.N., Bekker A., Simarov B.V. *Genetika*, 2005, 41(12): 1617-1623 (in Russ.).
59. Yurgel S.N., Sharypova L.A., Simarov B.V., Soberon M., Miranda J., Morera C. Isolation of *Sinorhizobium meliloti* Tn5 mutants with altered cytochrome terminal oxidase expression and improved symbiotic performance. *FEMS Microbiology Letters*, 1998, 165(1): 167-173 (doi: 10.1111/j.1574-6968.1998.tb13142.x).
60. Crespo-Rivas J.C., Navarro-Gomez P., Alias-Villegas C., Shi J., Zhen T., Niu Y., Cuellar V., Moreno J., Cubo T., Vinardell J.M., Ruiz-Sainz J.E., Acosta-Jurado S., Soto M.J. *Sinorhizobium fredii* HH103 RirA is required for oxidative stress resistance and efficient symbiosis with soybean. *International Journal of Molecular Sciences*, 2019, 20(787): 1-16 (doi: 10.3390/ijms20030787).
61. Onishchuk O.P., Kurchak O.N., Chizhevskaya E.P., Provorov N.A., Simarov B.V. Population polymorphism of the alfalfa nodule bacteria (*Sinorhizobium meliloti*) for the genes encoding for symbiotic efficiency and competitiveness. *Agricultural Biology* [*Sel'skokhozyaistvennaya biologiya*], 2015, 50(3): 339-344 (doi: 10.15389/agrobiology.2015.3.339eng).
62. Deaker R., Roughley R.J., Kennedy I.R. Legume seed inoculation technology — a review. *Soil Biology and Biochemistry*, 2004: 36(8): 1275-1288 (doi: 10.1016/j.soilbio.2004.04.009).
63. Buntic A.V., Stajkovic-Srbinovic O.S., Knezevic M.M., Kuzmanovic D.Z., Rasulic N.V., Delic D.I. Development of liquid rhizobial inoculants and pre-inoculation of alfalfa seeds. *Archives of Biological Sciences*, 2018, 71 (doi: 10.2298/ABS181008062B).
64. Vriezen J.A., de Bruijn F.J., Nьsslein K. Responses of rhizobia to desiccation in relation to osmotic stress, oxygen, and temperature. *Applied and Environmental Microbiology*, 2007, 73(11):

3451-3459 (doi: 10.1128/AEM.02991-06).
65. Roumiantseva M.L., Muntyan V.S. *Mikrobiologiya*, 2015, 84(3): 263-278 (doi: 10.7868/S0026365615030179) (in Russ.).
66. Yakimenko M.V., Begun S.A., Sorokina A.I. *Vestnik DVORAN*, 2019, 3: 36-41 (doi: 10.25808/08697698.2019.205.3.006) (in Russ.).
67. Brechenmacher L., Lei Z., Libault M., Findley S., Sugawara M., Sadowsky M.J., Sumner L.W., Stacey G. Soybean metabolites regulated in root hairs in response to the symbiotic bacterium *Bradyrhizobium japonicum*. *Plant Physiology*, 2010, 153(4): 1808-1822 (doi: 10.1104/pp.110.157800).
68. Gourion B., Sulser S., Frunzke J., Francez-Charlot A., Stiefel P., Pessi G., Vorholt J.A., Fischer H.M. The PhyR-EcfG signalling cascade is involved in stress response and symbiotic efficiency in *Bradyrhizobium japonicum*. *Molecular Microbiology*, 2009, 73(2): 291-305 (doi: 10.1111/j.1365-2958.2009.06769.x).
69. Masloboeva N. Role of ECF factors in stress response of *Bradyrhizobium japonicum*. DISS. ETH No. 20849 (doi: 10.3929/ethz-a-007606640).
70. Gonzalez J.E., Marketon M.M. Quorum sensing in nitrogen-fixing rhizobia. *Microbiology and Molecular Biology Reviews*, 2003, 67(4): 574-592 (doi: 10.1128/MMBR.67.4.574-592.2003).
71. Perez-Montano F., Jimenez-Guerrero I., Sanchez-Matamoros R.C., Perez-Montano F., Jimenez-Guerrero I., Rodriguez-Carvajal M.A., Bellogin R.A., Espuny M.R. Rice and bean AHL-mimic quorum-sensing signals specifically interfere with the capacity to form biofilms by plant-associated bacteria. *Research in Microbiology*, 2013, 164(7): 749-760 (doi: 10.1016/j.resmic.2013.04.001).
72. Perez-Montano F., Jimenez-Guerrero I., Del Cerro P., Baena-Ropero I., Lopez-Baena I.J., Ollero F.J., Belloin R., Lloret J., Espuny R. The symbiotic biofilm of *Sinorhizobium fredii* SMH12, necessary for successful colonization and symbiosis of *Glycinemax* cv Osumi, is regulated by quorum sensing systems and inducing flavonoids via NodD1. *PLoS ONE*, 2014, 9(8): e105901 (doi: 10.1371/journal.pone.0105901).
73. Limoli D.H., Jones C.J., Wozniak D.J. Bacterial extracellular polysaccharides in biofilm formation and function. *Microbiology Spectrum*, 2015, 3(3): MB-0011-2014 (doi: 10.1128/microbiolspec.MB-0011-2014).
74. Bazaka K., Crawford R.J., Nazarenko E.L., Ivanova E.P. Bacterial extracellular polysaccharides. In: *Bacterial adhesion. Advances in experimental medicine and biology, vol. 715*. D. Linke, A. Goldman (eds.). Springer, Dordrecht, 2011: 213-226 (doi: 10.1007/978-94-007-0940-9_13).
75. Mendis H.C., Madzima T.F., Queiroux C., Jones K.M. Function of succinoglycan polysaccharide in *Sinorhizobium meliloti* host plant invasion depends on succinylation, not molecular weight. *mBio*, 2016 7(3): e00606-16 (doi: 10.1128/mBio.00606-16).
76. Janczarek M. Environmental signals and regulatory pathways that influence exopolysaccharide production in rhizobia. *International Journal of Molecular Sciences*, 2011, 12(11): 7898-7933 (doi: 10.3390/ijms12117898).
77. Marczak M., Mazur A., Koper P., ebrack I.K., Skorupska A. Synthesis of rhizobial exopolysaccharides and their importance for symbiosis with legume plants. *Genes*, 2017, 8(12): E360 (doi: 10.3390/genes8120360).
78. Noh J.G., Jeon H.E., So J.S., Chang W.S. Effects of the *Bradyrhizobium japonicum waa*L (*rfa*L) gene on hydrophobicity, motility, stress tolerance, and symbiotic relationship with soybeans. *International Journal of Molecular Sciences*, 2015, 16(8): 16778-16791 (doi: 10.3390/ijms160816778).
79. Yoshida K., Kim W.-S., Kinehara M., Mukai R., Ashida H., Ikeda H., Fujita Y., Krishnan H.B. Identification of a functional 2-keto-myo-inositol dehydratase gene of *Sinorhizobium fredii* USDA191 required for myo-inositol utilization. *Bioscience, Biotechnology and Biochemistry*, 2006, 70(12): 2957-2964 (doi: 10.1271/bbb.60362).
80. Sanchez S., Itakura M., Mitsui H., Minamisawa K. Linked expressions of *nap* and *nos* genes in a *Bradyrhizobium japonicum* mutant with increased $N_2O$ reductase activity. *Applied and Environmental Microbiology*, 2013, 79(13): 4178-4180 (doi: 10.1128/AEM.00703-13).
81. Bueno E., Mesa S., Sanchez C., Bedmar E.J., Delgado M.J. NifA is required for maximal expression of denitrification genes in *Bradyrhizobium japonicum*. *Environmental Microbiology*, 2010, 12(2): 393-400 (doi: 10.1111/j.1462-2920.2009.02076.x).
82. Itakura M., Tabata K., Eda S., Mitsui H., Murakami K., Yasuda J., Minamisawa K. Generation of *Bradyrhizobium japonicum* mutants with increased $N_2O$ reductase activity by selection after introduction of a mutated *dna*Q gene. *Applied and Environmental Microbiology*, 2008, 74(23): 7258-7264 (doi: 10.1128/AEM.01850-08).
83. Khmel' I.A., Belik A.S., ZaitsevaYu.V., Danilova N.N. *Vestnik Moskovskogo universiteta. Seriya 16: Biologiya*, 2008, 1: 28-35 (in Russ.).
84. Bogino P.C., Nievas F.L., Giordano W. A review: quorum sensing in *Bradyrhizobium*. *Applied Soil Ecology*, 2015, 94: 49-58 (doi: 10.1016/j.apsoil.2015.04.016).
85. Beerenwinkel N., Gunthard H.F., Roth V., Metzner K.J. Challenging and opportunities in estimating viral genetic diversity from next generating sequencing data. *Frontiers in Microbiology*, 2012, 3: 329 (doi: 10.3389/fmicb.2012.00329).