

BIOLOGICAL FEATURES OF GROUNDNUT MICROSYMBIONTS WIDESPREAD IN THE SOILS OF UKRAINE

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Objective. Study the presence of nodule bacteria in the soils of Ukraine, capable of nodulating groundnut, isolate new strains of rhizobia from peanut nodules, study their morphological, cultural and serological properties and the ability to form symbiosis with different legumes. **Methods.** Microbiological (isolation of nodule bacteria from nodules and cultivation of microorganisms, studying biological properties of strains), serological (producing antisera to *Bradyrhizobium lupini* 367a, studying rhizobia diversity in groundnut nodule populations, studying serological identity of new strains), vegetation experiment (studying formation and functioning of groundnut symbiotic systems with nodule bacteria, studying host specificity of new strains of groundnut rhizobia), gas chromatography (determining nitrogen-fixing activity of rhizobia in symbiosis with groundnut), mathematical and statistical. **Results.** Representatives of two species, *B. lupini* and *B. japonicum*, were found in nodule populations of rhizobia during the cultivation of groundnut plants on sod-podzolic soil and leached chernozem. The dominant microsymbionts of groundnut were lupine nodule bacteria of serogroup 367a (54.2 % and 45.8 % according to soils). Fewer nodules were formed by intensive growing soybean rhizobia of serogroup KB11 (16.7 % and 12.5 %). The share of nodule bacteria not classified in the studied serogroups was 21.9 % and 41.7 %. Fifteen new strains of nodule bacteria were isolated from groundnut nodules, which were morphologically, culturally and serologically identified as *B. lupini* serogroup 367a (7 of them), *B. japonicum* serogroup KB11 (4 of them) and *Bradyrhizobium* sp. (4 of them). New strains of *B. lupini* from groundnut nodules are able to infect white and yellow lupine but do not nodulate soybeans. Strains identified as *B. japonicum* form nodules on soybean roots but do not infect lupine. Serologically unidentified strain *Bradyrhizobium* sp. AR3, which is able to form a symbiosis with both lupine and soybean (phenotypes Nod^+Fix^+) was obtained. **Conclusion.** For the first time it was established that groups of nodule bacteria capable of nodulating groundnut are present in the agrocenoses of Ukraine. Fifteen new strains of groundnut rhizobia were obtained, which were identified as *B. lupini*, *B. japonicum* and *Bradyrhizobium* sp.

Key words: groundnut microsymbionts, rhizobia nodule populations, *Bradyrhizobium lupini*, *B. japonicum*, serogroups, soybean, lupine.

Introduction. Nodule bacteria (rhizobia) is a group of nitrogen-fixing microorganisms prevailing in almost all soil and climatic zones of the Earth and capable of forming specific symbiotic organs — nodules — on the roots of legumes [1; 2]. Due to the ability of rhizobia to fix nitrogen from the atmosphere, synthesize biologically active substances, improve plant nutrition, have an impact on plant resistance to path-

ogens and abiotic stresses, they are widely used as a basis for microbial preparations [1; 3; 4].

Analysis of the novel studies and publications. Most species of nodule bacteria that nodulate legumes belong to alpha-Proteobacteria of Rhizobiales (*Hyphomicrobiales*) and are grouped into four families: *Bradyrhizobiaceae* (genus *Bradyrhizobium*), *Rhizobiaceae* (genera *Rhizobium*, *Allorhizobium*, *Ensifer*, *Neorhizobi-*

um, *Pararhizobium*, *Sinorhizobium*), *Phyllobacteriaceae* (genus *Mesorhizobium*) and *Xanthobacteriaceae* (genus *Azorhizobium*) [5–7]. There have been numerous reports about isolation of nitrogen-fixing bacteria from legume nodules belonging to phylogenetically remote rhizobia genera: *Methylobacterium*, *Devosia*, *Ochrobactrum* and *Phyllobacterium* (*alpha-Proteobacteria* class), as well as *Burkholderia*, *Ralstonia* and *Cupriavidus* (*beta-Proteobacteria* class) [8].

Recently, much attention has been paid to the study of the diversity of nodule bacteria — microsymbionts of both traditional and rare legumes [2; 9]. The research of representatives of local groups of specific nodule bacteria may be important for understanding the processes of microevolution of rhizobia, as well as in the selection of potential bioagents of microbial preparations.

One of the economically valuable legumes is a groundnut (*Arachis hypogaea* L.). Its seeds contain 45–60 % fat, 25–37 % protein, 15–20 % carbohydrates. In addition, the product contain many minerals (sodium, potassium, calcium, phosphorus, iron) and vitamins (B1, B2, PP, D) [10; 11].

South America is considered as a homeland of groundnuts. Currently, this crop is grown in America, Africa, Australia, Asia and Europe on an area of about 20–25 million hectares. The main crops of groundnut are concentrated in Asia and Africa, and in Europe it is a rare crop [10; 11].

Groundnut was started to grown in Ukraine in the second half of the 19th century. The culture is successfully cultivated in the southern regions of the country – in the Regions of Mykolaiv, Odesa, Kherson, Zaporizhzhia and Dnipropetrovsk. The areas of groundnut under cultivation in Ukraine are small and concentrated mainly in the private sector and on farms. However, recently this culture has been intensively introduced into production, especially on irrigated lands [12].

In addition to the fact that groundnut is a valuable crop, it is widely used for scientific purposes as a trap-host in the study of the diversity of local groups of nodule bacteria [13].

The features of groundnut is that it can enter into a symbiotic relationship with both slow-growing and fast-growing nodule bacteria [14–16]. More than 30 species of *Bradyrhizobium* are currently known to be confirmed as ground-

nut microsymbionts [16–21]. Species such as *Rhizobium tropici*, *R. (Pararhizobium) giardinii*, *Neorhizobium galegae* and *N. huautlense* have been identified in different countries among fast-growing rhizobia in groundnut nodules [22; 23]. Slow-growing strains in general dominate in nodule populations of groundnut rhizobia [14]. It has been noted that in some regions of China, fast-growing nodule bacteria do not infect groundnut at all [24; 25].

The national literature contains no information on soil populations of groundnut nodule bacteria in agrocenoses of Ukraine. Due to the low prevalence of the crop, the species of local rhizobia that may form nodules on groundnut roots remain unidentified. Therefore, the objective of our work was to study the presence of nodule bacteria in Ukrainian soils capable of nodulating groundnut, to isolate new rhizobia strains from groundnut nodules, to study their morphological, cultural and serological properties and the ability to form symbiosis with different legumes.

Materials and methods. The objects of study were rhizobia isolated from groundnut nodules, as well as plants of groundnut (*Arachis hypogaea* L., Klynskyi variety), soybean (*Glycine max* (L.) Merr., Ustia variety), white lupine (*Lupinus albus* L., Lybid variety) and yellow lupine (*Lupinus luteus* L., Chernihivets variety). The seeds were provided by the National Scientific Centre “Institute of Agriculture of NAAS”, the Department of Scientific Support of Agroindustrial Manufacture of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS (NAAS IAMAM). New strains of groundnut rhizobia are stored in the collection of the Laboratory of Plant-Microbial Interactions of NAAS IAMAM.

The composition of nodule bacteria capable of entering into symbiotic relationships with groundnut plants was studied in sod-podzolic soil and leached chernozem. The study was conducted under the conditions of vegetation experiment. Soil has been sampled in the fields of NAAS IAMAM, where inoculated soybeans were periodically grown. Over the last 5–6 years, legumes have not been sown in the experimental plots, groundnut have never been grown.

Vegetation experiment was performed according to the generally accepted rules in 2 litre vessels. Before sowing, sterilized (96 % ethyl

alcohol) groundnut seeds were moistened with tap water, inoculation with nodule bacteria was not performed. The repetition of the experiment was quadruple. Humidity was maintained at 60 % of maximum water-holding capacity [26].

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) [27] using a set of specific immune antisera obtained to active strains of soybean nodule bacteria *B. japonicum* 46, M8, KB11, 634b, OR, HR, NR, microsymbionts of cowpea *Bradyrhizobium* sp. B1 and common bean *Rhizobium phaseoli* 700, ФБ1, ФД3 [28; 29].

We also used polyclonal immune O-antiserum to the strain of nodule bacteria of lupine *Bradyrhizobium lupini* 367a, which was obtained by the method of All-Russian Scientific and Research Institute of Agricultural Microbiology [30] in our modification. Rhizobia were grown on solid legume media at 28 °C. In the logarithmic growth phase, the bacterial mass was washed off the agar shoals, precipitated by centrifugation and washed twice with normal saline solution. 5 mL of normal saline solution and 5 mL of 2.5 % glutaraldehyde solution were added to the nodule bacteria cell precipitate (to release non-specific flagellar H-antigens) and left in the refrigerator for 24 hours. One day later, the bacterial cells (antigen) were washed three times to remove glutaraldehyde, the precipitate was resuspended in normal saline solution and the antigen titre was adjusted to $2 \cdot 10^9$ CFU/mL.

The rabbit immunization schedule included 6 injections (at weekly interval) with increasing

doses of antigen (Table 1). The antigen was administered subcutaneously using 0.5 mL of Complete Freund's Adjuvant (CFA). Blood sampling was performed three times one week after the last immunization from the ear vein.

The titre of 367a antiserum was determined by agglutination reaction. Its specificity was tested in a cross-agglutination reaction by the Gruber-Widal technique [27]. Slow- and fast-growing nodule bacteria of different species and genera, stored in the Collection of Beneficial Soil Microorganisms of NAAS IAMAM, were used as antigens. The titre of antigens for the agglutination reaction was 10^6 cells/mL.

Isolation of nodule bacteria from groundnut nodules was performed according to guidelines [31]. Morphological and cultural properties of the obtained isolates of nodule bacteria were studied according to generally accepted methods [30; 31].

The serological features of nodule bacteria isolated from groundnut nodules were studied using the Gruber-Widal agglutination test [27].

The ability of new strains of groundnut nodule bacteria to enter into a symbiotic relationship with cultivated soybeans, white and yellow lupine was studied in a vegetation experiment. Plants were grown in 2 L vessels on nitrogen-free substrate (sterile vermiculite) moistened with 0.2 % KH_2PO_4 solution. Before sowing, sterilized seeds were treated with a suspension of nodule bacteria (titre $2 \cdot 10^9$ CFU/mL). The inoculation load was 200–300 thousand cells per 1 seed. The repetition of the experiment was quadruple.

The activity of symbiotic nitrogen fixation was determined by the acetylene-ethylene method [32] on a gas chromatograph Chrom-4 with a flame ionization detector (column with β - β' -oxydipropionitrile).

Table 1. Rabbit immunization schedule with B. lupini 367a (antigen administration at weekly interval)

No. of injection	Amount of antigen, mL	Antigen titre, cell/mL	Method of antigen administration
1	1	$10^6 + \text{CFA}$	subcutaneously
2	1	10^6	intravenously
3	1	10^7	intravenously
4	2	10^7	intravenously
5	4	10^7	intravenously
6	1	10^8	intravenously

Statistical data processing was performed by conventional methods [33] using computer programs Microsoft Office Excel 2016 and Statistica 8.0.

Results and discussion. At the first stage of the work, vegetation experiment was used to find out whether nodule bacteria, which are able to enter into a symbiotic relationship with groundnut, are present in the agrocenoses of Ukraine.

The obtained data indicate that nodule bacteria that actively infect groundnut, forming numerous nodules, are present in both studied soils (sod-podzolic and leached chernozem) (Fig. 1).

When growing groundnuts on sod-podzolic soil, an average of 118 nodules were formed on

the roots of one plant. The soil population of groundnut microsymbionts in leached chernozem turned out to be more numerous, which is indirectly evidenced by the higher number of formed nodules — 163 units/plant (Table 2). The mass of root nodules per plant was at the level of 0.21 and 0.23 g, respectively. The nodules were red upon cutting, which indicates the active fixation of molecular nitrogen. Despite the lower number of root nodules in plants on sod-podzolic soil, their activity was higher (3.08 $\mu\text{g N/plant per hour}$), compared to plants grown on leached chernozem — 2.46 $\mu\text{g N/plant per hour}$. The dry aboveground mass of plants grown on different soil types correlated with the nitrogenase activity of the nodules: 1.55 g/plant and 1.49 g/plant, respectively.



Fig. 1. Nodules on groundnut roots upon cultivation on sod-podzolic soil (A) and leached chernozem (B).

Table 2. Symbiotic parameters of groundnut plants upon cultivation on different soils (vegetation experiment, flowering phase)

Type of soil	Dry aboveground mass, g/plant	Number of nodules, units/plant	Mass of nodules, g/plant	Activity of symbiotic nitrogen fixation, $\mu\text{g N/plant per hour}$
Sod-podzolic	1.55 \pm 0.07	118.17 \pm 7.73	0.21 \pm 0.01	3.08 \pm 0.28
Leached chernozem	1.49 \pm 0.05	162.58 \pm 5.45	0.23 \pm 0.01	2.46 \pm 0.23

The next step was to determine which species of nodule bacteria infected groundnut roots. To solve this task, we used the serological method. Typing of rhizobia in groundnut nodule populations was performed using specific antisera obtained to different strains of soybean rhizobia *B. japonicum* 46, M8, KB11, 634b, OR, HR, NR, microsymbionts of cowpea *Bradyrhizobium* sp. B1 and common beans *Rhizobium phaseoli* 700, ФБ1, ФД3.

Given that, according to the literature, groundnuts can be nodulated by representatives of different species of the genus *Bradyrhizobium* [14], we have developed a rabbit immunization schedule and obtained antiserum to standard strain *B. lupini* 367a to identify potential microsymbionts in the nodules — lupine rhizobia (*Bradyrhizobium lupini*). The titre of 367a antiserum in the agglutination reaction was 1:5,120, working dilution 1:500.

The specificity of the obtained antiserum was tested in the agglutination reaction with homo- and heterologous strains of nodule bacteria (Table 3).

As Table 3 shows, 367a antiserum did not react with any of the 20 strains of nodule bacteria belonging to the phylogenetically distant

genera *Rhizobium*, *Sinorhizobium*, *Neorhizobium* and *Mesorhizobium*. Representatives of the genus *Bradyrhizobium* — 13 strains of soybean rhizobia, 4 strains of microsymbionts of cowpea and 3 strains isolated from tick trefoil nodules, also had no common antigenic determinants with strain *B. lupini* 367a.

It should be noted that 10 strains of rhizobia isolated from nodules of different species of lupine were serologically similar to *B. lupini* 367a. That is, they all belong to one serogroup — 367a. Only strain *B. lupini* 3 did not react with the studied antiserum and can be assigned to another serogroup.

Thus, we obtained a group-specific antiserum to strain *B. lupini* 367a, which was also used to identify rhizobia of this species in groundnut nodule populations.

As a result of serological analysis of nodules, it was first established that rhizobia belonging to two species, *B. lupini* and *B. japonicum*, infected plant roots when cultivating groundnuts both on sod-podzolic soil and leached chernozem (Table 4). The dominant microsymbionts of groundnuts were lupine nodule bacteria of serogroup 367a (54.2 % and 45.8 %, respectively). The share of intensive growing

Table 3. Results of agglutination reaction of different species of rhizobia with 367a antiserum

Strains of microorganisms (antigens) / (host plant)	367a antiserum
<i>Rhizobium simplex</i> 820	–
<i>R. phaseoli</i> 700, ФБ1, Н6, ФБ2, ФБ3, ФБ4, ФА1, ФД1, ФД2, ФД3	–
<i>R. leguminosarum</i> <i>bv.</i> <i>viceae</i> 250a (pea), 0419 (bean), Ч14 (everlasting pea)	–
<i>R. trifolii</i> 1326	–
<i>Neorhizobium galegae</i> 0703	–
<i>Sinorhizobium meliloti</i> 425a	–
<i>Mesorhizobium ciceri</i> 522, 065, Н12	–
<i>B. japonicum</i> 1967 ^T , 46, M8, KB11, 634b, OR, HR, NR, KC22, KC23, CK1, CK5, KH10	–
<i>B. lupini</i> 367a	+++
<i>B. lupini</i> 30л	+++
<i>B. lupini</i> 4042 ₂ , 5500/4, 5854 ₃ , 3а, 5л, 8л	+++
<i>B. lupini</i> 1, 6 (ornamental lupine)	+++
<i>B. lupini</i> 3 (mutable lupine)	–
<i>B. lupini</i> 6 (mutable lupine)	+++
<i>Bradyrhizobium</i> sp. B1, B2, B3, B4 (cowpea)	–
<i>Bradyrhizobium</i> sp. 1, 2, 3 (tick trefoil)	–

Table 4. Ability of the representative of nodule bacteria soil populations to colonize groundnut roots (vegetation experiment)

Type of soil	Share of nodule bacteria strains in nodules, %												
	46	M8	KB11	634b	OR	HR	NR	367a	B1	700	Φ51	Φ13	Other*
Sod-podzolic	0	0	16.7	0	0	0	0	54.2	0	0	0	0	29.1
Leached chernozem	0	0	12.5	0	0	0	0	45.8	0	0	0	0	41.7

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

soybean rhizobia of serogroup KB11 in nodules was 16.7 % and 12.5 %. A significant number of nodules (21.9 % and 41.7 %) were formed by nodule bacteria, which do not belong to the studied serogroups. We believe that unidentified groundnut microsymbionts may be representatives of new serogroups of *B. lupini* and *B. japonicum* or may belong to other species of specific nodule bacteria.

The ability of groundnut to interact with soybean and lupine rhizobia present in soil populations can be explained by its symbiotic properties, namely compatibility with a wide range of microsymbionts, which is noted in the works of many researchers [14; 15]. Our data suggest that groundnut infection with different species of nodule bacteria occurs not only in tropical and subtropical countries [13; 14; 16], but also in moderate climates where groundnut has never been grown. Moreover, groundnut plants are able to select strains from the soil that belong to phylogenetically distant species. In our case, these are *B. lupini* and *B. japonicum*. Interestingly, the main host plants of these nodule bacteria (lupine and soybean) belong to different tribes of *Leguminosae* family: *Genisteae* and *Phaseoleae*, respectively, and groundnut is the member of *Dalbergieae* tribe [34]. The formation of nodules on groundnut roots by lupine and soybean nodule bacteria (*B. lupini* 367a and *B. japonicum* KB11) may indicate that they have a much wider range of hosts. And such a migration of microsymbionts between macrosymbionts may facilitate emergence of new genotypes of rhizobia capable to infect various leguminous plants.

To characterize nodule bacteria, which entered into a symbiotic relationship with groundnut plants, 15 isolates were isolated from root nodules.

The obtained cultures grow well at 26–

28 °C on a modified bean medium [31]. According to the growth rate on agar medium, isolates are divided into two groups: group I — colonies appear on Day 4 to 7 of growth; they are round, translucent, mucous, whitish, the diameter of the colonies is 2–4 mm (Fig. 2). Group II — colonies appear on Day 8 to 10 of growth; they are round, opaque, whitish, the diameter of the colonies is 1.0–1.5 mm.

According to the morphology, at Day 7 of culture growth, the cells are motile, have the shape of slightly curved rods, are gram-negative, do not form spores (Fig. 3). As cultures aging, cells lose mobility.

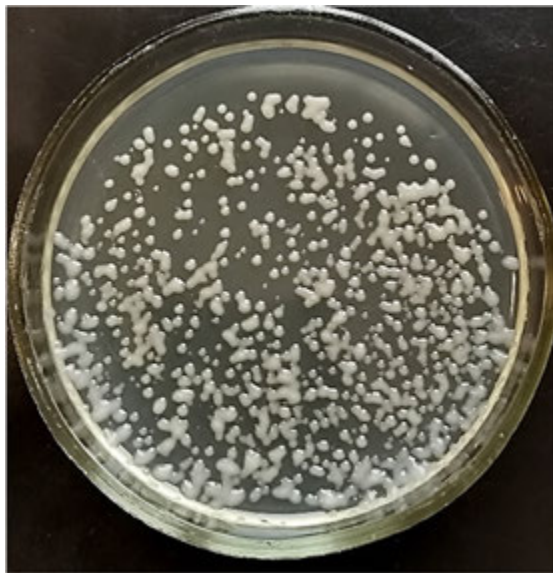
All isolates of groundnut nodule bacteria do not grow on MPA. Litmus milk is not peptonized, changing the reaction of the medium to alkaline. Most isolates on the surface of milk do not form a transparent zone. Only three isolates such as *Bradyrhizobium* sp. AR3, AR4 and AR5 are characterized by the formation of a small (1 mm) mucous ring.

According to morphological and cultural properties (cell shape, colony size and growth rate on agar bean medium with mannitol, growth on MPA and milk with litmus), the new strains belong to the genus *Bradyrhizobium*.

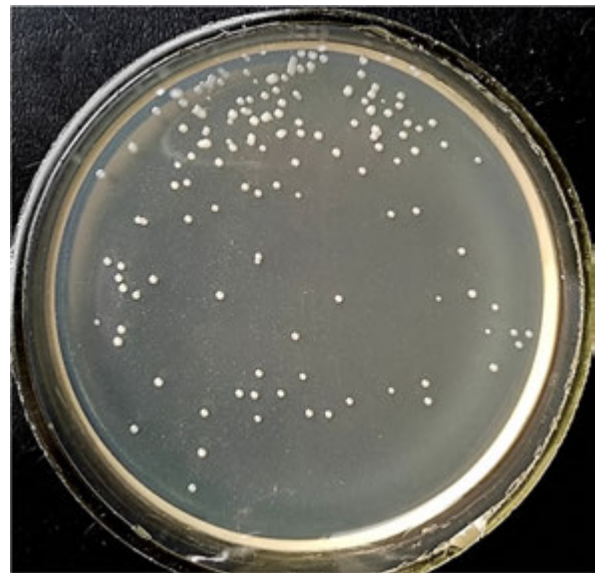
In the final portion of the experiments, the identity of new strains of groundnut microsymbionts with collectible cultures of nodule bacteria and their ability to enter into symbiosis with soybean and lupine plants were studied.

Serological identification of groundnut rhizobia was performed using the agglutination reaction with 9 specific antisera: 46, M8, KB11, 364b, OR, HR, NR, 367a and B1. The results are given in Table 5.

It was found that 7 new strains: *Bradyrhizobium* sp. AR1, AR2, AR7, AR9, AR12, AR13 and AR15 reacted positively with antiserum to

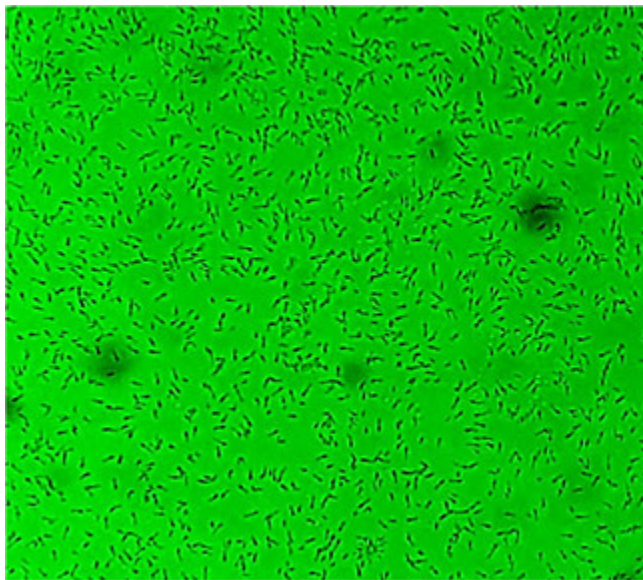


A

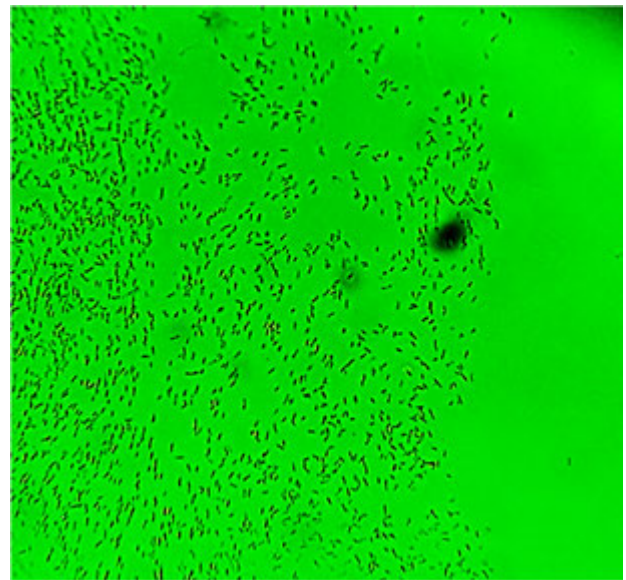


B

Fig. 2. Morphology of the colonies isolated from groundnut nodule bacteria on solid bean medium: A — group I, B — group II.



A



B

Fig. 3. Morphology of cells isolated from groundnut nodule bacteria ($\times 600$): A — group I, B — group II.

the strain of nodule bacteria of lupine *B. lupini* 367a. Four cultures, namely *Bradyrhizobium* sp. AR6, AR8, AR10 and AR14, were included in the serogroup KB11, which consists of intensive growing soybean nodule bacteria. Four strains of *Bradyrhizobium* sp. AR3, AR4, AR5 and AR11 did not interact with used antisera.

According to the main morphological, cultural and serological characteristics, new strains of groundnut microsymbionts can be classified as *B. lupini* and *B. japonicum*. The taxonomic status of the four cultures needs to be clarified.

Under the conditions of vegetation experiment, it was found that all strains of groundnut nodule bacteria belonging to serogroup 367a (*Bradyrhizobium* sp. AR1, AR2, AR7, AR9, AR12, AR13 and AR15) entered into an active symbiotic relationship with lupine (phenotype Nod⁺Fix⁺) but did not nodulate soybean (phenotype Nod⁻) (Table 5). Strains of *Bradyrhizobium* sp. AR6, AR8, AR10 and AR14, serologically related to *B. japonicum* KB11, in contrast, infected soybeans (phenotype Nod⁺Fix⁺) and did not form nodules on the roots of white and yellow lupine (phenotype Nod⁻).

Table 5. Characteristics of nodule bacteria isolated from groundnut nodules

Strains of nodule bacteria	Serogroup	Symbiotic phenotypes		
		<i>Glycine max</i>	<i>Lupinus albus</i>	<i>Lupinus luteus</i>
<i>B. lupini</i> 367a	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>B. japonicum</i> KB11	KB11	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR1	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR2	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR3	X	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR4	X	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR5	X	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR6	KB11	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR7	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR8	KB11	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR9	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR10	KB11	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR11	X	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR12	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR13	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺
<i>Bradyrhizobium</i> sp. AR14	KB11	Nod ⁺ Fix ⁺	Nod ⁻	Nod ⁻
<i>Bradyrhizobium</i> sp. AR15	367a	Nod ⁻	Nod ⁺ Fix ⁺	Nod ⁺ Fix ⁺

Note: Nod⁺Fix⁺ — formation of nitrogen-fixing nodules; Nod⁻ — nodules are not formed, X — unidentified serogroups.

Among the group of serologically unidentified strains, rhizobia which formed nodules on the roots of lupine, but did not infect soybeans (*Bradyrhizobium* sp. AR4 and AR11), and bacteria that infected soybeans, but were unable to initiate nodule formation on the roots of different species of lupine (*Bradyrhizobium* sp. AR5) were also found. It is noteworthy that the nodule bacteria of groundnut *Bradyrhizobium* sp. AR3 formed an active symbiosis with both soybean plants (Nod⁺Fix⁺ phenotypes) and lupine plants (Nod⁺Fix⁺ phenotypes). A strain of *B. japonicum* 631 isolated from soybean nodules is known from the literature, and it can form nitrogen-fixing nodules on the roots of soybean and lupine plants [4]. Further study of a new strain of *Bradyrhizobium* sp. AR3 using modern molecular genetic methods will allow species identification.

Conclusion. It was established for the first time that communities of nodule bacteria capable of nodulating groundnut are present in the agroecosystems of Ukraine. Representatives of two species, *B. lupini* and *B. japonicum*, were found in nodule populations of rhizobia during the cul-

tivation of groundnut plants on sod-podzolic soil and leached chernozem. The dominant microsymbionts of groundnut were lupine nodule bacteria of serogroup 367a (54.2 % and 45.8 %, respectively). Fewer nodules were formed by intensive growing soybean rhizobia of serogroup KB11 (16.7 % and 12.5 %). The share of nodule bacteria not classified in the studied serogroups was 21.9 % and 41.7 %.

Fifteen new strains were isolated from groundnut nodules, which were morphologically, culturally and serologically identified as *B. lupini* serogroup 367a (7 of them), *B. japonicum* serogroup KB11 (4 of them) and *Bradyrhizobium* sp. (4 of them).

New strains of *B. lupini* from groundnut nodules are able to infect white and yellow lupine but do not nodulate soybeans. Strains identified as *B. japonicum* form nodules on soybean roots but do not infect lupine. Serologically unidentified strain *Bradyrhizobium* sp. AR3, which is able to form a symbiosis with both lupine and soybean (phenotypes Nod⁺Fix⁺) was obtained.

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БІОЛОГІЧНІ ВЛАСТИВОСТІ МІКРОСИМБІОНТІВ АРАХІСУ, ПОШИРЕНИХ У ҐРУНТАХ УКРАЇНИ

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Мета. Дослідити наявність у ґрунтах України бульбочкових бактерій, здатних нодулювати арахіс, виділити нові штами ризобій із бульбочок арахісу, вивчити їхні морфолого-культуральні й серологічні властивості та здатність формувати симбіоз із різними бобовими культурами. **Методи.** Мікробіологічні (виділення бульбочкових бактерій із бульбочок та культивування мікроорганізмів, вивчення біологічних властивостей штампів), серологічні

(отримання антисироватки до штаму *Bradyrhizobium lupini* 367a, вивчення різноманіття ризобій у бульбочкових популяціях арахісу, дослідження серологічної належності нових штамів), вегетаційного дослідження (вивчення формування та функціонування симбіотичних систем арахісу з бульбочковими бактеріями, дослідження хазяйської специфічності нових штамів ризобій арахісу), газохроматографічний (визначення азотфіксувальної активності ризобій у симбіозі з арахісом), математично-статистичні. **Результати.** За вирощування рослин арахісу на дерново-підзолистому ґрунті та чорноземі вилугуваному в бульбочкових популяціях ризобій виявлено представників двох видів — *B. lupini* і *B. japonicum*. Домінуючими мікросимбіонтами арахісу були бульбочкові бактерії люпину серогрупи 367a (54,2 % і 45,8% відповідно до ґрунтів). Меншу кількість бульбочок формували інтенсивнорослі ризобії сої серогрупи KB11 (16,7 % і 12,5%). Частка бульбочкових бактерій, не зарахованих до досліджуваних серогруп, становила 21,9 % та 41,7%. Із бульбочок арахісу виділено 15 нових штамів бульбочкових бактерій, які за морфолого-культуральними та серологічними ознаками ідентифіковані як *B. lupini* серогрупи 367a (7 од.), *B. japonicum* серогрупи KB11 (4 од.) та *Bradyrhizobium* sp. (4 од.). Нові штами *B. lupini* з бульбочок арахісу здатні інфікувати люпин білий та жовтий, проте не нодують сою. Штами, ідентифіковані як *B. japonicum*, утворюють бульбочки на коренях сої, але не заражають люпин. Отримано серологічно не ідентифікований штам *Bradyrhizobium* sp. AR3, спроможний формувати симбіоз як з люпином, так і з соєю (фенотипи Nod^+Fix^+). **Висновки.** Уперше встановлено, що в агроценозах України наявні угруповання бульбочкових бактерій, здатних нодувати арахіс. Отримано 15 нових штамів ризобій арахісу, ідентифікованих як *B. lupini*, *B. japonicum* та *Bradyrhizobium* sp.

Ключові слова: мікросимбіонти арахісу, бульбочкові популяції ризобій, *Bradyrhizobium lupini*, *B. japonicum*, серогрупи, соя, люпин.

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