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КЛУБЕНЬКОВЫЕ АЗОТФИКСИРУЮЩИЕ БАКТЕРИИ

NODULE NITROGEN-FIXING BACTERIA

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ANNOTATION:
This research paper is devoted to the study of the history of discovery, morphology and physiology of nodule bacteria. In the theoretical part, the significance of nodule bacteria and their types, properties, and functions performed by themare considered.

Keywords: atmospheric nitrogen, ammonium salts, symbiosis, legumes, bacteroids, root nodules, nitrogen fixation, polymorphism, microaerophiles, cultivation, inoculation, infectious thread, free-living microorganisms.

Objective: to study the origin, physiology, and application of nodule nitrogen-fixing bacteria.

Nitrogen as an element is necessary for all living organisms to exist: it is part of almost all biological macromolecules. However, the first link in all food chains – plants-are not able to bind molecular nitrogen from the air. To do this, there is a whole group of very diverse bacteria that can convert molecular nitrogen (N2) into biologically available (ammonium compounds).

The importance of the existence of nitrogen-fixing bacteria on Earth is very difficult to overestimate. They belong to different taxonomic groups, but they are united precisely on the basis of the ability to absorb atmospheric nitrogen and convert it to the ammonium form.

Opening history

The problem of biological nitrogen arose with the development of agricultural culture. It has long been known from the practical agronomic activity of humans that legumes increase soil fertility. Back in the third and first centuries BC, the Greek

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philosopher Theophrastus and the Romans Cato, Varro, Pliny and Virgil wrote about this.

The first scientific explanation of the ability of legumes to store nitrogen belongs to the French agrochemist J. R. R. Tolkien. Bussengo (1838). He found that alfalfa and clover enrich the soil with nitrogen, while cereals and root vegetables deplete it. These facts he associated with the ability of legumes to fix nitrogen from the air. However, Bussengo mistakenly imagined that the fixing agent is the leaves of a legume plant. It was this incorrect conclusion that led Boussengo to deny his discovery 15 years later.

In an effort to prove more strongly the correctness of his idea, Bussengo conducted a series of experiments. In contrast to his earlier experiments, he no longer grew sprouts of legumes transplanted from the field on calcined sand, but carefully washed seeds (lupines and beans); vessels with plants were placed under a glass cap. Under such conditions, the plants were not enriched with" air nitrogen". Now it is clear and easily explained.

It was the thoroughness of the experiment, which excluded the possibility of infection of the root system of seedlings with nodule bacteria, that led Bussengo to the results that refuted his previous data. СчитаяNevertheless, considering the latest experiments more reliable and not even assuming the existence of nodule bacteria, he recognized the resultsof previous experiments as erroneous and disavowed them.

It took several decades before it was possible to establish that legumes fix molecular nitrogen only in symbiosis with microorganisms that cause nodule formation on their roots.

Russian naturalists of the 19th century M. S. Voronin, P. S. Kossovich, and K. A. Timiryazev made a great contribution to the discovery of the causes of nitrogen enrichment in the soil during the cultivation of legumes. Agronomic and microbiological studies conducted in the same years by a number of foreign authors also contributedto establishing this truth.

Currently, more than 200 different species of other plants are also known, for which the ability to fix nitrogen in symbiosis with microorganisms that form nodules on the root system or on their leaves is fully proven. Most of them belong to trees and shrubs.

About 80 years ago, S. N. Vinogradsky isolated the anaerobic bacterium Clostridiumpasteurianum from the soil, which fixes nitrogen gas. Somewhat later, Dutch researcher M. Beyerink discovered the aerobic nitrogen-fixing bacterium Azotobacter.

In the future, the list of nitrogen fixers was supplemented with new types of microorganisms-representatives of other systematic groups.

The activity of all free-living nitrogen-fixing bacteria in the soil is limited by a lack of organic substances. Therefore, they cannot provide significant nitrogen accumulation (on average, they accumulate no more than 5 kg of nitrogen per 1 ha). Their activity can be activated by adding fresh organic matter.

The full use of both symbiotic and free-living nitrogen-fixing microorganisms in agriculture is possible only with a comprehensive knowledge of the factors that determine the fixation of molecular nitrogen, and the establishment of ways to intensify this process. There are two possible ways to intensify the accumulation of biological nitrogen — expansion of legume crops and creation of agrotechnical conditions that ensure the maximum nitrogen-fixing activity of symbiotic and free-living nitrogen fixers.

To successfully solve the problem of intensifying the accumulation of biological nitrogen, it is necessary to increase the acreage for legumes, conduct in-depth research to study the issues of effective nitrogen fixation by symbiotic and free-living microorganisms.

Nodule bacteria of legumes

Paleontological data indicate that the oldest legume crops with nodules were some plants belonging to the Eucaesalpinioideae groupEucaesalpinioideae.

In modern legume species, nodules are found on the roots of many members of the family Papilijilijaeae.

Phylogenetically more primitive members of families such as Caesalpiniaceae MimosaceaeCaesalpiniaceae Mimosaceae do not form nodules in most cases.

Out of 13,000 species (550 genera) of legumes, the presence of nodules has so far been detected only in approximately 1,300 species (243 genera). This primarily includes plant species used in agriculture (more than 200).

By forming nodules, legumes acquire the ability to absorb atmospheric nitrogen. However, they are also able to feed on bound forms of nitrogen-ammonium and nitric acid salts. Only one plant, Hedysarumcoronarium, Hedysarumcoronariumassimilates only molecular nitrogen. Therefore, without nodules in nature, this plant is not found.

Nodule bacteria supply the legume with nitrogen, which is fixed from the air. Plants, in turn, supply bacteria with carbohydrate metabolism products and mineral salts, which they need for growth and development.

In 1866, the famous botanist and soil scientist M. S. Voronin saw the smallest "corpuscles"in the nodules on the roots of legumes. Voronin put forward bold assumptions for that time: he connected the formation of nodules with the activity of bacteria, and the increased division of root tissue cells with the plant's reaction to bacteria that had penetrated the root.

20 years later, Dutch scientist Beyerink isolated bacteria from the nodules of peas, vetch, chyna, beans, seradella and лядвенцаliadwentz and studied their properties, testing their ability to infect plants and cause nodule formation. He named these microorganisms Bacillus Radicicola. Since the genus Bacillus includes bacteria that form spores, and nodule bacteria lack this ability, A. Prazhmovsky renamed them Bacteriumradiicola. B. Frank proposed a more successful generic name for nodule bacteria — Rhizobium (from the Greek rhizo-root, bio-life; life on the roots). This name was instilled and is still used in the literature.

Morphology and physiology

Nodule bacteria are characterized by a striking variety of forms-polymorphism. This was noticed by many researchers when studying nodule bacteria in pure culture in laboratory conditions and soil. Nodule bacteria can be rod-shaped or oval. Among these bacteria, there are also filterable forms, L-forms, coccoid immobile and motile organisms.

Young nodule bacteria in pure culture on nutrient media usually have a rod—shaped shape, the size of the rods is approximately 0.5—0.9 X 1.2-3.0 microns, mobile, multiply by division. In rod-shaped cells of nodule bacteria of clover, division by lacing is observed. With age, rod-shaped cells can pass to budding. According to Gram, the cells are negatively colored, and their ultra-thin structure is typical for gram-negative bacteria.

With aging, nodule bacteria lose their mobility and go into a state of so-called shingled rods. They received this name due to the alternation of dense and loose sections of protoplasm in cells. Probably, with age, the bacterial cell is filled with fat inclusions that do not perceive color and, as a result, cause striation of the cell. The stage of" girdled rods " precedes the stage of formation of bacteroids-cells of irregular shape: thickened, branched, spherical, pear-shaped and bulb-shaped. The

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term "bacteroids" was introduced into the literature by J. Brunhorst in 1885, applying it to unusual-shaped formations, much larger than the rod-shaped bacterial cells found in nodule tissues.

Bacteroids contain more volutin granules and are characterized by a higher content of glycogen and fat than rod cells. Bacteroids grown in artificial nutrient media and formed in nodule tissues are physiologically of the same type. It is believed that bacteroids are forms of bacteria with an incomplete division process. With incomplete cell division of nodule bacteria, dichotomically branching formsof bacteroides arise. The amount of bacteroids increases with the aging of the culture; their appearance is promoted by the depletion of the nutrient medium, the accumulation of metabolic products, and the introduction of alkaloids into the medium.

On nutrient media, nodule bacteria of different legume plant species grow at different rates. The fast — growing onesinclude nodule bacteria of peas, clover, alfalfa, forage beans, vetch, lentils, chyna, sweet clover, fenugreek, beans, chickpeas, lyadvenets; the slow-growing ones include nodule bacteria of lupine, soy, peanuts, seradella, mash, вигнывigna, sainfoin, gorse. Fully formed colonies of fast — growing crops can be obtained on the 3rd-4th day of incubation, colonies of slow — growing ones - on the 7th-8th.

Colonies of fast-growing nodule bacteria are the color of melted milk, often translucent, slimy, with smooth edges, moderately convex, and eventually grow on the surface of the agar medium. Colonies of slow-growing bacteria are more convex, small, dry, dense and, as a rule, do not grow on the surface of the medium. The mucus produced by nodule bacteria is a complex polysaccharide-type compound, which includes hexose, pentose and uronic acids.

Nodule bacteria are microaerophiles (they develop with small amounts of oxygen in the environment), but they prefer aerobic conditions.

Nodule bacteria use carbohydrates and organic acids as a carbon source in nutrient media, and various mineral and organic nitrogen — containing compounds as a nitrogen source. When cultivated on media with a high content of nitrogen-containing substances, nodule bacteria may lose their ability to enter the plant and form nodules. Therefore, usually nodule bacteria are grown on plant extracts (bean, pea broth) or soil extracts. Nodule bacteria can obtain phosphorus necessary for development from mineral and organic phosphorus-containing compounds; mineral compounds can serve as a source of calcium, potassium and other mineral elements.

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To suppress extraneous saprophytic microflora when isolating nodule bacteria from nodules or directly from the soil, nutrient media with the addition of crystal violet, tannin or antibiotics are recommended.

For the development of most cultures of nodule bacteria, an optimal temperature of 24-26°C is required. At 0°C and 37°C, growth stops. Usually, cultures of nodule bacteria are stored in the laboratory at low temperatures (2-4-°C).

Many types of nodule bacteria are able to synthesize B vitamins, as well as growth substances such as heteroauxin (beta-indolylacetic acid).

All nodule bacteria are approximately equally resistant to the alkaline reaction of the medium (pH = 8.0), but they are not equally sensitive to the acidic one.

Specificity, virulence, competitiveness and activity

The concept of specificity of nodule bacteria is a collective one. It characterizes the ability of bacteria to form nodules in plants. If we talk about nodule bacteria in general, then for them the formation of nodules only in a group of legumes is already specific in itself — they have selectivity to legumes.

However, if we consider individual cultures of nodule bacteria, it turns out that among them there are some that are able to infect only a certain, sometimes larger, sometimes smaller, group of legumes, and in this sense, the specificity of nodule bacteria is the selective ability in relation to the host plant. The specificity of nodule bacteria can be narrow (nodule clover bacteria infect only a group of clovers — species specificity, and lupine nodule bacteria can even be characterized by varietal specificity — infect only alkaloid or non-alkaloid lupine varieties). With broad specificity, pea nodule bacteria can infect pea, chyna, and bean plants, while chyna and bean nodule bacteria can infect pea plants, i.e. all of them are characterized by the ability to "cross-infect". The specificity of nodule bacteria underlies their classification.

The specificity of nodule bacteria arose as a result of their long-term adaptation to one plant or to a group of them and the genetic transmission of this property. In this regard, different adaptations of nodule bacteria to plants are also present within the cross-infection group. Thus, alfalfa nodule bacteria can form nodules in sweet clover. But, nevertheless, they are more adapted to alfalfa, and sweet clover bacteria - to sweet clover.

In the process of infection of the root system of legumes with nodule bacteria, the virulence of microorganisms is of great importance. If specificity determines the spectrum of action of bacteria, then the virulence of nodule bacteria characterizes the activity of their action within this spectrum. Virulence refers to the ability of nodule bacteria to enter the root tissue, multiply there and cause nodule formation.

An important role is played not only by the ability to penetrate into the roots of the plant, but also by the speed of this penetration.

To determine the virulence of a strain of nodule bacteria, it is necessary to establish its ability to cause nodule formation. The criterion of virulence of any strain can be the minimum number of bacteria that provides a more vigorous infection of the roots in comparison with other strains, resulting in the formation of nodules.

In soil, in the presence of other strains, it is not always the more virulent strain that will infect the plant first. In this case, it is necessary to take into account its competitive ability, which often masks the property of virulence in natural conditions.

It is necessary that virulent strainsare also competitive, i.e. they can successfully compete not only with representatives of the local saprophytic microflora, but also with other strains of nodule bacteria. An indicator of the competitiveness of a strain is the number of nodules formed by it as a percentage of the total number of nodules on plant roots.

An important property of nodule bacteria is their activity (efficiency), i.e. the ability to assimilate molecular nitrogen in symbiosis with legumes and meet the needs of the host plant. Depending on the extent to which nodule bacteria contribute to increasing the yield of legumes, they are usually divided into active (effective), inactive (ineffective) and inactive (ineffective).

A bacterial strain that is inactive for one host plant in symbiosis with another legume species can be quite effective. Therefore, when describing a strain from the point of view of its effectiveness, it should always be indicated in relation to which type of host plant its action is manifested.

The activity of nodule bacteria is not their permanent property. Often in laboratory practice, there is a loss of activity in cultures of nodule bacteria. In this case, either the activity of the entire culture is lost, or individual cells with low activity appear. A decrease in the degree of activity of nodule bacteria occurs in the presence of certain antibiotics and amino acids. One of the reasons for the loss of activity of nodule bacteria may be the influence of phage. By passing, i.e. by repeatedly passing bacteria through the host plant (by adapting to a particular plant species), it is possible to obtain effective strains from ineffective ones.

Exposure to gamma rays makes it possible to obtain strains with increased efficiency. There are known cases of highly active radiomutants of alfalfa nodule bacteria from an inactive strain. The use of ionizing radiation, which has a direct effect on changing the genetic characteristics of the cell, is likely to be a promising technique in the selection of highly active strains of nodule bacteria.

Infection of legumes with nodule bacteria

To ensure the normal process of infection of the root system with nodule bacteria, it is necessary to have a fairly large number of viable bacterial cells in the root zone. Researchers ' opinions on the number of cells needed to support the inoculation process vary. Thus, according to the American scientist O. Allen (1966), 500-1000 cells are required for inoculation of small — seeded plants, and at least 70,000 cells per 1 seed are required for inoculation of large-seeded plants. According to the Australian researcher J. R. R. Tolkien, According to Vincent (1966), at the time of inoculation, each seed should contain at least several hundred viable and active nodule bacterial cells. There is evidence that single cells can also be embedded in the root tissue.

During the development of the legume root system, the growth of nodule bacteria on the root surface is stimulated by root secretions. Products of destruction of root covers and hairs also play an important role in providing nodule bacteria with a suitable substrate.

In the rhizosphere of the legume plant, the development of nodule bacteria is sharply stimulated, but this phenomenon is not observed for cereals.

There are a number of hypotheses about the mechanism of penetration of nodule bacteria into the plant root.

It is known that nodule bacteria cause softening of the walls of root hairs. Howeverцеллюлазы, they do not form either cellulase or pectinolytic enzymes. In this regard, it was suggested that nodule bacteria penetrate the root due to the release of mucus of a polysaccharide nature, which causes the synthesis of the enzyme polygalacturonase by plants. This enzyme, destroying pectin substances, affects the shell of root hairs, making it more plastic and permeable. In small amounts, polygalacturonase is always present in the root hairs and, obviously, causing partial dissolution of the corresponding components of the shell, allows the cell to stretch.

Some researchers believe that nodule bacteria enter the root due to companion bacteria that produce pectinolytic enzymes. This hypothesis was put forward on the basis of the following facts. When microscoping root hairs, many researchers noted the presence of a light spot, near which nodule bacteria accumulate. This spot may be a sign of the beginning of maceration (destruction) of the tissue протопектиназойby protopectinase, similar to the same sign observed in plants with many bacterial diseases. In addition, it was found that avirulent cultures of nodule bacteria in the presence of bacteria that produce pectinolytic enzymes are able to penetrate into the root.

The process of introduction of nodule bacteria into the root tissue is the same for all types of legumes and consists of two phases. In the first phase, infection of the root hairs occurs. In the second phase, the process of nodule formation is intensive. The duration of the phases varies between different plant species: in Trifoliumfragiferum, the first phase lasts 6 days, and in Trifoliumnigrescens, it lasts 3 days. In some cases, it is very difficult to detect the boundaries between phases. The most intensive introduction of nodule bacteria into root hairs occurs in the early stages of plant development. The second phase ends in the period of mass nodule formation. Often, the introduction of nodule bacteria into the root hairs continues even after the nodules have formed on the roots. This so-called excess or additional infection occurs because the infection of the hairs does not stop for a long time. At a later stage of infection, nodules are usually placed lower along the root.

The type of development, structure and density of root hairs do not affect the rate of introduction of nodule bacteria. Nodule formation sites are not always associated with the locations of infected hairs.

Having penetrated the root (through the root hair, epidermal cell, places of root damage), nodule bacteria then move to the root tissues of the plant. Most easily, bacteria pass through the intercellular spaces.

Either a single cell or a group of bacterial cells can enter the root tissue. If a single cell has been implanted,it can continue to move through the tissue as a loner. The path of infection of the root with single cells is characteristic of lupine plants.

However, in most cases, the invaded cell, actively multiplying, forms so-called infectious threads and already in the form of such threads moves into the plant tissues.

The term "infectious thread" originated from the study of the infection process in a light microscope. Starting with Beyerink's work, the infectious thread was considered as a slimy hyphoid mass with multiplying bacteria enclosed in it.

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In essence, an infectious thread is a colony of multiplied bacteria. The beginning of it is the place where a single cell or group of cells has penetrated. It is possible that the bacterial colony (and, consequently, the future infectious thread) begins to form on the root surface before the introduction of bacteria into the root.

The number of infected root hairs varies significantly between individual plants. Usually, infectious filaments appear in deformed, twisted root hairs. However, there are indications that similar filaments are sometimes found in straight hairs. More often, one branching thread is observed in the root hairs, less often two. In some cases, there are several threads in one root hair, or several have common infection threads that give rise to a single nodule.

The percentage of infected root hairs in the total number of deformed ones is inexplicably low. It usually ranges from 0.6 to 3.2, occasionally reaching 8.0. The proportion of successful infections is even lower, since there are many (up to 80%) infectious threads so-called abortive threads that have stopped developing. The rate of movement of normally developing infectious filaments in the plant is 5-8 microns per hour. At this rate, the path through the root hair length of 100-200 microns infectious thread can pass within one day.

Free-living nitrogen-fixing microorganisms.

Until recently, it was assumed that the monopoly of nitrogen fixation belongs only to representatives of the genera Azotobacter, Clostridium and Rhizobium.

However, even before M. Beyerink (1901) isolatedthe ktera azotobe and almost simultaneously with S.N. Vinogradsky (1895) isolated clostridium, the French researcher Berthelot (1885-1892) described a large number of different soil microorganisms with a nitrogen-fixing function. Some of them increased the nitrogen content in the medium by 80% (from the initial one). However, the lack of identification of the selected crops in some cases and the imperfection ofthe methods used by BertLo did not allow us to agree with the statements about the significant spread of the nitrogen-fixing function ofsoil microorganisms.

Recently, the use of a highly sensitive isotope method and other precise methods of research has made it possible to establish the ability of many soil microorganisms to bind atmospheric nitrogen. The list of nitrogen digesters has been expanded with new bacterial species: Azotomonasinsolita, Azotomonas Fluorescens, Pseudomonasazotogensis. A number of well-known bacteria, such as actinomycetes (Actinomycetes), photosynthetic bacteria (Rhodospirillumrubrum), representatives эубактерийof Eubacteria (families Thiorhodaceae, Athiorhodaceae, as well as genera: Pseudomonas, Bacterium, Bacillus), were found to have nitrogen-fixing ability. Finally, it was found that some types of microorganisms that do not fix nitrogen under normal cultivation conditions can become energetic nitrogen fixers in associations with other types of microorganisms.

Currently, more than 80 species and varieties of bacteria, several types of actinomycetes, yeasts, yeast-like organisms and molds that can fix nitrogen are known. They inhabit the soil, the bottom of the seas and fresh water bodies.

Small amounts of molecular nitrogen can be absorbed by soil mycobacteria. Nitrogen assimilated by mycobacteria, combined with nitrogen assimilated by other oligonitrophilic microorganisms, blue-green algae, lichens and mosses, so enriches the primitive soil with nitrogen that it can be inhabited by higher plants.

Conclusion:

Biological nitrogen can serve as a significant addition to the nitrogen fund of the soil, contributing to an increase in its fertility and thereby providing a more economical use of technical nitrogen-fertilizer nitrogen.

Nitrogen deficiency is largely compensated by biological means, mainly due to the nitrogen reserve accumulated in the soil by microorganisms, primarily nitrogen-fixing ones.

There are two groups of microorganisms that fix atmospheric nitrogen. One of them is in symbiosis with higher plants, forming nodules on the roots. This group includes nodule bacteria. Microorganisms of another group live in the soil independently of plants. These include Azotobacter, clostridium, beyerinkia, and other free-living microorganisms. The potential of symbiotic nitrogen fixers is significantly higher than that of free-living ones.

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