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D.V. KRUTYLO*, O.V. NADKERNYCHNA

Institute of Agricultural Microbiology and Agroindustrial Manufacture, NAAS,
97 Shevchenka Str., Chernihiv, 14027, Ukraine

* Corresponding author; e-mail: krutylodv@gmail.com

FEATURES OF LOCAL BRADYRHIZOBIA POPULATIONS AFTER LONG-TERM PERIOD IN THE SOIL WITHOUT A HOST PLANT

In previous years, the serological and genetic diversities of soybean nodule bacteria in agrocenoses of Ukraine have been researched. Less attention was paid to the study of their survival in the soil. Taking into account the natural heterogeneity of bacteria of the genus Bradyrhizobium, the ***aim*** *of this work was to evaluate the diversity of bradyrhizobia in local populations of different soils after a long-term period without leguminous plants, to obtain new isolates of nodule bacteria and to study their properties.* ***Methods.*** *Microbiological (isolation of bradyrhizobia from the nodules of trap plants, study of the properties of strains), serological (study of the diversity of rhizobia in nodule populations, study of the serological affiliation of strains), vegetation and field experiments (study of plant infecting with bradyrhizobia).* ***Results.*** *Local populations of bradyrhizobia in sod-podzolic soil and leached chornozem were studied using trap plants of the genera Glycine, Vigna, and Lupinus. It was established that after a 7 to 8-year period without leguminous plants, active nodule bacteria remained in both types of soil, which nodulated cultivated and wild soybeans, cowpeas, mung beans, adzuki beans, and lupine. The main microsymbionts of plants of the genera Glycine and Vigna on different types of soil were soybean bradyrhizobia belonging to 6 serological groups: 46, M8, KB11, 634b, HR, and B1. The representatives of 4 serogroups corresponded to the inoculant strains of Bradyrhizobium japonicum 46, M8, 634b, and KB11, which were periodically used in the studied areas. In addition to B. japonicum, cowpea plants trapped microsymbionts of B. lupini serogroup 367a (4.2%) from the soil. Bradyrhizobia of serogroup B1 were detected both in nodules of cowpea (6.3%) and wild soybean (12.5%). 45.8% of lupine nodules were formed by bacteria B. lupini of serogroup 367a. The appearance in populations of representatives of serogroups HR and B1 along with a group of unidentified microsymbionts requires further research. Cultivation of trap plants of wild soybeans and various types of cowpea made it possible to identify saprophytic strain B. japonicum M8 (formed 25.0% to 83.4% of nodules) in the sod-podzolic soil, which did not infect the roots of cultivated soybeans. 70 isolates of bradyrhizobia were obtained from nodules of trap plants, which were preliminarily identified as B. japonicum, B. lupini, and Bradyrhizobium sp.* ***Conclusions.*** *The results confirm the importance of using different leguminous trap plants for a more complete characterization of the local rhizobial community. Cultiva-*

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tion of plants of the genera Glycine, Vigna, and Lupinus, capable of cross-infection, made it possible to detect bacteria B. japonicum (serogroups 46, M8, KB11, 634b, HR), B. lupini (serogroup 367a), and Bradyrhizobium sp. (serogroup B1), which exist for a long-term period as saprophytes in sod-podzolic soil and leached chornozem. 70 isolates of bradyrhizobia were obtained, 35 of which were serologically related to the inoculant strains of B. japonicum introduced into the agrocenosis at the beginning of the research.

Keywords: *Bradyrhizobium japonicum, B. lupini, bradyrhizobia populations, trap hosts, wild soybean, cowpea, mung bean, adzuki bean, lupine.*

An important feature of legumes is their ability to form symbiosis with nitrogen-fixing microorganisms — nodule bacteria. The use of active strains of rhizobia to improve the growth and nutrition of legumes is considered one of the promising approaches in modern agriculture. As the basis of biological preparations, they provide an increase in yield, improve the quality of the obtained products, and contribute to the formation of local populations of specific nodule bacteria in the soil [1, 2].

Scientists around the world pay considerable attention to the study of the diversity of microsymbionts of various leguminous plants [3, 4]. However, there are relatively few studies of long-term survival of rhizobia in the absence of a host plant [5, 6]. Evaluation of the polymorphism of soil communities of nodule bacteria contributes to the understanding of the processes of formation of local populations and the emergence of new rhizobia genotypes. It is also important for the search for highly effective strains — potential bioagents of microbial preparations.

In Ukraine, the research of microsymbionts of various leguminous plants is at an initial stage. Several types of slow-growing nodule bacteria of the genus *Bradyrhizobium*, widespread in the country's soils, are described in domestic literature. These are soybean microsymbionts — *Bradyrhizobium japonicum* and *B. diazoefficiens* [7] and lupine microsymbionts — *B. lupini* [8]. Their soil populations have different densities, which is related to the duration of cultivation of specified leguminous crops and the use of appropriate biological preparations in cultivation technologies [1, 3]. In previous years, we have investigated the serological and genetic diversity of soybean microsymbionts in the agrocenoses of Ukraine [9, 10]. However, not enough attention has been paid to studying the long-term survival of bradyrhizobia in the absence of a host plant. Considering the natural heterogeneity of the *Bradyrhizobium* genus [11], it is important to study the survival and qualitative composition of local communities of these microorganisms after a long-term saprophytic mode in the soil.

A promising method for detecting individual strains of nodule bacteria in soil populations and assessing their diversity is the use of the so-called 'trap plants' capable of forming symbiotic relationships with many types of rhizobia [12, 13]. Representatives of *Glycine*, *Vigna* and *Lupinus* genera can be used as trap plants for the analysis of local communities of bacteria of *Bradyrhizobium* genus in the agrocenoses of Ukraine.

Considering the above, the aim of our work was to evaluate the diversity of bradyrhizobia in local populations of different soils after a long-term period without leguminous plants in order to obtain new isolates of nodule bacteria and to study their properties.

Materials and Methods. The objects of the research were local populations of bradyrhizobia in the soils of the Chernihiv oblast of Ukraine, strains of nodule bacteria obtained from root nodules of plants of the genera *Glycine*, *Vigna,* and *Lupinus*; plants of cultivated soybean (*Glycine max* (L.) Merr., Smolyanka variety) and wild soybean (*Glycine soja*), cowpea (*Vigna unguiculata* (L.) Walp., UD0301857), mung bean (*Vigna radiata* (L.) Wilczek, Tadzhytskyi 1 variety), adzuki bean (*Vigna angularis* (Willd.).

Ohwi &Ohashi), and white lupine (*Lupinus albus* L., Lybid variety). New strains of rhizobia are stored in the collection of the Laboratory of Plant-Microbial Interactions of the Institute of Agricultural Microbiology and Agroindustrial Manufacture, NAAS (IAMAM NAAS) of Ukraine.

Research of the composition of local bradyrhizobia populations in different soils.

The research was conducted under the conditions of small-scale field and vegetation experiments on a sod-podzolic soil and leached chornozem, respectively.

Soybeans cultivation started 20 years before on the sod-podzolic soil of the IAMAM NAAS experimental field. The use of inoculant strains of *B. japonicum* (slow- and fast-growing) contributed to the formation of a local population of soybean microsymbionts in the soil. During the last 7 years, legumes had not been sown in this area. To characterize the population of nodule bacteria in the year of the research, cultivated soybeans and trap plants for bradyrhizobia, such as wild soybean, cowpea, mung bean, and adzuki bean, were grown in the field. In Ukraine, representatives of *Vigna* genus are less common or not cultivated at all. Before sowing, the seeds were moistened with tap water; inoculation with nodule bacteria was not performed. The area of the registration plot was 3 m^2. The experiment had four repeats.

In the flowering phase, nodules were selected from the roots of plants. The share of different strains of rhizobia in nodule populations was determined by analysis of nodule homogenates (48 units) in the agglutination reaction (Gruber-Widal technique) [10, 14]. We used a set of specific immune antisera obtained to active strains of soybean nodule bacteria *B. japonicum* 46, M8, KB11, 634b, OR, HR, and NR, microsymbionts of cowpea *Bradyrhizobium* sp. B1 and lupine *B. lupini* 367a.

The diversity of rhizobia in the nodules was estimated by the Shannon diversity index (H), calculated according to the formula [15]:

$$H = -\sum P_i \ln P_i,$$

where P_i is the relative incidence of the *i*-th strain, calculated as n_i/N, where N is the total number of nodules formed by different strains of soybean nodule bacteria and n_i is the number of nodules formed with the involvement of the rhizobia strain of a specific serogroup.

The research of the local bradyrhizobia population in leached chornozem was conducted under the conditions of vegetation experiment. Soil was sampled in the field of IAMAM NAAS. Soybean cultivation started 14 years before on this field using *B. japonicum* strains with different growth rate. Over the last 8 years, legumes had not been sown in the experimental plot.

The vegetation experiment was performed according to the generally accepted rules in 2-litre vessels. Wild soybean, cowpea, mung bean, and adzuki bean were used as leguminous trap plants. Cultivated soybean and white lupine (*Lupinus* genus representatives are common in Ukraine) were also grown. Before sowing, the seeds had been moistened with tap water; no inoculation with nodule bacteria was performed. Humidity was maintained at 60% of the maximum water-holding capacity. The experiment had four repeats.

In the flowering phase, nodules were selected from the roots of plants. The share of different strains of rhizobia in nodule populations was determined by analysis of nodule homogenates (48 units) in the agglutination reaction [10, 14].

Isolation of nodule bacteria and determination of their morphological and cultural properties.

Isolation of nodule bacteria from soybean, cowpea, mung bean, adzuki bean, and lupine nodules was performed according to the guidelines [16]. Morphological and cultural properties of the obtained isolates were studied according to the generally accepted methods [16].

Study of the serological properties of the obtained bradyrhizobia.

The serological properties of nodule bacteria isolated from nodules were studied using the Gruber-Widal agglutination test [10, 14].

Statistical analysis of the results.

Statistical processing of data was performed by standard methods, and the software Statistica 7.0 was used.

Results. At the first stage of the work, we investigated the population of bradyrhizobia, which lived as saprophytes in sod-podzolic soil for 7 years. When growing leguminous trap plants on this field, numerous nodules were registered on their roots: 47.58 ± 1.80 items/plant in cultivated soybean, 39.58 ± 2.51 items/plant in wild soybean, 18.67 ± 1.05 items/plant in cowpea, 16.58 ± 1.11 items/plant in mung bean, and 15.17 ± 1.47 items/plant in adzuki bean. This fact confirmed that virulent nodule bacteria capable of entering a symbiotic relationship with these leguminous plants persisted in the soil.

The serological analysis of nodules proved that the dominant microsymbionts of cultivated and wild soybeans were nodule bacteria *B. japonicum*, serogroup KB11, with an increased growth rate. They formed 58.3% and 37. 5% of nodules, respectively (Table 1). The slow-growing strain *B. japonicum* 46 (18.7—18.8%) and the nodule bacteria not assigned to the studied serogroups (16.7—22.9%) were found in significantly smaller amounts in the nodule populations. It should be noted that wild soybean roots were also infected by representatives of serogroups M8 (14.6%) and B1 (12.5%), absent in cultivated soybean nodules. The results confirm literature data on the ability of wild soybeans to select from the soil and maintain a greater diversity of microsymbionts in nodules compared to cultivated soybeans [17]. The proof of this is the maximum value of the calculated Shannon diversity index (H = 1.52).

As can be seen from the data (Table 1), local nodule bacteria of the species *B. japonicum* entered symbiosis with plants of the genus *Vigna*. Cultivation of cowpea, mung bean and adzuki bean, as well as wild soybean, allowed detection of serogroup M8 in the rhizobial community of the soil, despite the fact that these rhizobia had not been identified in cultivated soybean nodules. On the roots of trap plants, they formed a significant number of nodules (25.0—83.4%). Soybean rhizobia of serogroup KB11 actively infected cowpea (33.3% of nodules) and adzuki bean (50.0% of nodules), but they were not detected in mung bean nodules. A smaller share of nodules (8.3—16.7%) was

Table 1. **Ability of representatives of nodule bacteria soil populations to colonize the roots of different leguminous plants (small-plot field experiment, IAMAM NAAS, 2021, sod-podzolic soil)**

Trap-hosts	Share of nodule bacteria strains in nodules,%										
	46	M8	KB11	634b	OR	HR	NR	367a	B1	Other *	Shannon index (H)
Soybean (*Glycine max*)	18.8	0	58.3	0	0	0	0	0	0	22.9	0.97
Wild soybean (*Glycine soja*)	18.7	14.6	37.5	0	0	0	0	0	12.5	16.7	1.52
Cowpea (*Vigna unguiculata*)	0	45.8	33.3	0	0	0	0	4.2	6.3	10.4	1.27
Mung bean (*Vigna radiata*)	8.3	83.4	0	0	0	0	0	0	0	8.3	0.57
Adzuki bean (*Vigna angularis*)	16.7	25.0	50.0	0	0	0	0	0	0	8.3	1.20

Note: * are nodule bacteria are not assigned to studied serogroups.

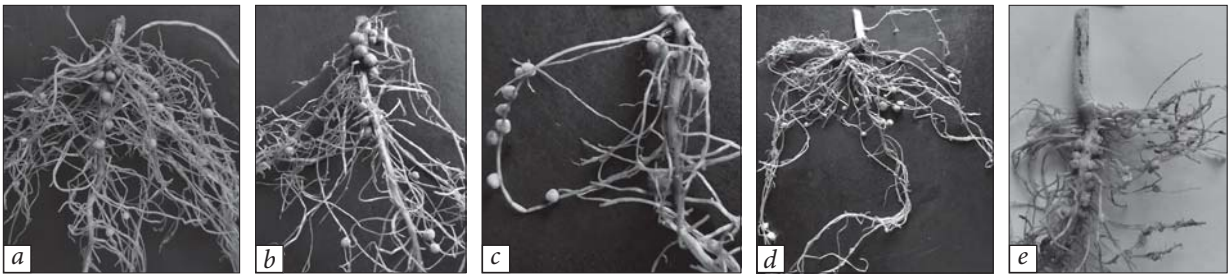

Fig. 1. Nodules on soybean (*a*), wild soybean (*b*), cowpea (*c*), adzuki bean (*d*) and white lupine (*e*) roots upon cultivation on leached chornozem (vegetation experiment)

formed by nodule bacteria of serogroup 46 and rhizobia not assigned to the studied serogroups (8.3—10.4%).

It is worth noting that cowpea was also characterized by a significant diversity of nodule populations (H = 1.27). In addition to soybean rhizobia, lupine microsymbionts (*B. lupini*) belonging to serogroup 367a (4.2%) were found on lupine roots. A minor amount of cowpea nodules (6.3%) was formed by bradyrhizobia related to *Bradyrhizobium* sp. B1 strain, previously isolated from the nodules of this plant.

At the second stage of work (under vegetation conditions), we investigated the population of bradyrhizobia in leached chornozem. It was formed in the soil 14 years before, and over the last 8 years, legumes had not been sown on this field.

In the flowering phase, numerous nodules were formed on the roots of all trap plants: 23.92 ± 1.46 items/plant in cultivated soybean and 31.58 ± 0.80 items/plant in wild soybean, 17.75 ± 1.14 items/plant in cowpea, 10.92 ± 0.76 items/plant in mung bean and 7.92 ± 0.61 items/plant in adzuki bean, and 31.75 ± 2.81 items/plant in white lupine (Fig. 1).

As evidenced in Table 2, nodule bacteria *B. japonicum* belonging to 5 serogroups, namely 46, M8, KB11, 634b, and HR, were identified in the root nodules of both types of soybean (*Glycine max* and *Glycine soja*). The dominant microsymbionts of these plants were bacteria serologically

Table 2. **Ability of the representative of rhizobia soil populations to colonize the roots of different leguminous plants (vegetation experiment, leached chornozem)**

Trap-hosts	Share of nodule bacteria strains in nodules,%										
	46	M8	KB11	634b	OR	HR	NR	367a	B1	Other*	Shannon index (H)
Soybean (*Glycine max*)	14.6	35.4	25.0	12.5	0	12.5	0	0	0	0	1.51
Wild soybean (*Glycine soja*)	12.5	16.6	54.2	12.5	0	4.2	0	0	0	0	1.28
Cowpea (*Vigna unguiculata*)	0	43.7	8.3	16.7	0	0	0	0	0	31.3	1.23
Mung bean (*Vigna radiata*)	0	66.7	0	0	0	0	0	0	0	33.3	0.64
Adzuki bean (*Vigna angularis*)	0	12.5	0	6.3	0	0	0	0	0	81.2	0.60
White lupine (*Lupinus albus*)	0	0	0	0	0	0	0	45.8	0	54.2	0.69

*nodule bacteria not assigned to studied serogroups.

related to *B. japonicum* M8 and *B. japonicum* KB11 strains. They formed 35.4% and 25.0% of nodules on cultivated soybean roots and 16.6% and 54.2% on wild soybean roots, respectively. Bradyrhizobia of serogroups 46, 634b, and HR were found to be minor representatives of nodule populations of plants of the genus *Glycine* and formed 4.2—12.5% of nodules.

Representatives of the local population of bradyrhizobia also infected the cowpea. Bacteria belonging to 3 serogroups, namely M8, KB11, and 634b, were identified in its nodules. At the same time, nodule bacteria related to *B. japonicum* M8, which formed 43.7% of nodules, were the dominant cowpea microsymbionts. A significant share of the nodule population (31.3%) also included rhizobia, which did not react with the antisera. Other representatives of the genus *Vigna* — mung bean and adzuki bean — were infected with smaller numbers of bradyrhizobia of different serogroups. For example, 66.7% of nodules on mung bean roots were formed by M8 serogroup bacteria, the remaining 33.3% — by rhizobia not assigned to studied serogroups. In adzuki bean nodules, the majority of microsymbionts (81.2%) were not identified. Bacteria serologically similar to *B. japonicum* M8 were detected in only 12.5% of nodules, and to *B. japonicum* 634b — in 6.3% of the nodules.

According to literature data, plants of the genus *Lupinus* can form a symbiosis with *B. japonicum* [11]. In our experiment, none of the known strains of this species present in the soil infected white lupine. Almost half the nodules on its roots (45.8%) were formed by nodule bacteria *B. lupini* belonging to serogroup 367a; the other lupine microsymbionts (54.2%) did not react with the antisera.

To characterize bradyrhizobia, which acted for a long time in the soil as saprophytes, 70 isolates were obtained from the nodules of leguminous plants of the genera *Glycine*, *Vigna*, and *Lupinus* (Table 3).

The obtained isolates grew well at a temperature of 26—28 °C on agar bean medium [16]. According to the growth rate, all isolates were divided into two groups. Group I — rounded, translucent, slimy, and whitish colonies appeared on day 4 to 7 of growth. The diameter of the colonies was 2.0—4.0 mm. Group II — round, opaque, and whitish colonies appeared on day 8 to 10 of growth. The diameter of the colonies was 1.0—1.5 mm.

In terms of morphology, on day 7 of culture growth, the cells were mobile, had the shape of slightly bent rods, were Gram-negative, and did not form spores. As the cultures aged, the cells lost their mobility.

All isolates did not grow on MPA (Table 3). When growing cultures on litmus milk, the reaction changed to alkaline, but the serum zone did not form on the surface.

In terms of morphological and cultural properties (cell shape, colony size and growth rate on agar bean medium with mannitol, growth on

Table 3. **Cultural properties of nodule bacteria isolates obtained from nodules of different leguminous plants**

Host-plants	No. of isolates, units	Growth on meat-peptone agar (MPA)	Reaction in litmus milk
Wild soybean (*Glycine soja*)	13	no growth	no serum zone, alkaline
Cowpea (*Vigna unguiculata*)	18	» »	» »
Mung bean (*Vigna radiata*)	13	» »	» »
Adzuki bean (*Vigna angularis*)	12	» »	» »
White lupine (*Lupinus albus*)	14	» »	» »

MPA, and litmus milk), the new strains were assigned to the genus *Bradyrhizobium*.

To study the affinity of new isolates to known strains of soybean and lupine nodule bacteria, we carried out their serological identification using the agglutination reaction with 9 specific antisera: 46, M8, KB11, 364b, OR, HR, NR, 367a, and B1.

It was established that 20 isolates reacted positively with antiserum to the slow-growing soybean rhizobia *B. japonicum* M8, 5 isolates were related to *B. japonicum* 46, and 1 isolate — to *B. japonicum* HR (Table 4). 10 new cultures are assigned to KB11 serogroup, which includes soybean rhizobia with an increased growth rate. They are isolated from nodules of wild soybeans, cowpea, and mung bean. 10 isolates were obtained from white lupine nodules, which turned out to be related to the nodule bacteria *B. lupini* 367a. It should also be noted that 10 isolates, microsymbionts of wild soybeans and cowpea, reacted with antiserum B1 obtained for *Bradyrhizobium* sp. B1. 14 new isolates showed a negative result in the agglutination reaction with antisera; their species affiliation needs clarification.

Thus, based on the main morphological, cultural, and serological characteristics, 35 new strains were found to be related to nodule bacteria introduced into agrocenoses 14—20 years before and were identified as *B. japonicum* (25 slow-growing strains and 10 strains with an increased growth rate). 10 strains are classified as *B. lupini*, and 24 strains — as *Bradyrhizobium* sp. and require additional identification.

Discussion. Cultivation of leguminous crops using nodule bacteria-based biological preparations leads to the formation of local populations of these microorganisms in agrocenoses [1—3]. The density of rhizobial communities depends on the duration of cultivation of the host plant and the influence of abiotic and biotic factors. The diversity of populations is largely determined by the spectrum of used inoculant strains and their survival in the soil [1, 7, 18]. In addition, when local nodule bacteria interact with each other and introduced strains, new genotypes of microsymbionts may arise, which also causes their heterogeneity. In view of this, soil populations are a convenient object for studying microevolutionary processes, the result of which is the formation of the diversity of rhizobia [19].

Wide usage of leguminous trap plants when studying survival and polymorphism of nodule bacteria in soil populations is known from the

Table 4. **Serological affiliation of rhizobia isolates allocated from nodules of leguminous plants of the genera *Glycine*, *Vigna,* and *Lupinus***

Host-plants	No. of isolates, units	Belonging of isolates (units) to serogroups							
		46	M8	KB11	634b	HR	367a	B1	Other *
Wild soybean (*Glycine soja*)	13	1	0	4	0	1	0	6	1
Cowpea (*Vigna unguiculata*)	18	2	7	2	0	0	0	4	3
Mung bean (*Vigna radiata*)	13	1	7	4	0	0	0	0	1
Adzuki bean (*Vigna angularis*)	12	1	6	0	0	0	0	0	5
White lupine (*Lupinus albus*)	14	0	0	0	0	0	10	0	4
In total	70	5	20	10	0	1	10	10	14

Note: * are nodule bacteria are not assigned to studied serogroups.

literature. Some of these plants can simultaneously form nodules with both slow-growing and fast-growing nodule bacteria [12, 13]. In our opinion, growing different trap plants in a certain field will allow one to more fully characterize the bradyrhizobia community, since it is known that the genotype of the host plant can affect the spectrum of rhizobia trapped from the soil [1, 5].

This work investigated the diversity of local populations of bradyrhizobia in sod-podzolic soil and leached chornozem after a long saprophytic period without a host plant. Legumes of the genera *Glycine*, *Vigna,* and *Lupinus*, capable of cross-infection by nodule bacteria of the genus *Bradyrhizobium*, were chosen as trap plants [11—13].

The studies have shown that after 7—8 legume-free years, both types of soil retained nodule bacteria that nodulated cultivated and wild soybeans, cowpeas, mung beans, adzuki beans, and lupines. Their groups differed in quantitative and qualitative compositions.

The main microsymbionts of plants of the genera *Glycine* and *Vigna* on different types of soil were soybean nodule bacteria belonging to 6 serological groups, namely 46, M8, KB11, 634b, HR, and B1. The representatives of 4 serogroups corresponded to *B. japonicum* 46, M8, 634b, and KB11 inoculant strains, which were previously periodically used in the studied areas. It should be noted that nodule bacteria of serogroup 634b were maintained only in the more fertile leached chornozem and were not detected on the roots of any of the trap plants on the sod-podzolic soil. This may be due to their lower adaptability to the conditions in the soil environment. In addition to *B. japonicum* (2—3 serogroups depending on the type of soil), cowpea plants trapped from the soil the microsymbionts of *B. lupini* serogroup 367a (4.2%). *Bradyrhizobium* sp. serogroup B1 nodule bacteria were identified in both cowpea nodules (6.3%) and wild soybeans (12.5%), but they did not infect cultivated soybeans. 45.8% of white lupine nodules were formed by *B. lupini* of serogroup 367a. The rest belonged to unknown serogroups.

The appearance of other representatives of nodule bacteria (serogroups HR, B1, and groups of unidentified microsymbionts) in populations may be associated with various factors such as their transfer with seeds, soil particles, or microevolutionary processes [19, 20]. Similar results were obtained by other researchers. For example, Giongo A. et al. [5] found significant genetic diversity of soybean nodulating bacteria *Bradyrhizobium* spp. after a 30-year period without a host plant. They showed the presence of bradyrhizobia in the soil, which differ from the parental strains. There are also reports when after several years of growing soybeans, researchers noted the formation of nodules by strains serologically different from the original introduced microorganisms [21—23].

Comparing the two studied types of soil, the larger proportion of rhizobia of unknown serogroups in the nodules of plants of the genus *Vigna* when growing them on leached chornozem (31.3—81.2% to 8.3—10.4% in sod-podzolic soil) should be highlighted. These microsymbionts competed with known strains of nodule bacteria, partially or completely displacing them from nodule populations. In our opinion, they may be representatives of other *B. japonicum* and *B. lupini* serogroups or may belong to other species of slow- and fast-growing nodule bacteria, which requires additional research. Special attention should be paid to this fact when selecting potential inoculant strains for the corresponding leguminous crops.

Both types of soybean and cowpea (Shannon's index H = 0.97—1.52) were characterized by the greatest diversity of rhizobia in nodule populations. In addition, the cultivation of trap plants of wild soybeans and various types of cowpea made it possible to detect *B. japonicum* M8 serogroup (formed 25.0—83.4% of nodules) in the saprophytic state in the sod-podzolic soil, which

did not infect the roots of cultivated soybeans. This may be related to the high competition between *B. japonicum* strains when interacting with the host plant and the greater complementarity of serogroup M8 bacteria to wild and rare legume species. The results confirm the importance of using different leguminous trap plants for a more complete characterization of the local rhizobial community.

It should also be noted that after a long saprophytic period in the soil, all representatives of local populations of nodule bacteria maintained their nitrogen-fixing activity, forming red nodules on the roots of trap plants. Soybean bradyrhizobia of serogroup KB11 maintained the property of the original strain *B. japonicum* KB11 to form white inactive nodules on the roots of cowpea and red active nodules on the roots of other leguminous plants [24].

We have previously established that nodule bacteria of serogroup KB11 are characterized by increased saprophytic competence (surviving in the soil) and are related to the *B. japonicum* USDA 123 strain by the nucleotide sequence of the ITS region [25, 26]. The dominance of bradyrhizobia 123 serogroup in nodules just after several years of soybean cultivation was demonstrated by scientists from the USA [27] and Brazil [28, 29]. In contrast, Narożna D. et al. [6] showed high survival of two strains of *B. japonicum* USDA 123 and USDA 110 in Polish soils after 17 years of saprophytic condition. Batista J.S.S. et al. [29] associated the ability of serogroup 123 bacteria to adapt to environmental conditions and their high saprophytic competence with a higher mucoidity (production of a significant amount of extracellular polysaccharides, EPSs) of these microorganisms. Basing on the results of our own research on the production of exopolysaccharides by inoculant strains, we also believe that this may be one of the factors of their successful survival in the soil.

70 new isolates of nodule bacteria were obtained from the nodules of trap plants, which were previously identified as *B. japonicum, B. lupini*, and *Bradyrhizobium* sp.

Conclusions. The results confirm the importance of using different leguminous trap plants for a more complete characterization of the local rhizobial community. Cultivation of plants of the genera *Glycine*, *Vigna,* and *Lupinus*, capable of cross-infection, made it possible to detect *B. japonicum* (serogroups 46, M8, KB11, 634b, HR), *B. lupini* (serogroup 367a), and *Bradyrhizobium* sp. (serogroup B1), which exist for a long-term period as saprophytes in sod-podzolic soil and leached chornozem.

70 isolates of bradyrhizobia have been obtained, 35 of which were serologically related to *B. japonicum* 46, M8, KB11, and 634b inoculant strains, introduced into the agrocenosis at the beginning of the research.

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Д.В. Крутило, О.В. Надкернична

Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН
вул. Шевченка, 97, Чернігів, 14027, Україна

ХАРАКТЕРИСТИКА МІСЦЕВИХ ПОПУЛЯЦІЙ БРАДІРИЗОБІЙ
ПІСЛЯ ТРИВАЛОГО ІСНУВАННЯ В ГРУНТІ БЕЗ РОСЛИНИ-ГОСПОДАРЯ

У попередні роки досліджено серологічне та генетичне різноманіття бульбочкових бактерій сої в агроценозах України. Менше уваги приділялося вивченню виживання їх у грунті. Враховуючи природну неоднорідність бактерій роду *Bradyrhizobium*, **метою** даної роботи було: оцінити різноманіття брадіризобій у місцевих популяціях різних ґрунтів після тривалого існування без бобових рослин, отримати нові ізоляти бульбочкових бактерій та вивчити їх властивості. **Методи.** Мікробіологічні (виділення брадіризобій із бульбочок рослин-пасток, вивчення властивостей штамів), серологічні (дослідження різноманіття ризобій у бульбочкових популяціях, вивчення серологічної належності штамів), вегетаційні, польові (вивчення інфікування рослин брадіризобіями). **Результати.** За використання рослин-пасток родів *Glycine*, *Vigna* та *Lupinus* досліджено місцеві популяції брадіризобій у дерново-підзолистому грунті та вилугуваному чорноземі. Встановлено, що після 7—8 років існування без бобових рослин в обох типах ґрунтів збереглися активні бульбочкові бактерії, які нодулювали культурну та дику сою, вигну, маш, квасолю адзукі та люпин. Основними мікросимбіонтами рослин родів *Glycine* і *Vigna* на різних типах ґрунту були брадіризобії сої, які належать до 6 серологічних груп: 46, М8, КВ11, 634б, HR та В1. Представники 4 серогруп відповідали штамам-інокулянтам *Bradyrhizobium japonicum* 46, М8, 634б та КВ11, які періодично використовувались на досліджуваних ділянках. Рослини вигни, окрім бактерій *B. japonicum,* відбирали з ґрунту мікросимбіонтів *B. lupini* серогрупи 367а (4,2%). Брадіризобії серогрупи В1 виявлено як у бульбочках вигни (6,3%), так і дикої сої (12,5%). 45,8% бульбочок люпину формували бактерії *B. lupini* серогрупи 367а. Поява в популяціях представників серогруп HR, В1 та групи неідентифікованих мікросимбіонтів потребує додаткових досліджень. Вирощування рослин-пасток дикої сої та різних видів вигни дозволило виявити сапрофітно присутній у дерново-підзолистому грунті штам *B. japonicum* М8 (формував 25,0—83,4% бульбочок), який не інфікував корені культурної сої. Із бульбочок рослин-пасток отримано 70 ізолятів брадіризобій, які за морфолого-культуральними і серологічними ознаками попередньо ідентифіковані як *B. japonicum*, *B. lupini*, *Bradyrhizobium* sp. **Висновки.** Отримані дані підтверджують важливість використання різних бобових рослин-пасток для повнішої характеристики місцевого ризобіального угруповання. Вирощування рослин родів *Glycine*, *Vigna* та *Lupinus*, здатних до перехресного інфікування, дозволило виявити у дерново-підзолистому грунті та вилугуваному чорноземі сапрофітно існуючі протягом тривалого часу бактерії *B. japonicum* (серогрупи 46, М8, КВ11, 634b, HR), *B. lupini* (серогрупи 367а) та *Bradyrhizobium* sp. (серогрупи В1). Отримано 70 ізолятів брадіризобій, 35 з яких виявилися серологічно спорідненими зі штамами-інокулянтами *B. japonicum*, інтродукованими в агроценози на початку досліджень.

Ключові слова: *Bradyrhizobium japonicum, B. lupini,* популяції брадіризобій, рослини-пастки, дика соя, вигна, маш, квасоля адзукі, люпин.