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THE SEARCH OF THE NATIVE SOIL BACTERIA BRADYRHIZOBIUM

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The use of inoculants is a necessary and inalienable agrotechnology for the cultivation of soybeans and other leguminous crops in the most countries of the world. An important property of nodule bacteria is the ability to fix molecular nitrogen in symbiosis with legumes. Obtaining of the effective rhizobia is possible by the direct isolation from the soil or with modification by applying genetic engineering techniques. We isolated 34 bacterial isolates. As a result of biochemical tests, three isolates (LG2, LG3 and LG5) possible to allocate them to *Bradyrhizobium* genus. *Key words:* symbiotic system, nodule bacteria, nitrogen fixation, soybean, Bradyrhizobium japonicum.

Пошук аборигенних бактерій Bradyrhizobium з ґрунту. Гуменюк І.І., Грузінський С.Ю., Бровко І.С., Чабанюк Я.В. Використання інокулянтів є обов'язковою та невід'ємною агротехнологією вирощування сої та інших бобових культур більшості країн світу. Важливою властивістю бульбочкових бактерій є здатність фіксувати молекулярний азот у симбіозі з бобовими культурами. Одержання ефективних ризобій можливе завдяки безпосередньому виділенню з ґрунту або модифікуванню при застосуванні методів генетичної інженерії. Нами було виділено 34 бактеріальних ізоляти. Внаслідок проведення біохімічних тестів з'ясовано, що три ізоляти (LG2, LG3 та LG5) належать до бактерій роду *Bradyrhizobium. Ключові слова:* симбіотична система, бульбочкові бактерії, азотфіксація, соя, Bradyrhizobium japonicum.

Поиск аборигенных бактерий Bradyrhizobium из почвы. Гуменюк И.И., Грузинский С.Ю., Бровко И.С., Чабанюк Я.В. Использование инокулянтов является обязательной и неотъемлемой агротехнологией выращивания сои и других бобовых культур в большинстве стран мира. Важным свойством клубеньковых бактерий является способность фиксировать молекулярный азот в симбиозе с бобовыми культурами. Получение эффективных ризобий возможно благодаря непосредственному выделению таковых из почвы или модифицирование при помощи методов генетической инженерии. Нами было выделено 34 бактериальных изолята. В результате проведения биохимических тестов три изолята (LG2, LG3 и LG5) были классифицированые как род Bradyrhizobium. *Ключевые слова:* симбиотическая система, клубеньковые бактерии, азотфиксация, соя, Bradyrhizobium japonicum.

Introduction. Lately in Ukraine there is a tendency to increase sowing of soybean, and at the same time – increasing demand of the inoculants. These biopreparations, based on one or more useful strains of microorganisms that use molecular nitrogen as a food source and convert it into a form that is accessible to plants, are gaining popularity due to their relatively simple application and high efficiency. In general, the use of inoculants is an indispensable and integral agricultural technology for growing soybeans and other leguminous crops in most foreign countries of the world. Soybean inoculation improves crop yields and leads to a reduction in financial risks, due to lower cost compared to mineral nitrogen fertilizers. One of important properties of nodule bacteria is ability to fix molecular nitrogen from the atmosphere, it is possible only in symbiosis with legume crops. Under enabling conditions this legume crop can accumulate in the soil up to 320 kg / ha of biological nitrogen (50-80 kg/ha on average). While being in symbiotic relations, bacteria get all elements needed for growth and development from the host plant [1−3].

So, it's important to use inoculants based on highly active strains of nodule bacteria because of absence of effective local microbiota in Ukrainian soils. The obtaining of the useful soil rhizobia is possible by the direct isolation from the soil or with modification by applying genetic engineering techniques (transposonal mutagenesis, for example).

Market of biological products is actively developing and is full of biological preparation of both foreign and national production. Leading productions are concentrated in Argentina, Brazil, Germany, the United States and Canada. Guided by general stereotypes, agrarians prefer foreign brands. However, actual researches confirm the efficiency and expediency of using Ukrainian biologics, which have goof results and are not less effective than foreign analogue. Local biologics have a number of advantages, among which the most important thing is considered to be adaptation to weather circumstances, climate, operating environment on the territory of Ukraine [4].

Literary review. Majority of symbiotic nitrogen-fixing bacteria is classified as *Rhizobiacae* fam-

ily, it's *Alphaproteobacteria*, representatives of *Rhizobium, Mezorhizobium, Ensifer (Sinorhizobium)* and *Bradyrhizobium* genus. Effective symbiosis can be formed by sowing legumes in a new area of the earth due to the presence in the soil of adapted strains of nodule bacteria [5; 6].

Now in soybean growing technologies, pre-sowing seed treatment with microbial preparations is widely used based on highly effective strains of nodule bacteria. The productivity of symbiosis with nitrogen-fixing bacteria is determined by the activity and competitiveness of the strain in specific soil and climatic conditions, its complementarity to a certain plant variety, and the genetic characteristics of the macrosymbiont. Therefore, the creation of highly effective nitrogen fixing system *Bradyrhizobium japonicum* – soybean *Glycine max* (L.) Merril has a great theoretical and practical value.

Thus, it is necessary to search for new highly effective and competitive strains of rhizobia – potential components of microbial preparations. Creation of this preparations based on effective nodule bacteria will allow to obtain environmentally safe and economically profitable high quality products [7].

Materials and methods. The search for and selection of effective strains of soybean rhizobia was carried out using methods of analytical selection in the log area, which was used as a pasture and for 30 years did not grow crops. Nodules on soybean plants were selected during the flowering phase. Sterilization of soybean nodules was carried out using ethanol and Microbac (BODE Cheimie GmbH, Germany).

The isolation of bacterial isolates from sterile nodules was carried out by sowing on yeast-agar medium (YAM) of the following composition (g/l): mannitol – 8,0; yeast extract – 2,0; glucose – 2,0; $(NH_4)_2SO_4$ – 0,5; K_2HPO_4 – 0,35; KH_2PO_4 – 0,35; $MgSO_4$ – 0,2; agar – 20,0; pH 7,2.

Determination of the main culture-morphological and physiological properties of new rhizobial strains was carried out using express systems for the bacterial identification API® (bioMérieux, USA) and identifying features of Bergey`s manual [8, 9].

The technological parameters of the growth of cultures of the strains of the nodule soybean bacteria were determined by Nikitin [10].

The activity of the symbiotic apparatus of soy was studied in a laboratory experiment. For this purpose, the length and weight of the stem and root of plants, the number and weight of nodules were measured [11].

The statistical analysis of the results was carried out using the recommendations of statistical analysis manuals and standard statistical software Statistica 10.0, Microsoft Excel 16.

Results of the research and discussion. Thus, during the vegetative period, we isolated 34 bacterial isolates. Making morphological studies of colonies and individual microorganisms, their coloring by Gram allowed to divide the bacteria into several groups. Among them there were endophytic microorganisms, fast-growing and slow-growing nodule bacteria.

As a result of done research, we came to the conclusion, that ten isolates, conditionally marked as SF (12-21) were gram-negative aerobic rod-shaped bacteria and without spores, therefore they can be classified as *Ensifer (Sinorhizobium)* genus. Whereas in 3-day culture we could observe bacteria with the size 2,3-2,5 x 0,4-0,5 μm, which were agile and formed 7 mm diameter cream-white colonies on solid nutrient medium yeast-mannitol agar (YMA) and meat-peptone agar (MPA) and over time have the property of merging.

Other thirteen isolates, conditionally marked as BP (22-34) were classified as endophytes, since they are gram-positive aerobic rod-shaped bacteria and with endospore. This gave the opportunity to make a conclusion about their affiliation to *Bacillus* genus. Isolates SF12-SF21 and BP22-BP34 were not used in following research.

For our research we matched only 11 isolates. They were classified as *Rhizobium* genera, according to preliminary estimation, since all of them are gram-negative, obligatory aerobic rod-shaped bacteria and do not produce spores. In 3-day culture rods in the size 0,5−0,9 x 1,2−3,0 μm are agile and form two types of colonies: cream-white, round, convex, 1 mm and white, slimy, round, 2 mm, don't grow on MPA. By morphological features of the bacteria and characteristics of their colonies (form, color, size, surface) more than a half of isolates were bigger and had other color at the next sowing on YMA were larger in size, different in color and over time had the ability to merge, which does not look like *Bradyrhizobium* genus [9].

By studying the ability to metabolize sugar, it was shown that LG2, LG3, LG5 isolates possessed the ability to use arabinose, galactose, glucose, rhamnose, sucrose and mannitol as a substrate, and do not use sorbitol and inositol at all, which is typical of the reference strain.

Selected bacterial isolates didn't have the ability to produce gelatinase and were characterized by negative Voges-Proskauer test, which is classical for rhizobia. Also they didn't excrete H_2S, didn't synthesize indole and didn't utilize citrate.

Owing to making biochemical tests, three isolates (LG2, LG3 and LG5) can really be referred to *Bradyrhizobium* genus. Therefore, these isolates isolated from soybean plants *Glycine max* (L.) Merril can be classified as rhizobia due to the evaluation of their morphological and biochemical characteristics. Similar data were obtained and characterized by the indian researchers Khansole and Gachande, who isolated isolates of nodule bacteria from the rhizosphere of *Glycine max* (L.) Merril soil and characterized them as *Bradyrhizobium japonicum sp*. on the basis of morphological, cultural and biochemical features. It is advisable to carry out a genetic analysis of the presented isolates, and now is possible to allocate them to *Bradyrhizobium sp.* genus [12].

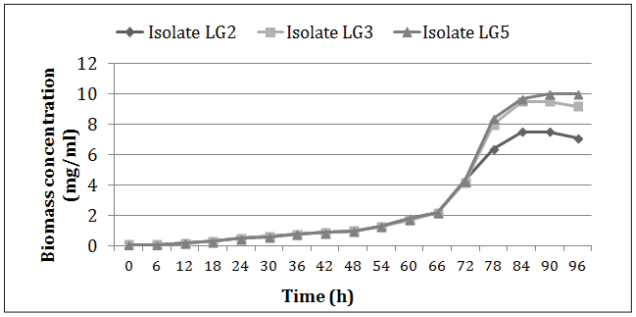

Fig. 1. Dynamics of growth of isolates Bradyrhizobium sp.

The other two isolates LG2 and LG3 also showed a good result. In particular, there was an increase in the vegetation index of soybean plants compared to control. Root length increased by 18 and 25%, root weight by 12 and 33% relative to LG2 and LG3 isolates accordingly. The stem height has increased by 15 and 17%, and the weight – 13 and 14%, accordingly. The weight of the stem and its height increased by 2-3% compared to the variant with *Bradyrhizobium japonicum eko/001*. The results of the root weight and length were somewhat lower than in this variant. The number of nodules

Table 1

The effect of B. japonicum isolates on the formation of a symbiotic apparatus and the development of soybean plants of Moravia variety

Variant	Stem length, cm	Stem weight, g	Root length, cm	Root weight, g	Nodules Quantity units/plant	Nodules weight, g/plant
Control	15,7 ±0,36	1,83±0,06	6,5±0,61	0,42±0,03	-	-
Bradyrhizobium japonicum eko/001 (Rizoaktiv R)	17,8 ±0,47	2,02±0,01	8,2±0,56	0,59±0,02	22,5±0,5	0,44±0,05
Isolate LG2	18,1±0,23	2,08±0,08	7,7±0,44	0,47±0,01	31,5±0,5	0,62±0,03
Isolate LG3	18,3±0,81	2,06±0,04	8,1±0,25	0,56±0,02	20,7±0,4	0,46±0,02
Isolate LG5	17,4 ±0,98	2,11±0,11	8,7±0,53	0,54±0,02	38,6±0,5	0,68±0,02

Studies on the dynamics of biomass growth of nodules soybean bacteria have shown that they are slowly growing bacteria (Fig. 1). In the exponential phase of isolates LG2 and LG3 went out on 3.5th day, and LG5 − on 4th day of cultivation.

The analysis of growth of studied isolates allows us to affirm that the optimum for cultivation of these soybean nodule bacteria is 90-96 hours. At the same moment the substrate of the nutrient medium is almost completely consumed by the studied microorganisms under investigation.

The pot experiment takes place in these studies to verify the efficiency of our isolates. The obtained data indicate that vegetative parameters of soybean plants are improved in all variants with bacterization (Table 1).

The isolate LG5 produced the largest number of nodules, which exceeded positive control by 72% and weight – by 55%, respectively. The development of plants in this variant was the best. For example, the length and mass of the root increased by 29 and 34% compared to the control variant without bacteria. The root length was 6%, and the stem weight was 4% higher than in the variant with the professional strain *Bradyrhizobium japonicum eko/001*.

and their weight while in variant with isolate LG2 was 1.4 times higher than that number in variant with professional strain *Bradyrhizobium japonicum eko/001*. And isolate LG3 index were at the level of the professional strain. In the control variant without bacterization, the formation of nodules was not noted.

While searching we isolated active bacterial isolates, which require a verification of competitiveness, virulence and other important features in field conditions and in the future they can be used as a base for microbiological preparations.

Conclusions. Thus, due to the search of nodule soybean bacteria and the study of their properties, the evaluation of the morphological and cultural characteristics of new isolates and the implementation of generally accepted physiological and biochemical tests for the identification of three isolates (LG2, LG3 and LG5) can be allocated to the genus *Bradyrhizobium sp.* These isolates are slowly growing bacteria, with an output of 3.5-4 days in the exponential phase. The largest nodulation activity was shown by isolate *Bradirhizobium sp*. LG5, which exceeded the positive control by 72% by number and by 55% by the weight of the tubers. Further study and evaluation of these isolates, their use as bio-agents of modern biological products is all the more necessary.

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