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ENDOPHYTIC MICROORGANISMS IN FUNDAMENTAL RESEARCH AND AGRICULTURE

© E.N. Vasileva^{1,2}, G.A. Akhtemova², V.A. Zhukov², I.A. Tikhonovich^{1,2}¹ Saint Petersburg State University, Saint Petersburg, Russia;² All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg, Russia

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✿ The ubiquity of endophytic microorganisms is an accepted fact nowadays and the possibility of using it in agriculture keeps attracting attention of scientific community. In contrast to rhizospheric (living on root surface) and phyllospheric (colonizing aerial parts of plants) members of plant-microbial interactions endophytes are able to establish closer relationships with host-plant, in some cases strongly influencing its phenotype, bringing benefits. However, these microorganisms do not form any specific structures like nodules in case of symbiosis between legumes and rhizobium bacteria. Having a great amount of functions including phytohormone level modulation, vitamins production and nutrient supply improving, endophytes could serve as a basis for biofertilizer, which could potentially minimize the necessity of mineral fertilizers, thus reducing the negative impact of the latter on soil fertility, biodiversity and human health. Our main aim here is to highlight the question of functional significance of endophytes and endophytic bacteria in particular, as well as the way of its application in agriculture and to identify key points in understanding biology of these organisms. In this review we will consider such aspects of plant-endophytic symbiosis as biodiversity of legume and non-legume endophytes, ecology of endophytes and some ways which are commonly in use by studying these microorganisms.

✿ **Keywords:** agriculture; bacteria; endophytes; symbiosis; peas.

ЭНДОФИТНЫЕ МИКРООРГАНИЗМЫ В ФУНДАМЕНТАЛЬНЫХ ИССЛЕДОВАНИЯХ И СЕЛЬСКОМ ХОЗЯЙСТВЕ

© Е.Н. Васильева^{1,2}, Г.А. Ахтемова², В.А. Жуков², И.А. Тихонович^{1,2}¹ ФГБОУ ВО «Санкт-Петербургский государственный университет», Санкт-Петербург;² ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Санкт-ПетербургДля цитирования: Васильева Е.Н., Ахтемова Г.А., Жуков В.А., Тихонович И.А. Эндوفитные микроорганизмы в фундаментальных исследованиях и сельском хозяйстве // Экологическая генетика. – 2019. – Т. 17. – № 1. – С. 19–32. <https://doi.org/10.17816/ecogen17119-32>.

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✿ Повсеместное распространение эндوفитных микроорганизмов является общепризнанным фактом, а открывающиеся возможности использования их в сельском хозяйстве вызывают огромный интерес к ним со стороны научного сообщества. В отличие от ризосферных (населяющих поверхность корней) и филлосферных (колонирующих надземные органы) представителей растительно-микробного сообщества, эндوفиты способны вступать с хозяином в более тесные взаимоотношения, в некоторых случаях сильно влияя на его фенотип и в целом принося определенную пользу, не формируя, однако, специфических структур, таких как клубеньки, в случае бобово-ризобияльного симбиоза. Выполняя целый набор функций, среди которых модуляция уровней фитогормонов, продукция витаминов и улучшение снабжения питательными веществами, эндوفиты могут служить основой для биопрепаратов, что позволит в перспективе снизить необходимость использования минеральных удобрений в практике сельского хозяйства и вследствие этого негативное влияние последних на плодородие почв, биоразнообразие и здоровье человека. В этом обзоре рассмотрены такие аспекты растительно-эндوفитного симбиоза, как биоразнообразие эндوفитов бобовых и небобовых культур, экология данных микроорганизмов, вопросы их функциональной значимости, распространенные способы изучения, а также возможности их применения в сельском хозяйстве.

✿ **Ключевые слова:** сельское хозяйство; бактерии; эндوفиты; симбиоз; горох.

INTRODUCTION

The widespread distribution of microorganisms in the environment is well known, and the presence of close relationships between these organisms and

other inhabitants of the biosphere is of interest to researchers and encourages both the study of the fundamentals of symbiosis of microorganisms and higher plants, as well as the search for practical ap-

plications of such relationships. The possibility of using plant-bacterial interactions in the field of agriculture is particularly noteworthy, given that plants often serve as a target for pathogenic microorganisms, but more interestingly, they also participate in symbiosis with beneficial microflora, including bacteria and fungi that can stimulate the growth and development of a plant [1]. Understanding that microbial communities represented in the internal environment of the plant are not just indifferent “passengers” but play an important role in the development of a host plant and its resistance to different stresses has become one of the most exciting discoveries over the past decade [2].

Currently, the relationship between plants and rhizosphere microorganisms is the most studied [3–5]. Rhizosphere microorganisms consist of fungi of arbuscular mycorrhiza, capable of assimilating poorly soluble phosphates and other nutrients of the soil, nodule bacteria from the *Rhizobiaceae* family, and associative nitrogen fixers of the genera *Azospirillum*, *Azotobacter*, and *Klebsiella*, which can give an advantage to the host plant in the absence of bound nitrogen. In addition, beneficial bacteria of the PGPR group (Plant Growth Promoting Rhizobacteria) are also present in this broad group [6, 7]. The latter group of rhizosphere microorganisms is heterogeneous, and representatives are able to synthesize vitamins, phytohormones (auxins, cytokinins, and gibberellins), and other biologically active substances that can contribute to plant adaptation to external stress factors [8, 9].

However, in addition to rhizosphere bacteria colonizing the surface of the roots, as well as phyllospheric bacteria that inhabit the aerial parts of plants, there exists an entire community of endophytes, which are non-pathogenic microorganisms that live inside the plant and are able to coexist without harm and can bring certain benefits [1, 10]. Endophytic bacteria do not form specific anatomical structures like nodules and plant galls but are in closer relationships with plants than free-living microorganisms. Bacterial endophytes are able to increase the supply of nutrients, modulate hormone levels, and produce vitamins, thereby positively affecting the growth, development, and resistance of plants to stress [11]. This may be the reason for higher yield of plants inhabited by endophytes. The efficiency of endophytes has been revealed in the case of abiotic stresses, including drought [12], salinity [13, 14], excessive irrigation, low temperature [15], and the presence of toxic organic compounds and heavy met-

als in the soil [16]. In addition, some endophytes have the ability to convert nitrogen and phosphorus into forms that are easily digestible for plants [17, 18].

Using microorganisms isolated from the internal environment of plants, researchers have the opportunity to create highly efficient biological preparations, which are already widely used in agriculture [19–21]. Unlike mineral fertilizers, which are the most energy-intensive process in agricultural practices, the production of microbiological products is not as expensive. In addition, the use of microbiological products does not adversely affect human health, soil fertility, and/or biodiversity [22]. In some cases, the use of such biological preparations obviates the need for plants to be treated with pesticides [20].

This review highlights the role of endophytes in plant and microbial symbiosis, both in terms of basic research and in the case of practical applications. The biodiversity of endophytes of leguminous and non-leguminous plants is considered, as well as the pathways of microorganisms entering the plant, the colonization of internal tissues and the maintenance (persistence) of bacteria in them, various aspects of the functional significance of endophytic bacteria, and methods of using endophytes in agriculture.

DIVERSITY OF ENDOPHYTIC BACTERIA

The study of biodiversity of endophytic microorganisms is a relatively new field. However, based on previous studies, it can be argued that almost all plants contain endophytes, including cultivated and wild-growing, herbaceous, namely sugar beet, corn, sorghum, soy, wheat, rice; tree crops, namely oak, pear, poplar, spruce, and also sphagnum mosses [10, 23–30]. Endophytes have also been found in various parts of plants, and have been isolated from leaves, stems, roots, seeds of various plant species, and in some cases, fruits and flowers (i. e., grape plants from which *Pseudomonas* and *Bacillus* were isolated) [1, 31, 32]. Previous studies of the biodiversity of endophytic bacteria were based on the characterization of isolates obtained from the internal tissues of surface-sterilized plants [33, 34]. However, currently, the study of the taxonomic diversity of bacteria, based on the methods of high-throughput, next-generation sequencing (NGS), is gaining increasing importance, which, unlike the traditional methods of cultivation, biochemical tests, and microscopy, provides information not only of the cultivated but also of the uncultivated taxa (Table 1).

Bacterial communities of various organs largely overlap. Thus, endophytes of the root zone, as a rule, are represented by bacteria from the phylum *Proteobacteria* (approximately 50% of the community), *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* (10% of the community each). Representatives of other phyla, whose appearance in a role of endophyte was often unexpected, were also found in the root endosphere, although their share in the community was much smaller. Such microorganisms include bacteria from the phyla of *Cyanobacteria*, *Chloroflexi*, *Verrucomicrobiae*, *Planctomycetes*, *Fusobacteria*, and *Nitrospirae* [35, 36]. In addition, it was possible to establish, in the internal tissues of the root, the presence of archaea and bacteria of the phylum *Acidobacteria*, although their numbers were significantly lower than that of the above-mentioned representatives (about 1%) [35].

Since one method of endophytic colonization is the penetration of bacteria into the plant root and their further spread through xylem vessels or intercellular spaces [37, 38], it is not surprising that *Proteobacteria*, *Firmicutes*, and *Actinobacteria* are the dominant phyla in stems and leaves. In particular, this has been demonstrated on beans (*Phaseolus vulgaris*) [39].

It is known that plants have the ability to provide for themselves a specific microbiome. That is, to selectively allow only certain necessary microorganisms into their endosphere [1, 11]. Recent studies of root endospheres have shown that only a few bacterial groups dominate in this environment, which supports the above statement. This

has been demonstrated on potato plants [40] and rice [35, 41, 42]. In both cases, *Enterobacter*, *Pseudomonas*, and *Stenotrophomonas* from the *Gammaproteobacteria* phylum dominated the bacterial community, and in rice they accounted for up to 98% of observed OTUs (operational taxonomic units, or the groups of microorganisms characterized by similarity of diagnostic fragments used in molecular-genetic analysis). Sometimes, only one of the most-represented groups was found in the root. For example, in the case of *Pseudomonas*-type bacteria in the roots of poplar *Populus deltoids* [43], or in wheat germs and arabidopsis, the typical dominant family is *Streptomyetaceae* from the *Actinobacteria* phylum [2, 44, 45].

ENDOPHYTIC BACTERIA OF LEGUMINOUS CROPS

Bacteria belonging to various taxa, including the genera *Aerobacter*, *Aeromonas*, *Agrobacterium*, *Bacillus*, *Chryseomonas*, *Curtobacterium*, *Enterobacter*, *Erwinia*, *Flavimonas*, *Pseudomonas*, *Sphingomonas*, and *Rhizobium* [24, 31, 46–50] were possible to isolate from roots and nodules of leguminous crops. In the work of López-López et al. (2010), 99 bacterial isolates from beans (*Phaseolus vulgaris*) were identified. All of these were classified as *Firmicutes*, *Actinobacteria*, and/or *Proteobacteria* phyla, and approximately 50 species were identified. The phylum *Firmicutes* was most diverse. In addition to the well-known species from the genera *Enterococcus*, *Nocardioides*, *Roseomonas*, *Leptothrix*, *Cohnella*, *Rhizobium*, *Phyllobacterium*, *Microbacterium*, *Janibacter*, *Knoellia*, *Macrocooccus*,

Table 1

Diversity of endophytes from non-legume plants

Plant	Endophytes	Source
Cannabis (<i>Cannabis sativa</i>)	<i>Achromobacter</i> , <i>Pseudomonas</i> , <i>Alcaligenes</i> , <i>Enterobacter</i> , <i>Acinetobacter</i> , and <i>Bacillus</i>	[61]
Grape (<i>Vitis vinifera</i> L.)	<i>Pseudomonas</i> , <i>Bacillus</i>	[1, 31, 32]
Potato (<i>Solanum tuberosum</i>)	<i>Enterobacter</i> , <i>Pseudomonas</i> , and <i>Stenotrophomonas</i>	[40]
Rice (<i>Oryza sativa</i>)	<i>Enterobacter</i> , <i>Pseudomonas</i> , and <i>Stenotrophomonas</i>	[35, 41, 42]
Poplar (<i>Populus deltoids</i>)	<i>Pseudomonas</i>	[43]
Wheat (<i>Triticum sp.</i>)	<i>Streptomyetaceae</i>	[2, 44]
Arabidopsis (<i>Arabidopsis thaliana</i>)	<i>Streptomyetaceae</i>	[2, 45]

Brachy bacterium, and *Streptomyces*, new types of genera *Acinetobacter*, *Bacillus*, *Enterococcus*, *Nocardioidea*, *Paracoccus*, *Phyllobacterium*, and *Sphingomonas* were observed [51].

The pea is a representative of the legume family, a model organism, and an important crop. It has also been studied for its endophytic community. In 2013, a study was conducted in which 75 bacterial isolates were isolated from surface-sterilized roots and nodules. Most of them (approximately 67%) belonged to gram-positive bacteria, of which 70% and 90% of the nodule and root isolates, respectively, were spore-forming and were attributed to the genus *Bacillus* [31]. Particular attention should be given to the fact that, in addition to bacteria of the genus *Rhizobium*, various non-rhizobial bacteria can also be widely represented in the nodules of legumes (e. g., *Pantoea*, *Escherichia*, *Bosea*, *Phyllobacterium*, *Sphingomonas*, *Pseudomonas*, *Agromyces*, *Microbacterium*, *Paenibacillus*, *Aerobacter*, *Agrobacterium*, *Chryseomonas*, *Curtobacterium*, *Erwinia*, *Flavimonas*, *Sphingomonas*, *Methylobacterium*, *Blastobacter*, *Devosia*, *Rhodopseudomonas*, *Paracraurococcus*, *Phyllobacterium*, *Ochrobactrum*, *Cupriavidus*, *Herbaspirillum*, *Pseudomonas*, *Enterobacter*, *Leclercia*, *Ochrobactrum*, *Starkeya*, *Azotobacter*, *Azospirillum*, *Ornithinococcus*, *Bacillus*, and also *Serratia*), although they do not belong to typical representatives of the nodule microflora [52–55]. Due to the possibility of using endophytes in agriculture, studies were conducted to determine the identification of plant growth promoting rhizobacteria (PGPB) in pea

plants. By analyzing the 16S rRNA gene sequence, it was possible to establish the taxonomic identity of the isolated organisms, namely *Ochrobactrum* and *Enterobacter* [56]. In addition, a large number of members of the genus *Micromonospora* were recently found in the roots of the garden pea (*M. aurantiaca*, *M. auratinigra*, *M. chaiyapumensis*, *M. chersina*, *M. coerulea*, *M. coriariae*, *M. coxensis*, *M. fulvoviridis*, *M. lupini*, *M. matsumotoense*, *M. pattaloongensis*, *M. saelicesensis*, *M. sagramiensis*, *M. Siamensis*, “*Micromonospora zae*”, “*Micromonospora jinlongensis*”, and *Micromonospora zamorensis*) [57, 58]. Initially, *Actinobacteria* of this genus were detected in the internal tissues of the legume *Lupinus angustifolius* [57].

Other legumes have also been studied to determine their endophytic community composition. Chickpea root bacterial endophytes (*Cicer arietinum* L.), grown in saline soils, were represented by 40 observed isolates. Moreover, some of these significantly improved the general condition of the host plant under conditions of high salt content, while protecting it from the pathogenic fungus *Fusarium solani*. Such useful endophytes included *Bacillus cereus*, *Achromobacter xylosoxidans*, *Bacillus thuringiensis* и, and *Bacillus subtilis* [14]. A model organism for genetic experiments, *Medicago truncatula*, is no exception to the general rule and may contain endophytic bacteria. In particular, human pathogens *Klebsiella pneumonia* and *Salmonella enterica ser. Typhimurium*, in the role of PGPB, may be the inhabitants of the endosphere of this plant. [59].

Table 2

Diversity of endophytes from legume plants

Plant	Endophytes	Source
Mung bean (<i>Vigna radiata</i> L.)	<i>Bacillus</i> , <i>Agrobacterium</i> , <i>Bradyrhizobium</i>	[54]
Clover (<i>Trifolium pretense</i> L.)	<i>Agrobacterium</i> , <i>Bacillus</i> , <i>Bortedella</i> , <i>Comamonas</i> , <i>Curtobacterium</i> , <i>Enterobacter</i> , <i>Methylobacterium</i> , <i>Pantoea</i> , <i>Pasteurella</i> , <i>Pseudomonas</i> , <i>Rhizobium</i> , <i>Xanthomonas</i>	[52]
East Asian arrowroot (<i>Pueraria thunbergiana</i>)	<i>Sinorhizobium</i> , <i>Mesorhizobium</i> , <i>Bacillus</i> , <i>Serratia</i> , <i>Enterobacter</i> , <i>Pantoea</i>	[53]
Common bean (<i>Phaseolus vulgaris</i>)	<i>Enterococcus</i> , <i>Nocardioidea</i> , <i>Roseomonas</i> , <i>Leptothrix</i> , <i>Cohnella</i> , <i>Rhizobium</i> , <i>Phyllobacterium</i> , <i>Microbacterium</i> , <i>Janibacter</i> , <i>Knoellia</i> , <i>Macrocooccus</i> , <i>Brachy bacterium</i> , <i>Streptomyces</i> , <i>Acinetobacter</i> , <i>Bacillus</i> , <i>Enterococcus</i> , <i>Nocardioidea</i> , <i>Paracoccus</i> , <i>Phyllobacterium</i> , and <i>Sphingomonas</i>	[51]
Pea (<i>Pisum sativum</i> L.)	<i>Bacillus</i> , <i>Micromonospora</i> , <i>Ochrobactrum</i> , <i>Enterobacter</i> , <i>Pantoea</i> , <i>Pseudomonas</i> , <i>Serratia</i>	[48, 54, 55, 57, 58]

Table 2 (continued)

Plant	Endophytes	Source
Lupine (<i>Lupinus angustifolius</i>)	<i>Micromonospora</i>	[58]
Chickpea (<i>Cicer arietinum</i> L.)	<i>Bacillus cereus</i> , <i>Achromobacter xylosoxidans</i> , <i>Bacillus thuringiensis</i> , and <i>Bacillus subtilis</i>	[14]
Alfalfa (<i>Medicago truncatula</i>)	<i>Bacillus cereus</i> , <i>Achromobacter xylosoxidans</i> , <i>Bacillus thuringiensis</i> , and <i>Bacillus subtilis</i>	[59]
Faba bean (<i>Vicia faba</i>)	<i>Rahnella</i> , <i>Stenotrophomonas</i> , and <i>Enterobacter</i>	[60]

Several species of bacteria from the genus *Pseudomonas*, as well as the genera *Rahnella*, *Stenotrophomonas*, and *Enterobacter*, were found in the nodule community of common beans (*Vicia faba*), which may confirm that nodules of legume bacteria may not be inhabited only by rhizobial nodule-forming bacteria [60].

The aboveground organs of leguminous plants, stems, and leaves, are studied less often. In particular, using the fatty acid profiles, it was possible to establish that the most widespread endophytes of the stems and leaves of garden pea are bacteria from the genera *Pantoea*, *Pseudomonas*, and *Bacillus* [48] (Table 2).

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA

Methods for determining the localization of endophytic bacteria in plant tissues can be divided into quantitative and qualitative. In the case of quantitative methods, the researcher has the opportunity to accurately determine the number of bacterial cells in the plant. Usually, a qPCR method (quantitative polymerase chain reaction, or real-time PCR, which is based on amplification and measurement of the number of DNA molecules in real time after each amplification cycle) is used for this, which enables the determination of numbers within a cell [62]. Sometimes, quantitative methods include *de novo* isolation, but in this case it is necessary to distinguish inoculated strains from the rest of the microbiome (e. g., by selecting for previously established antibiotic resistance) [63].

There are also bioengineering approaches based on visualization of bacteria (i. e., qualitative methods). Microorganisms containing a plasmid with a reporter gene are investigated. In particular, GFP (Green Fluorescent Protein), RFP (Red Fluorescent Protein), and GUS-tagged cells are used [37, 64]. However, the use of GFP as a signaling molecule is

limited due to the presence of autofluorescence in plant tissues. In addition, work with immunomarker-labeled bacteria can be performed, as well as fluorescent *in situ* hybridization in combination with laser scanning confocal microscopy. In the latter case, labeled oligonucleotide probes are used based on a 16S rRNA gene sequence, so it is possible to detect rRNA in morphologically intact cells [65]. Visualization methods, to some extent, can be attributed to quantitative, as in each field of view, the number of cells of the strain of interest can be counted. Another simple, fast method for qualitative evaluation is classical PCR. With PCR, setting up controls in order to avoid false-positive results is necessary, and quantitative assessment in this case is impossible [62].

Immunological instruments can also be used to localize and count endophytes. These methods imply a qualitative and/or quantitative assessment of interactions of the antigen-antibody type. The signal molecule, which can be a fluorochrome, interacts with the antibody, which makes visualization possible [65].

Traditional methods of cultivation with corresponding biochemical tests and microscopy, previously widely used to study taxonomic diversity, have given way to NGS methods. New technologies enable identification of both cultivated and uncultivated groups of microorganisms, as well as characterization of not only individual representatives of the internal community, but the entire plant microbiome. This has opened up new opportunities for the study of relationships between host plants and bacterial symbionts and between microorganisms of the plant endosphere, which is necessary when studying plant and microbial symbiosis.

ECOLOGY OF ENDOPHYTIC MICROORGANISMS

Endophytic microorganisms are widespread, found in stems, leaves, roots, and seeds of various plant species [1, 31]. The presence of bacteria in

the tissues of generative organs, such as flowers and fruits, has also been reported, although this phenomenon was observed relatively rarely, and the amount of endophytes observed was small [23, 32]. Microorganisms provide many benefits to their host plants by providing vital functions. For bacteria, the plant serves as a kind of reservoir or nutrient medium. The plant, in turn, is able to select the most effective complexes of microorganisms, providing its individual microbiome [1, 11].

Bacterial endophytes usually inhabit the intercellular space of plant tissues, and it was previously assumed that their distribution is possible only through intercellular spaces [23, 37, 66]. However, it was later shown that some endophytes are able to move through the xylem lumen, which is a characteristic method for spreading pathogens. The transition is made from one xylem element to another through the holes of perforation plates [37].

Bacteria that inhabit plant tissues can colonize the *de novo* endosphere from the environment or can be transferred from seed to seed [13, 67, 68]. The mode of transfer is influenced by the ecological and evolutionary aspects of the relationship, as the path of vertical transmission ensures the transfer of a useful symbiont from generation to generation [69]. This variant is common in cases where the bacterial symbiont is responsible for an important function [70].

Most bacterial endophytes are likely to be transmitted horizontally. This is demonstrated by the diversity of bacteria in seeds and seedlings grown under field conditions, which is usually wider than the diversity of endophytes in plants grown under sterile conditions. This indicates that most endophytes come from the environment [68, 71]. In addition, bacterial endophytes often do not have high specificity to the host plant [19, 72, 73], and microorganisms that infect many plant species may spread horizontally between them and are unlikely to be inherited strictly vertically.

Many believe that most endophytic bacteria penetrate plants through the root system and then spread throughout the plant via a stream of water and nutrients [37, 74]. However, there are opinions that the colonization of the aboveground portion of plants can occur through airborne transmission via stomata and cortical pores [11, 75–77].

Bacterial cells primarily colonize the rhizosphere of a plant, “recognizing” substances of root exudates. Communicating with signaling molecules, plants can attract mutualists and limit the penetra-

tion of pathogenic microorganisms [8]. For this, plants can use salicylic and jasmonic acids, as well as ethylene [59, 78]. Many bacteria do not stop at the rhizosphere and rhizoplane but penetrate inside the plant through cracks (passively) or actively, causing positive effects for the host plant. In passive penetration, bacteria form biofilms [16]. With active penetration, the plant reacts to endophytic microorganisms (e. g., by strengthening cell walls and resin extraction). However, the action of these mechanisms in response to endophytic colonization is less pronounced than in the case of an attack of pathogens [19, 78, 79].

As described above, not only vegetative, but also generative organs of plants can undergo endophytic colonization. In particular, this phenomenon has been described in grapes. Namely, bacteria of the genera *Pseudomonas* and *Bacillus* were isolated from the fruits, flowers, and seeds of this plant [32]. In some cases, flowers and fruits of plants have been inhabited by unique species of endophytes that are not found in the roots, which suggest that endophytes are present in almost all plant organs [1, 37, 80]. Reports on the presence of endophytes on the surface and inside the pollen of various plant species are extremely interesting [81–84]. Moreover, experimental data reported by Madmony et al. indicate that endophytes isolated from pollen originated from the parent plant (i. e., vertical inheritance).

FUNCTIONS OF ENDOPHYTIC BACTERIA

It is thought that the associations of bacteria with plants could arise and be a result of a positive selection in favor of endophytes [85]. This implies the existence of mutually-beneficial cooperation, and, indeed, in the study of the functional activity of endophytic strains, it was found that they have a positive effect on the growth and development of the plant and can improve nutrient supply. Their presence has a positive effect on resistance to various stresses, and in addition, during the long-term co-evolution of plants and endophytes, the latter acquired the ability to synthesize chemical compounds originally produced by the host plant [11, 86]. In this regard, the fact that under stress conditions, the frequency of infection with endophytes increases deserves special attention [17].

The ability of endophytic microorganisms to produce vitamins and phytohormones explains why plants inhabited by endophytes are generally more resistant to diseases and produce high yields. For example, the endophytes *Rahnella aquatilis* and

Pseudomonas putida, which are able to synthesize indoleacetic acid, have a positive effect on the growth and development of some cereals and radishes [87]. The endophyte *Bacillus subtilis*, which produces gibberellins, has a positive effect on plants [88].

The effect of stress resistance due to the presence of endophytes in plant tissues is of particular interest. It has been reported that some microorganisms are able to increase their tolerance to stresses caused by drought, excessive irrigation, salinity, presence of heavy metals, toxic organic compounds, and pathogens due to modulation of ethylene levels. Ethylene is a stress hormone responsible for many processes. Its biosynthesis is tightly regulated by a number of biotic and abiotic factors. Some endophytic bacteria produce a certain enzyme (1-aminocyclopropane-1-carboxylate-deaminase) that causes degradation of the ethylene precursor, thereby reducing its content in the plant, as a result of which the influence of many stresses decreases [16].

The fact that phytopathogens and endophytic bacteria occupy similar ecological niches cannot be ignored, as this indicates the existence of competition between these organisms and the possible place of endophytes in biocontrol [80]. Many endophytes are able to control pathogen numbers, including nematodes and insects [89, 90].

Endophytic bacteria can also produce antibiotics. For example, most bacteria from the genus *Bacillus* synthesize compounds such as circulin, colistin, and polymyxin, which suppress the growth of gram-positive and gram-negative bacteria, as well as many pathogenic fungi [91]. In addition, almost all bacteria are capable of producing bacteriocins, specific proteins that suppress the activity of cells of other strains of the same species or related species of bacteria [92].

Representatives of the endophytic community often have capabilities of synthesis of antifungal metabolites. In particular, the bacterium *Pseudomonas viridiflava*, commonly inhabitant of the aboveground portion of herbaceous plants, produces ecomycin that can act against human pathogens, such as *Cryptococcus neoformans* and *Candida albicans*. The pseudomycin produced by endophytes is effective against *Ceratocystis ulmi* and *Mycosphaerella fijiensis* [93]. Antifungal activity of endophytes of peas and beans, with respect to *Bipolaris sorokiniana* and *Fusarium oxysporum*, has also been reported [94].

There is evidence that endophytes are capable of producing siderophores (low molecular weight substances that chelate Fe^{3+} ions) and vitamins, the

presence of which can enhance plant immunity and resistance to pathogens [90, 95–97].

There are endophytes that produce immunosuppressants, anti-tumor, and antiviral compounds [90], regulate osmotic pressure, stomata work, and modify the development of the root system [19], triggering an induced system of resistance of plants [98]. In addition, we must not forget about one of the most basic functions of endophytic microorganisms, namely providing the host with nutrients, such as nitrogen and phosphorus, through their conversion into easily digestible forms [17].

ENDOPHYTIC MICROORGANISMS IN AGRICULTURE

In traditional agriculture practices, the most common, but also the most energy-intensive process, is the production of mineral fertilizers, which, when used in high doses, have a negative impact on human health, soil fertility, and biodiversity [22]. Microbiological preparations used in the practice of eco-oriented farming, which involves the use of well-established symbiotic bonds, represent a good alternative to chemical fertilizers. The possibility of using microorganisms that inhabit the internal tissues of plants for the production of highly-effective biological preparations makes this topic increasingly attractive for research. There are studies demonstrating the higher efficiency of this type of fertilizer compared to mineral fertilizers. In particular, studies on *Bacillus subtilis* strains obtained from hogweed, have shown that productivity of spring barley (*Hordeum vulgare* L.) was higher than with the use of mineral fertilizers [21]. A number of microbial preparations based on the bacteria *Azospirillum*, *Pseudomonas*, *Bacillus*, *Herbaspirillum*, and *Acetobacter* have been developed [19, 20]. Their use often enables avoiding the need for treatment with pesticides [20]. In experiments, Garipovaya et al. demonstrated the feasibility of bacterial treatments, given the disease manifestations in beans treated with *Bacillus subtilis* and *Rhizobium leguminosarum* were significantly reduced. Inoculation also increased plant mass, the number of beans and seeds, and the mass of seeds from a single plant [99]. The role of endophytes in the field of soil bioremediation and basic research on plant and microbial interactions cannot be underestimated [4, 7, 80, 100–103].

Plants growing on soils contaminated with xenobiotics usually contain microorganisms that are not only resistant to such compounds, but also capable of degrading them [104]. Endophytic bacteria from white poplar trees growing on a site contaminated

with toluene increased the effect of phytoremediation of volatile organic substances and herbicides [105], while bacteria from the genera *Achromobacter*, *Pseudomonas*, *Alcaligenes*, *Enterobacter*, *Acinetobacter*, and *Bacillus*, isolated from *Cannabis sativa*, had the ability to degrade phenol and benzene [61]. A particularly interesting and important fact in the field of bioremediation is the ability of these endophytic “utilizers” to degrade xenobiotics inside the plant, thereby reducing the phytotoxic effect [90].

However, it is important to keep in mind that the internal environment of the plant is inhabited by many different endophytes. A study of plant microbiomes has shown how great the contribution of microorganisms is to the phenotype and physiological characteristics of the host. Currently, properties of endophytic microorganisms are widely known as growth-promoting activity, for nutrient supply (especially supply of nitrogen and phosphorus), increases in resistance to various stresses, and modulation of hormone levels.

It is not surprising that topic of microbial community manipulation is becoming increasingly relevant in the field of agriculture. However, the so-called MAP (microbiome-associated phenotype) was based on taxonomy, rather than on existing traits [106, 107]. For this reason, for a long time, the transition from fundamental knowledge to practical application, namely the development of effective communities and manipulation of the microbiome, caused considerable difficulties. This is evidenced by the repeated ineffective attempts to use one strain as a bio-fertilizer in various climatic and geographical conditions [107]. However, in 2018, researchers proposed a new concept of a “modular microbiome,” which is a microbial consortium developed in accordance with the plant genotype, which imparts different, but complementary, MAPs to a separate host plant or entire population [106]. Since, in fact, the relative importance of the microbiome for plant growth, development, and health has not been studied experimentally for most types of crops, new MAPs-first approach is highly interesting, as it implies the choice of consortia and implements a specific MAP based on mathematical models [106]. Data obtained from such studies will form the necessary foundation for experiments, which will enable researchers to select strategies for the development of synthetic endophytic communities.

CONCLUSION

The widespread distribution of endophytes is well known and occurred as a result of long-term

co-evolution and selection in favor of these microorganisms. Most bacterial endophytes enter into close relationships with their host plant and provide significant advantages, such as producing a number of biologically active substances that can serve as growth stimulants. They are able to improve the immune status of plants, increase the stress-tolerance, and provide protection against diseases by competing with phytopathogens. Endophytic bacteria are active participants in multi-component symbiotic systems, interacting not only with the plant, but also with other microorganisms in the community. It is not surprising that these organisms attract more and more attention from the point of view of not only basic research of plant and microbial interactions, but also practical applications in agriculture. Growing interest is evidenced by improvement of research methods and new proposals on methods of studying not only specific representatives of one or another part of the community, but also the multi-component network as a whole. Integration of various approaches will allow for a deeper understanding of the interaction of microorganisms and plants, which will be the foundation for more promising studies and strategies for using the data obtained in practice.

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☞ Information about the authors

Ekaterina N. Vasileva — Technician, Laboratory of Genetics of Plant-Microbe Interactions, All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia; Student, Faculty of Biology, Department of Microbiology, Saint Petersburg State University, St. Petersburg, Russia. E-mail: evasilieva@arriam.ru.

Gulnar A. Akhtemova — PhD, Senior Scientist, Laboratory of Genetics of Plant-Microbe Interactions. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: ahgulya@yandex.ru.

Vladimir A. Zhukov — PhD, Head of the Lab, Laboratory of Genetics of Plant-Microbe Interactions. All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia. E-mail: vzhukov@arriam.ru.

Igor A. Tikhonovich — Sc.D., Professor PI, Academician of RAS, All-Russian Research Institute for Agricultural Microbiology, Pushkin, St. Petersburg, Russia; Dean of the Faculty, Faculty of Biology, Saint Petersburg State University, St. Petersburg, Russia. E-mail: arriam2008@yandex.ru.

☞ Информация об авторах

Екатерина Николаевна Васильева — техник I-й категории, лаборатория генетики растительно-микробных взаимодействий, ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург; студент, кафедра микробиологии, биологический факультет, ФГБУ ВПО «Санкт-Петербургский государственный университет», Санкт-Петербург. E-mail: evasilieva@arriam.ru.

Гульнар Асановна Ахтемова — канд. биол. наук, старший научный сотрудник, лаборатория генетики растительно-микробных взаимодействий. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: ahgulya@yandex.ru.

Владимир Александрович Жуков — канд. биол. наук, заведующий лабораторией, лаборатория генетики растительно-микробных взаимодействий. ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург. E-mail: vzhukov@arriam.ru.

Игорь Анатольевич Тихонович — д-р биол. наук, научный руководитель института, академик РАН, ФГБНУ «Всероссийский научно-исследовательский институт сельскохозяйственной микробиологии», Пушкин, Санкт-Петербург; декан, биологический факультет, ФГБУ ВПО «Санкт-Петербургский государственный университет», Санкт-Петербург. E-mail: arriam2008@yandex.ru.