Methods of increasing the microbial diversity of agricultural soils and plant protection

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Abstract. The paper analyzes an array of scientific literature data in the field of studying methods for increasing microbial diversity of agricultural soils. The variety of biological products used in agriculture has been studied. Some biological products approved for use in the Russian Federation are given. The importance of hub species for the biodiversity of the rhizobiome of plants has been revealed. Approaches to obtaining of high-efficiency bioproducts are considered. The necessity of creating biological products to restore the microbial diversity of agricultural soils using hub species is substantiated.

1 Introduction

In the context of the growing demand of the population for high-quality agricultural products and the inexpediency of further application of traditional methods of increasing production volumes, a number of issues are need to be solved at the present time. Among them are increasing agricultural productivity, maintaining soil fertility and using effective ways to improve the quality of agricultural products. The area of land used in agriculture is increasing, but this does not contribute to an increase in the share of arable land [1]. At the same time, in many countries, an extensive method of land cultivation remains, and the use of agrochemicals and other chemical plant protection products (pesticides) prevails [2-4]. The greatest impact is on the soil, the condition of which depends on many factors [5-7]. Therefore, researchers are actively searching for more environmentally friendly methods to increase soil fertility, and one of such methods is the use of biological products based on microorganisms with various properties useful both for the soil and for plants.

1.1 The possibilities of using beneficial bacterial species and hub taxa

"Beneficial bacteria" or PGPR (plant growth promoting rhizobacteria) can be used as components of a biological product. They are also used as remediators in the purification of eutrophied reservoirs or soils contaminated with pesticides, and biological products are created on their basis to increase crop yields and maintain systemic plant resistance [8]. For example, the use of biological products containing strains of *Bacillus* and *Pseudomonas* as "biological pesticides" has been proposed as a substitute for some chemical pesticides due to their properties of suppressing the development of pathogens by producing antibiotics;

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Paenibacillus polymyxa, *Bacillus amyloliquefaciens*, *Bacillus licheniformis* are also used to combat stress caused by exposure to cultures of various bacterial pathogens, *Bacillus subtilis*, *Pseudomonas cepacia*, *Pseudomonas gladioli*, and others [8-9].

Also, in addition to the use of "beneficial bacteria", it is currently promising to identify "key species", or hub taxa, study their properties and use in agrobiotechnology. The key species of the bacterial community, or hub taxa, are species capable of creating a consortium around themselves by forming functional connections with other organisms, they have a significant impact on the structure and functioning of the microbiome, regardless of their distribution in space and time. These species are an essential component of the rhizosphere microbial community, and their disappearance can lead to drastic changes in the structure and functioning of the microbiome [10]. Hub taxa can have a positive impact on soil quality, carbon transformation, and degradation of organic compounds [11]. They also play a role in biochemical communication and can be markers of changes in the composition of communities, as well as influence the functioning of entire ecosystems [12]. The composition of key taxa varies in different ecosystems. For example, in forest communities these can be: *Actinomycetales, Acidobacteria, Rhizobiales, Burkholderiales, Clostridiales, Sphingobacteriales, Rhodobacterales, Verrucomicrobia*, and soils of agrocenoses are characterized by *Gemmatimonas, Acidobacteria GP17, Xanthomonadales, Rhizobiales, Burkholderiales, Solirubrobacterales, Verrucomicrobia* [13].

It can be assumed that if hub taxa have a significant impact on the functioning of the microbiome, then they should be a dominant species in the structure of the community. However, this is not always the case. Hub taxa are not always dominant in the community, there is a difference between these terms. It consists in the functional characteristics of a particular species [14]. The dominant species often has a significant impact on the functioning of an ecosystem or a specific process solely due to its predominance in biomass or abundance, while key taxa can have a significant impact on the functioning of the microbiome regardless of these parameters [15]. For example, dominant taxa or taxa actively involved in major energy conversion processes can influence global soil processes such as denitrification or decomposition of organic matter. At the same time, the influence of minor, but at the same time key, taxa may be more pronounced if the process is specific or simple in terms of its course (for example, nitrogen fixation or ammonia oxidation), and is carried out by a small group of specialized microorganisms stimulated by the presence of a key taxa [16]. Simply put, the influence of key taxa on various processes involving microorganisms is sometimes inversely proportional to the scale of these processes [13]. But if a key taxa dominates a community in terms of biomass or abundance, then it becomes more difficult to draw a line between key and dominant species. Thus, hub taxa are microorganisms that can be present in the rhizobiome in both minor and dominant positions, but their main characteristic is the ability to form consortia with other species (satellites), affecting their abundance and functions [15].

The number and distribution of key species in the soil is determined by spatial and temporal heterogeneity. The influence of a key taxon may be more significant if it belongs to the core microbiome [17]. Key taxa can function both independently and in a group, thus forming a consortium capable of changing the structure and dynamics of the ecosystem in which they are located [18].

Key taxa can be relevant only under certain conditions [10]. The presence of such species in the community underlines the importance of small taxa for the functioning of the microbiome [19]. Their existence only confirms that the number of a species is not a factor determining its contribution to the community [3]. For example, the presence of such an important organism as a sulfate-reducing bacterium (*Desulfosporosinus)*, even in minor amounts, is extremely important for peat bog ecosystems in terms of regulating carbon cycle [19]. Many key species can, for example, contribute to the implementation of functions important for the ecosystem, such as nitrification; or support the vital activity of organisms with narrow substrate specificity (methanotrophs, methylotrophs); or stimulate the course of such complex processes as, for example, the degradation of specific chemicals, etc. [3].

As can be noted, rhizobacteria are extremely important for maintaining "soil health", increasing yields and improving the quality of crop production [10]. In this sense, maintaining soil fertility using the species present in the rhizobiome of plants is a powerful mechanism for solving the problem. The evidence of the existence of a negative effect of using traditional methods of increasing yields has led to the need to develop a more ecosystem-friendly way of farming. Therefore, recently, scientifically based approaches have been developed to achieve high yields and minimize the negative impact on various components of the environment. That is why it is important to develop biological methods for maintaining soil "health". This will make it possible to achieve high quality agricultural products without damage to the environment, natural maintenance of a high level of soil fertility and the biological diversity of soil microbiomes necessary for successful agricultural activities.

2 Diversity of biological products

The possibilities of using biological products are quite high: they are used both for cereals and for melons, fodder, and vegetables [20-23]. By having a positive effect on soil fertility, biological products increase the productivity of cultivated crops, help reduce the chemical pressure on the agrocenosis and ensure environmentally safe farming without adversely affecting representatives of local flora and fauna [24].

For these purposes, groups of microorganisms such as, for example, phosphate-mobilizing, ammonifying and nitrifying bacteria can be used, since they supply available phosphorus and nitrogen compounds to plants. The vital activity of denitrifiers in the soil is suppressed by inhibitors, since it contributes to the emission of nitrogen, which returns to the atmosphere [25].

Currently, biological products are used, in the vast majority of cases containing a high content of a single microorganism with targeted beneficial properties. Examples of such microorganisms are bacteria of the genera *Bacillus, Pseudomonas, Azotobacter, Bradyrhizobium*, as well as micromycetes of the genus *Trichoderma*. An alternative to such biological products are biological products with 1 organism enhanced with active products of microbial synthesis, or products applied to carriers that improve soil properties and at the same time contribute to better survival of biological product (biochar, for example [26]).

However, there are also multifunctional biological product that have several goals of action (simultaneously and effectively combining different directions of action). The multifunctional effect of such biological products achieved both by the individual characteristics of the producing strain and by a combination of several cultures of microorganisms with different directions of biological action [27].

Complex biological products containing a microbial consortium of beneficial strains of microorganisms are also effective [28]. They are more stable in changing climatic conditions due to ensuring the greatest ecological and physiological compatibility of bacteria and careful selection of organisms that form the basis for the product. In the conditions of vital activity of the host plant, its effect on representatives of the rhizosphere microbiome is much greater than the effect of abiotic factors. This is due to the special composition of root exudates and its effect on the characteristics of the soil and its inhabitants. Therefore, it is necessary to take into account many factors when selecting strains of microorganisms that can potentially be included in the composition of a biological product. Well-chosen microbial consortia and specific organisms that make up the consortia can adapt more quickly to a variety of conditions, creating effective associations from the point of view of agrochemistry, for example, biofilms and microbial mats [29]. The use of such consortia has a number of advantages compared to inoculation of a single strain, since they will have greater survival and combine the properties of biofertilizers, fungicides, biostimulators of growth, etc., will improve the quality of agricultural products and soil conditions, i.e. will ensure the proper level of fertility. Thanks to the use of biological products, the chemical pressure on phytocenoses and on the ecosystem as a whole is reduced, since such an approach in agriculture involves reducing the use of chemical plant protection products. Examples of biological products currently used in the Russian Federation are shown in Table 1.

Table 1. Examples of microbiological products approved for use in the Russian Federation [30].

Name of the microbiological product and the state registration number	Direction of action of the biological product	The effect of the biological product	Bacteria included in the biological product
Agrinos 1 ("Agrinos Inc", Norway) 344-19-933-1	Complex biological product	Nitrogen fixation, nitrification, ammonification, stimulation of plant growth and development of beneficial microflora, mobilization of P, K, Ca, Zn	*Azotobacter vinelandii, Clostridium pasteurianum,* et al (27 strains)
Bacterial fertilizer Azotovit ("Industrial Innovations", Russia) 461-19-1666-1	Biological product with 1 bacteria	Nitrogen fixation, conversion of nitrogen into ammonium, nitrite and nitrate forms	*Beijerinckia fluminensis Bf 2806*
Aqua (FRAGARIA, Argentina) 472-19-1834-1	Biological product with 1 bacteria	Nitrogen fixation	*Bradyrhizobium japonicum*
BioAzFK (IP Kuznetsova Maria Vyacheslavovna, "BashInkom", Russia) 585-19-3467-1	Multifunctional biological product	Improvement of nitrogen, phosphorus and potassium nutrition with anti-stress, growth-accelerating, immunostimulating properties	*Azotobacter chroococcum*, *Bacillus megaterium*, *Bacillus mucilaginosus*
Italpollina microbio Coveron (Italpololina group, Italy) 484-19-2004-1	Complex biological product	Stimulating plant growth and stress resistance, fighting pathogens, improving plant nutrition	*Trichoderma atroviride MUCL45632*, *Glomus intra-radices BEG 72*, *Glomus mosseae BEG 234*, *Bacillus pumilus*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Paenibacillus polymyxa*, *Paenibacillus macerans*
Ligabact for soy ("CropBio SAS", Argentina) 755-19-3262-1	Multifunctional biological product	Nitrogen fixation, increased yield, control of phytopathogens	*Bradyrhizobium japonicum E109*, *Bradyrhizobium diazoefficiens USDA 110*, *Bradyrhizobium elkanii E123*
Biofertilizer Nitragin KM (OOO "R&D Center BIO", Russia) 232-19-200-1	Biological product with 1 bacteria	Stimulation of the root nodules formation with bacteria as intracellular symbionts capable of forming ammonium nitrogen due to fixation of atmospheric nitrogen	*Bradyrhizobium japonicum*
Microbial fertilizer Panoramix: Panoramix wheat ("KOPPERT RUS", Russia) 548-19-2205-1	Complex biological product	Seed protection, increasing the resistance of plants and seeds to pathogens, stimulating the growth of the root system, stimulating plant resistance to drought and other types of abiotic stress	*Bacillus megaterium*, *Bacillus amyloliquefaciens*, *Trichoderma asperellum*, *Rhizophagus intraradices*

Complex biological products are considered the most effective means of increasing soil fertility and agricultural product quality without harming the environment. However, not all microorganisms inoculated as a result of introducing a biological product into the soil are able to form an effective community with local biota. Microorganisms in the microbial consortia should have the ability to form colonies, as well as stably coexist with representatives of the rhizobiome of the host plant [28]. Cultures of such microorganisms must have good growth, high colonizing activity, produce biologicaly active substances, and can also form systemic plant resistance, play an important role in plant physiological processes (for example, fix nitrogen) and much more. No less important is also the high adaptive ability and competitiveness in microbiocenoses, due to low sensitivity to biocenotic factors; and long-term preservation of the stability of the phytoprotective effect will ensure the possibility of long-term use of the resulting product [31].

3 Approaches to the creation of a high-efficiency biological product

There are several approaches aimed at forming an effective microbial consortium in these environmental conditions [31]. One of them is a thorough analysis of the native microbial community of a wild plant closely related to the cultivated one, and based on this, the creation of a microbial consortium. This approach is quite effective because it takes into account the connections formed as a result of the evolution process between microorganisms in the community. The taxa that make up the microbiome play an important role in the adaptation process of inoculated consortia, and also carry the most important functional characteristics. Such microbiomes may also contain rare taxa that have one or more specific but vital functions, which should also be taken into account when designing a consortium. Thus, the creation of a simplified microbial consortium (simplified microbial consortium – SMC) based on native organisms is able to ensure high survival of inoculated drugs, but requires a detailed analysis of the microbial network of the rhizobiome of the plant [29].

The next approach in designing a synthetic microbial consortium is to use microbial consortia with mutually reinforcing or complementary functions. A deep functional understanding of microbial genomes is important here. Secondary metabolites produced by endophyte bacteria, for example, play an important role in the formation of biofilms, the process of colonization of the root zone of plants and in suppressing the vital activity of pathogens, as well as in the formation of systemic resistance of plants. A more accurate prediction of the genomic functions of microorganisms in relation to plant productivity, as well as in relation to survival and vital activity in various environmental conditions will lead to a more specific understanding of the composition of potential biological product [32].

Another approach to the formation of microbial consortia is based on the possibility of combining closely related microorganisms with different functional characteristics. For example, it is possible to increase the survival rate of a biological product and thereby the diversity of the community by combining species of the same genus in a biological product that are similar in functionality. This can enhance plant resistance to pathogens [32]. This approach is promising and practically significant, but requires more research for various types of microorganisms.

Activation of microbial-plant interaction is an important factor in increasing the productivity of agricultural soils, which is insufficiently used in agricultural production due to the unstable effectiveness of applied microbiological products [33]. Biological product can not only increase the yield of grain crops, but also reduce the incidence of fungal diseases. It can also be effective in the process of decomposition of post-harvest plant residues, which increases the biological activity of the soil and improves its water and air regimes, which in turn has a beneficial effect on crop yields [34].

Since the activity of biological products depends on soil and climatic conditions, it is difficult to unlock their potential without additional resources, including the introduction of organic and mineral fertilizers. The solution may be pre-sowing seed treatment with liquid biological product and biologicaly active substances. Through treatment with microbiological products, it is possible to increase the stress resistance of plants in drought conditions by increasing the absorption of moisture and nutrients [24]. For example, pre-sowing treatment of seeds with biological products containing rhizospheric bacteria improved plant nutrition in the early stages of development, and adding a biological product to a conventional fertilizing can also help increase yields. [21].

When using microbiological products to restore and increase soil fertility, the humus content in the soil, its agrochemical properties, biological activity, soil structure and density increase, the supply of water and nutrients that can be absorbed is optimized, microbiological processes improve and soil fertility increases [35]. The advantage of biological product over pesticides and fertilizers also lies in the lower negative impact on the environment.

It is important to identify the strains that are most suitable under these conditions when creating a biological product. For the selection of microorganisms, significant factors are the rapid rate of biomass accumulation, preservation of viability under various temperature conditions, long storage periods, etc. [1]. The state of the microbial population of the rhizosphere of cultivated plants can be influenced by the weather conditions of the growing season, soil type, plant variety, and type of land use [23].

4 Conclusion

Thus, it became obvious that maintaining soil fertility, and consequently, high quality of agricultural products and crop yields, is possible only through the preservation of the diversity of the soil microbial community, in particular, the rhizobiome. The most effective and promising way to maintain the diversity of the microbiome of the rhizosphere of cultivated plants is the introduction of complex biological products containing hub species as components. These species are not always dominant and have beneficial properties in the community, but they are critically important for the diversity of the microbiome. They are able to form a consortium and ensure the diversity of the community by attracting microorganisms with beneficial properties into it. The use of such biological products will contribute to the preservation of the natural microbial diversity of the rhizosphere of cultivated plants and maintain soil fertility at the proper level.

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