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DIAZOTROPH ACTIVITY REGULATING STRATEGY UNDER THEIR INTRODUCTION IN AGROCENOSES

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Objective. *Investigate approaches to managing the activity of soil diazotrophs and propose a strategy for its regulation.* ***Methods****. Theoretical, vegetation and field experiments, microbiological, gas chromatographic, mathematical and statistical.* ***Results****. The activity of beneficial soil microorganisms can change under the action of temperature, humidity, chemical compounds of various origin, and other microorganisms. It was established that, taking into account a significant variety of factors, it is necessary to develop a set of specific ways to increase the growth and functional activity of nitrogen-fixing bacteria, as well as their viability. It has been proved that the combination of diazotrophs forms an effective symbiotic leguminous-rhizobial system, which provides additional biological nitrogen in agrocenoses. At the same time, there was an increase in plant mass, chlorophyll content in the leaves, protein and oil content in the products. The combined use of diazotrophs increases the yield, in particular, soybeans by 9–16 % compared with inoculation by pure bacterial culture.* ***Conclusion.*** *Based on the analysis and generalization of the obtained research results, a strategy for regulating the activity of diazotrophs for their effective introduction into agrocenoses is proposed, which consists in combining bacteria of different species, selecting conditions for their co-cultivation and application upon stabilisation of the number of viable bacterial cells. The proposed strategy involves solving the problem by obtaining an inoculant, which is characterized by a high titre and a stable number of viable cells, which allows to obtain an effective nitrogen-fixing system. The strategy is tried-and-tested on the example of regulating the growth and functional activity of soybean nodule bacteria by combining diazotrophs of different species, substantiating the conditions of their co-cultivation and application to ensure positive interaction in the form of commensalism, as well as by regulating viability of diazotrophs by adding stabilisers to the medium.*

Key words: *nitrogen-fixing bacteria, growth activity, nitrogen fixation, viability, diazotroph activity regulating strategy.*

Introduction. Modern ideas about the interaction of microorganisms and plants suggest that soil nitrogen-fixing bacteria play an important role in agrocenoses. The positive effect of diazotrophs is due to their ability to fix atmospheric nitrogen and supply it to plants, as well as to produce physiologically active compounds that stimulate the growth and development of crops. As specific beneficial bacteria are not always present in the soil in sufficient quantities, appropriate inoculants and methods of their use are being developed in Ukraine and other countries. At the same time, diazotrophs do not fully realize their potential under the influence of negative environmental factors, which can negatively affect the efficiency of their use. Therefore, it is important to develop and implement a strategy to regulate the activity of nitrogen-fixing bacteria during their introduction into agrocenoses of crops, which would solve this problem

Analysis of the novel studies and publications. The final objective of the use of selected highly effective nitrogen-fixing bacteria is to increase the productivity of crops and obtain quality plant products [1–3]. At the same time, it is necessary to achieve economic and energy efficiency of this agricultural measure, as well as maximum maintenance of soil resources.

Since the main function of introduced diazotrophs is the fixation of atmospheric nitrogen [4], it is necessary to ensure the formation of an effective nitrogen-fixing system, which occurs as a result of the interaction between bacteria and a particular crop. The formation of such a system is impossible without the introduction of a sufficient number of diazotrophs into the appropriate agrocenosis, which can be done by using inoculum with a high titre. However, even having such an inoculant, it is necessary to ensure maximum stability of viable bacterial cells during storage and use [5].

The titre of nitrogen-fixing cells in the inoculant depends on their growth activity during cultivation [6]; the stability of the number of diazotrophs that can be introduced into agrocenoses depends on the viability of these microorganisms [7–9], and the formation of an effective nitrogen-fixing system — also on the implementation of the functional activity of microorganisms.

In ecosystems, microorganisms cannot be considered in isolation, because there is a constant interaction between them [10–14], which can significantly affect their activity. Therefore, it is important to study mixed cultures of microorganisms, including soil nitrogen-fixing bacteria, for maximum implementation of their potential for practical purposes.

In view of the above, it is important to determine the functional activity and viability of diazotrophs under the action of biotic and abiotic factors, as these studies may be the basis of a strategy to regulate the activity of beneficial soil bacteria

Objective. Investigate approaches to managing the activity of soil diazotrophs and propose a strategy for its regulation.

Materials and methods. ***Bacteria used in thee work***. *Azospirillum brasilense* sp. 7 (typical strain), *Azospirillum brasilense* 410 (V. V. Volkohon), *A. brasilense* 18-2 (V. I. Lokhova, O. V. Nadkernychna), *Azotobacter chroococcum* M-70 (Yu. M. Mochalov, V. I. Kanivets), *Azotobacter vinelandii* M-X (Yu. M. Mochalov, V. I. Kanivets), *Bradyrhizobium japonicum* M-8 (M. Z. Tolkachev), *B. japonicum* 634б (A. T. Novikova), *Rhizobium leguminosarum bv. viceae* 248б, *Rh. leguminosarum bv. viceae* 250a (A. F. Antypchuk, A. T. Novikova), *Rh. leguminosarum* 31 (M. Z. Tolkachev), *Rhizobium radiobacter* = *Agrobacterium radiobacter* 204 (M. K. Sherstoboev, A. V. Khotianovych, V. P. Patyka), *Enterobacter aerogenes* 30-ф (O. O. Berestetskyi, A. V. Yermolina, V. P. Patyka), *Pseudomonas fluorescens* B-17 (typical strain). Microorganisms were obtained from the Collection of Beneficial Soil Microorganisms of the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS. We sincerely gratitude to the authors for their kindly provided strains

Agricultural crops. Soybean of the varieties Lehenda, Suziria, Poltava. Winter wheat of the variety Poliska 90. Winter rye of the variety Borotba. Winter barley of the variety Honar.

The experiments were performed at the Institute of Agricultural Microbiology and Agroindustrial Manufacture of the NAAS on leached light loam chernozem, which contains from 2.8 % to 3.4 % of humus (according to Tiurin), from 0.27 % to 0.31 % of total nitrogen, about 15 mg/100 g of soil P_2O_5 (according to Kirsanov), from 13 mg/100 g of soil to 16 mg/100 g of K_2O (according to Maslova), pH = 5.9–6.5. Planning and conducting field experiments were performed according to Dospiekhov [15].

The number and growth parameters of microorganisms in the bacterial suspension were determined by microbiological methods [16–19]. Studies of the nitrogen fixation activity of microorganisms were performed by the acetylene method [20; 21] on a Chrom-4 gas chromatograph with a flame ionization detector. Steel sorption columns were filled with sorbent Porapak Q 60–80 mesh. Thermostat temperature was 40 ºC. Consumption of gases: hydrogen — 15 cm^3/min, nitrogen — 100 cm^3/min, air — 500 cm^3/min.

The number of representatives of certain physiological and trophic groups of microorganisms in the rhizosphere soil of plants was determined by the plate method via deep seeding on agar media [21; 22]: meat-peptone agar (MPA) to account for microorganisms that use mainly organic forms of nitrogen, starch-ammonia agar (SAA) — for microorganisms that absorb mainly nitrogen of mineral compounds. The number of micromycetes was determined on acidified Czapek medium.

Statistical processing was performed according to generally accepted methods in mathematical statistics using Microsoft Excel. To assess the significance of the differences between the variants of the experiments, the least significant difference was calculated (HIP_{05}).

Results and discussion. The activity of nitrogen-fixing bacteria is not stable and unchangeable, as we have shown after a range of different experiments. In particular, the effect on diazotrophs was studied:

1) chemical substances of different nature [23–26];

2) soil microorganisms [27–33];

3) abiotic environmental factors [34–36].

Based on the analysis of the obtained results, a general strategy for regulating growth and functional activity, as well as the viability of diazotrophs is proposed. This strategy involves combination of diazotrophs of different species, selection of conditions for their co-cultivation and use upon stabilizing the number of viable bacterial cells (Fig. 1).

The strategy is developed on the pattern of regulating the activity of *B. japonicum* to increase productivity of a particular crop, namely soybeans.

Given the extremely large number of different factors that can potentially affect soybean nodule bacteria, it was necessary to identify specific factors that can significantly affect the activity and viability of the studied microorganisms. In our opinion, it is necessary to minimize the number of selected factors, as their increase may reduce the ability to manage them.

When selecting the factors that affect the introduction of nodule bacteria in soybean agrocenoses, it is necessary to take into account the fact that the regulation of growth and functional activity of diazotrophs necessitates activation of the vital processes of microorganisms, and improvement in viability is achieved by reducing bacterial activity, i. e. by bringing them to a state of rest and maintenance in such a state for the required period. Therefore, there is a need to divide the factors into two blocks.

Since in nature, soil diazotrophs function upon constant interaction with other bacteria [37–39], for example, nodule bacteria with associative and free-living nitrogen fixers, one of the effective biotic factors is the use of mixed cultures of microorganisms to increase their growth and functional activity. A feature of our proposed approach to the selection and combination of *B. japonicum* with other soil diazotrophs is the use of microorganisms complementary to nodule bacteria to manage their activity. At the same time, the known combinations of beneficial soil microorganisms in the inoculant aim to expand the spectrum of action of biological preparations: e. g. to improve nitrogen and phosphorus nutrition [40; 41].

Quite often these combinations are mechanistic in nature, without a comprehensive scientific substantiation and without answers to the

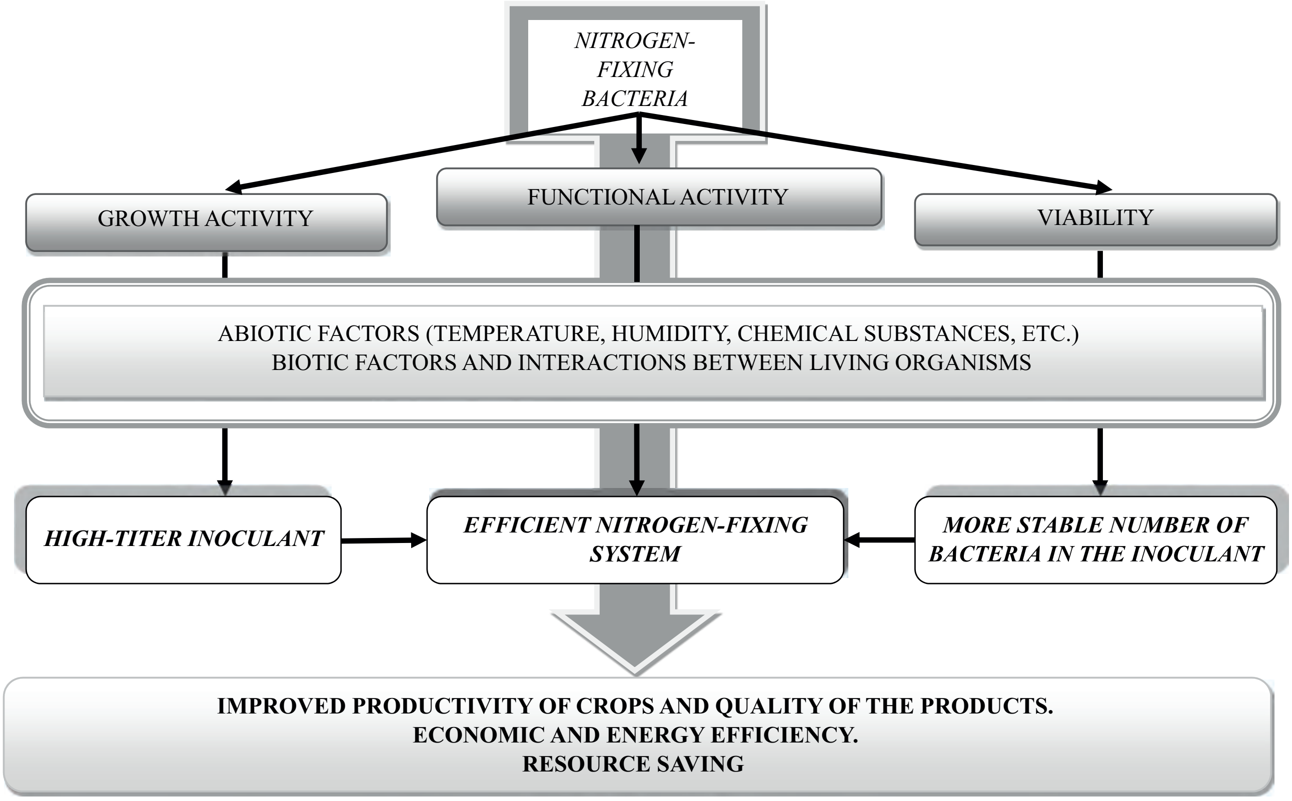

Fig. 1. General strategy for regulating activity of soil diazotrophs.

following questions: how does one microorganism affect the growth activity of another; what is the optimal quantitative ratio of the cells of different types of microorganisms; whether the effect of combining two or more microorganisms is greater than the simple sum of the effects of each individual microorganism, etc.

Furthermore, an important factor influencing diazotrophs during their introduction into agrocenoses is ensuring the viability of these bacteria [42; 43], which is influenced by a number of factors [44–47]: temperature, humidity, chemical substances of different nature, etc. The maintenance of diazotrophs can be achieved by transferring bacteria to a state of rest and protective action of certain substances due to the ability to form a protective cover [48; 49].

It is proposed to manage the activity of *B. japonicum* by regulating their growth and functional activity due to combining diazotrophs with the selection of conditions for their co-cultivation and use to ensure positive interaction in the form of commensalism, as well as by regulating the viability of diazotrophs by adding stabilizers to the medium (Fig. 2).

At the next stage, a number of studies were conducted to confirm the correctness of the selected strategy. Initially, the growth activity of soybean nodule bacteria under the action of suspensions containing metabolites of other diazotrophs was studied. No significant positive effect of suspensions of nitrogen-fixing bacteria belonging to rhizobia, azotobacter, pseudomonads, agrobacteria and enterobacter on the growth of soybean nodule bacteria was found. At the same time, high concentrations of suspensions of these microorganisms inhibited the growth of *B. japonicum* strains. Addition of the bacterial suspension with metabolites of *A. brasilense* in low concentrations to the culture medium for the cultivation of nodule bacteria had a positive effect on the growth activity of soybean nodule bacteria.

Upon mixed cultivation of *B. japonicum* and *A. brasilense,* microorganisms can directly affect the viability of bacterial cells of other species, resulting in an increase in both growth and functional activity. Given the different needs of the studied bacteria in food sources, we have developed a new semi-synthetic nutrient medium for their co-cultivation [50].

The production of physiologically active compounds by diazotrophs in pure and mixed culture has been studied. It has been shown that the amount of cytokinins and gibberellins increases, the content of abscisic acid and the auxin/cytokinin ratio decreases compared to variants with pure cultures of microorganisms in

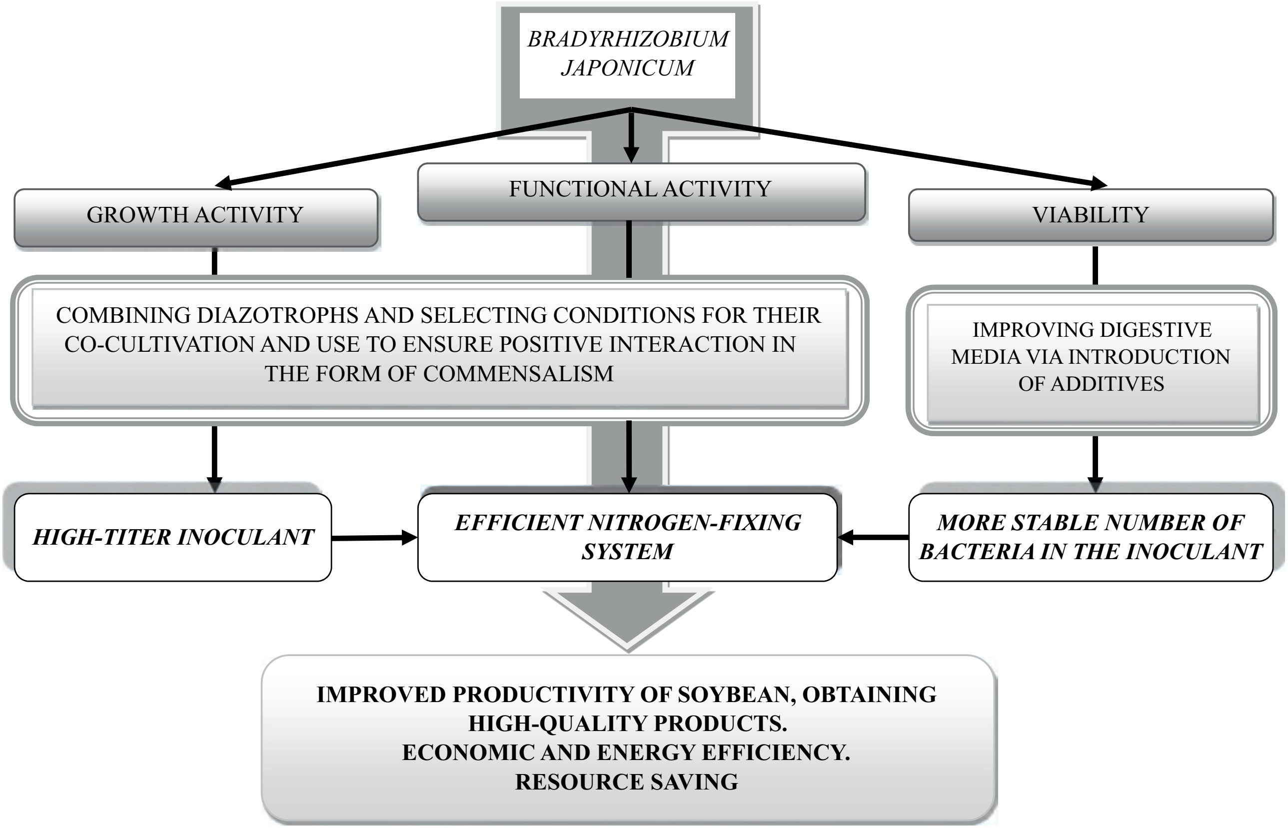

Fig. 2. Strategy for regulating Bradyrhizobium japonicum activity.

the culture fluid of nodule bacteria and azospirilla when they are co-cultivated [51]. The use of soybean nodule bacteria and azospirilla in a mixed culture promotes the formation of a more efficient symbiotic apparatus: the number and weight of nodules increases, nitrogen-fixing activity improves [52].

Various chemical compounds have been tested as stabilizers in the medium. During storage of the inoculum, the titre of bacteria grown with stabilizers decreases more slowly, which indicates the feasibility of using these substances to extend the shelf life of *B. japonicum*. The most effective stabilizer, which in our studies was sodium alginate, and its optimal concentration, which helps maintain the viability and functional activity of soybean nodule bacteria in a liquid medium for 4 months were selected [53; 54].

When using the inoculant based on nodule bacteria and azospirilla with sodium alginate, an increase in the mass of soybean plants in all phases of development, an increase in soybean yield by 0.23–0.4 t/ha, or 9–16 %, compared with inoculation with pure culture of nodule bacteria, was reported. At the same time, the protein and oil content in the products increased.

The combined use of *B. japonicum* and *A. brasilense* is characterized by a higher level of both economic and energy efficiency compared to the variant without inoculation and the variant with a pure culture of nodule bacteria. The implementation of the strategy for managing the activity of diazotrophs during their introduction into soybean agrocenoses is schematically presented in Fig. 3.

Conclusion. Based on the analysis and generalization of our study results, a strategy for regulating the activity of diazotrophs for their effective introduction into agrocenoses is proposed, which consists in combining bacteria of different species, selecting conditions for their co-cultivation and use upon stabilisation of the number of viable bacterial cells. The proposed strategy involves solving the problem by obtaining an inoculant, which is characterized by a high titre and a more stable number of viable cells, which allows to obtain an efficient nitrogen-fixing system

The strategy is tested on the pattern of regulating the growth and functional activity of soybean nodule bacteria by combining diazotrophs of different species, justifying the conditions of their co-cultivation and use to ensure positive interaction in the form of commensalism, and by regulating the viability of diazotrophs via adding stabilizers to the medium.

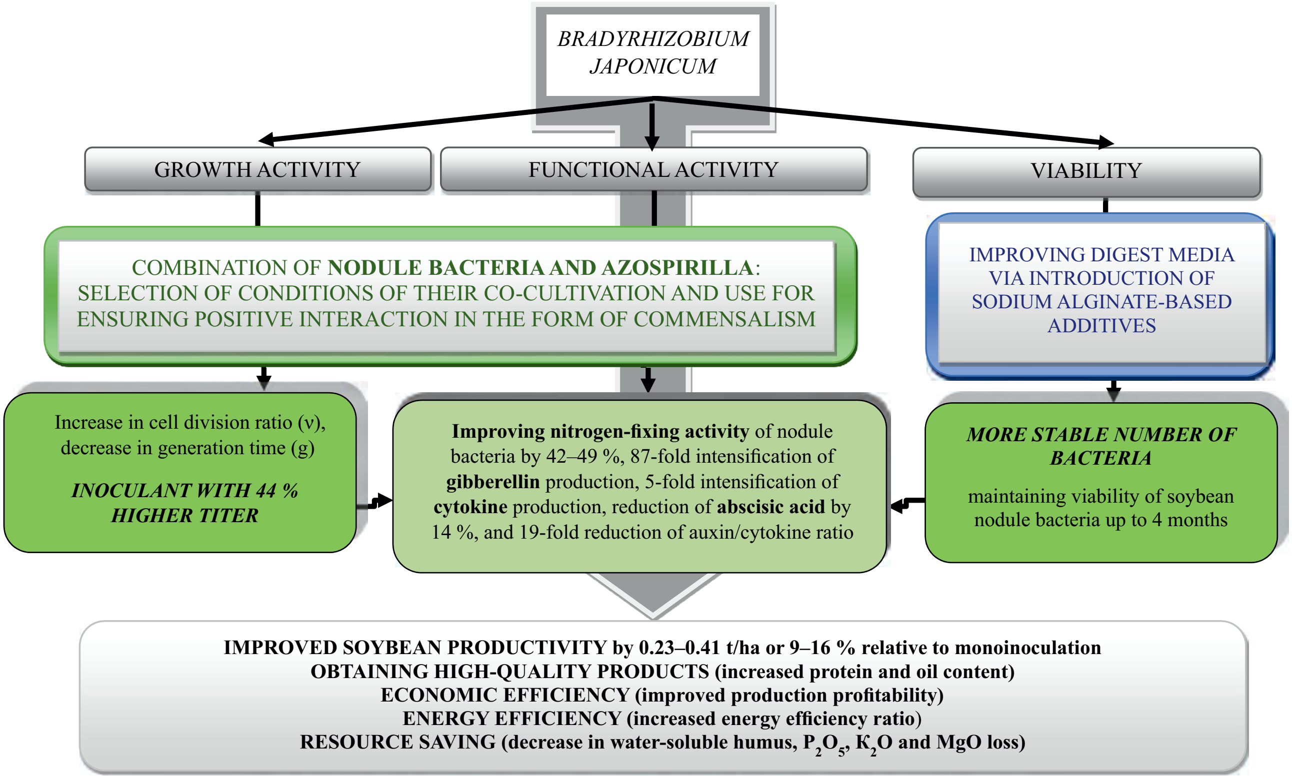

Fig. 3. Implementation of the strategy for regulating Bradyrhizobium japonicum activity.

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СТРАТЕГІЯ РЕГУЛЮВАННЯ АКТИВНОСТІ ДІАЗОТРОФІВ ЗА ЇХ ІНТРОДУКЦІЇ В АГРОЦЕНОЗИ СІЛЬСЬКОГОСПОДАРСЬКИХ КУЛЬТУР

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Мета. *Дослідити підходи щодо управління активністю ґрунтових діазотрофів та запропонувати стратегію її регулювання.* ***Методи.*** *Теоретичні, вегетаційного і польового досліду, мікробіологічні, газохроматографічні, математично-статистичні.* ***Результати.*** *Показано, що активність корисних ґрунтових мікроорганізмів може змінюватися за дії температури, вологості, хімічних сполук різної природи, інших мікроорганізмів. Встановлено, що зважаючи на значну різноманітність чинників, необхідна розробка комплексу конкретних способів підвищення ростової й функціональної активності азотфіксувальних бактерій, а також їхньої життєздатності. Доведено, що за поєднання діазотрофів формується ефективна симбіотична бобово-ризобіальна система, що забезпечує надходження додаткового біологічного азоту в агроценози. Водночас відзначено збільшення маси рослин, вмісту хлорофілів у листках, вмісту протеїну та олії в продукції. За сумісного застосування діазотрофів збільшується урожайність, зокрема сої — на 9–16 %, якщо порівняти з інокуляцією чистою культурою бактерій.* ***Висновки.*** *На основі аналізу й узагальнення отриманих результатів досліджень пропонується стратегія регулювання активності діазотрофів для їх ефективної інтродукції в агроценози, яка полягає у поєднанні бактерій різних видів, підборі умов їх сумісного культивування й застосування за стабілізації чисельності життєздатних клітин бактерій. Запропонована стратегія передбачає розв'язання поставленого завдання шляхом отримання інокулянту, який характеризується високим титром і більш стабільною кількістю життєздатних клітин, що дозволяє отримати ефективну азотфіксувальну систему. Стратегія опрацьована на прикладі регулювання ростової й функціональної активності бульбочкових бактерій сої за рахунок поєднання діазотрофів різних видів, обґрунтування умов їх сумісного культивування і застосування для забезпечення позитивної взаємодії за формою коменсалізму, а також шляхом регулювання життєздатності діазотрофів за рахунок внесення добавок-стабілізаторів у середовище.*

Ключові слова: *азотфіксувальні бактерії, ростова активність, азотфіксація, життєздатність, стратегія регулювання активності діазотрофів.*

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