**7.4. Phytopathogenic plant protective system**

Phytopathology studies the parasites, which may emerge on plants in the form of bacteria, fungi, viruses and several animalcules, as well as plant diseases that may be provoked by these organisms. There are more fungi phytopathogenes than bacteria phytopathogenenes. For example, the cultivated plant of rye *(Secale cereale)* may have up to 70 fungi plant diseases. Some phytopathogenes may be saprotrophic and some types of fungi phytopathogenes have the so called saprotrophic stage. The microorganisms that may switch from the saprotrophic stage to a parasitic one, are sometimes called «opportunistic» types. The *pseudomonas syringae* is thecommon representative of this type among bacteria. When conditions change, for example, when the plant is wounded or in other circumstances, under which such an «opportunist» may accidentally penetrate into the plant, it may «switch» to a parasitic stage. The mechanisms of these switches are poorly explored, though it is obvious that they can be provoked by sudden environmental changes, for example, abrupt temperature and humidity changes, i.е. shocks. There are many obligate parasites as well.

Parasite contacts with the host through phylloplane or rhizoplane respectively. The pathogene penetrates into the plant by different ways: through natural openinigs, for example, stomas and sites of lateral roots formation, after active enzymatic destruction of induviate covers, tissues and cell walls of plants (this destruction is caused by cellulases and pectinases), through wounds in case of mechanical impacts, for example, damages, induced by insects. Plants are commonly known to contain much pectin, especially some plant fruits. The pectinolytic enzymes secretion is one of the ways for the pathogene to penetrate into host plant. In this case pectin hydrolysis and plant cells and tissues fractionation take place.

The pectinolytic system of the phytopathogenic bacteria *Erwinia chrysanthemi* includes 16 genes that are involved in encoding and pectin-destructive enzymes synthesis management. The pathogenic impact on the plant may result in the plant tissues destruction (wound or molding areas emergence); in the toxigeriesis that may lead to local tissues necrosis; in hormonal balance changes (occurs a rapid local tissues growth that causes tumors formation, galls for example); in nutrient and energy intake that may be the reason for a decrease in the host plant growth or a complete cease of this growth; in disorders of the transport routes for nutrient substances and water or their capture, which may provoke the plant withering or substantial development violations. Finally, disorders or a total destruction of the transcriptional and translational processes system can occur, the consequences of such disorders are quite obvious [143].

*Agrobacterium tumefaciens* is the example of one of the most aggressive phytopathogenetic bacteria that can do a substantial harm to cultivated plants*.* These bacteria affect both monocotyledonous and dicotyledonous plants. The plants infection results in swellings on leaves. They are called galls. Galls are enlargements, more precisely, plant tissues tumors that contain *A. tumefaciens.*

The plants are considered not to have their own immune system. Nevertheless, they have various protective mechanisms. Some of these mechanisms are based on low and high- molecular compounds with antimicrobial activity, synthesized by plants. The first and actually all-round reaction of the plant on the contact with the pathogene is the implementation of the «hypersensitive death scenario» for the plant cells, as the result of which the cell produces active oxygen radicals that have negative impact both on pathogene and plant. Hypersensitive response of the plant cells is the first line of the protection from the pathogene. The plant cells death, connected with the hypersensitivity, is the part of a more common occurrence, to be exact, it reflects induced resistance response of the plant that facilitate strengthening of the plant protective means, which can be further used against pathogenes.

The two best known and explored types of induced resistance response are accumulation of the special proteins that are induced by pathogenesis and the accumulation of others nonproteous «protective» secondary metabolites. The proteins are synthesized both locally, at the site of the pathogene penetration and systematically in the whole plant. Thus, exist «local acquired resistance» (LAR) and «systematic acquired resistance». Glucanases and chitinases are the two largest groups of the pathogenic proteins, generated by a plant during systematic acquired resistance. Due to the impact of these enzymes on cell walls of the pathogenes (in this case we mean fungi) occurs the accumulation of glucosan and chitosan oligomers, which, in their turn, can function as the substances that stimulate further plant protective reaction that has nothing to do with the pathogenic proteins. These substances are called elicitors.

Another type of the plant protective reactions, connected with hypersensitivity, occurs when the plant accumulates some secondary metabolites, including phytoalexins. The phytoalexins are extremely specific not only in terms of species, but also in terms of organs and tissues for the same plant.

The positive forms of microbial-vegetative interactions can be used to protect plants from phytopathogenic microorganisms with the help of other microorganisms. Microorganisms, which are used to protect plants from phytopathogenes, are called biocontrol agents. The biocontrol doesn’t mean total elimination of the unfavorable microorganism - it means limitation of its prevalence and uncontrolled multiplication that may lead to the death of the host plant. When we deal with phytopathogenes, the advantages of biocontrol agents in comparison with pesticides are obvious. This method helps to avoid environmental and plant contamination. The phytopathogenes hardly adapt to the corresponding control agent or don’t adapt at all.

Nowadays many microorganisms are used as biocontrol agents. There are many methods of biocontrol influence of suppressor microorganism on the phytopathogene. Biocontrol agent and host plant are supposed to exchange «signals» in the form of the chemical substances, which help this «agent» to colonize the plant successfully. Biocontrol effect can take place as a consequence of the emission of antibiotic substances by the plant. For example — some bacteria of *Pseudomonas fluorescens* are able to produce 2,4-fluoroglucinol, which suppresses the growth of fungus *Gaeumannomyces gram inis,*, which causes the «withering» of wheat plants. Biocontrol agent can displace the phytopathogene, competing with it for similar substrata, lyse it, emitting lytic enzymes etc. Microorganisms-plant interaction triggers the emergence of «microbial-vegetative complexes» in different environmental regions. Many studies, conducted in different parts of the earth, proved that microbial-vegetative complexes exist everywhere, but they have different compositions, depending on environmental conditions. Over times of coexistence the pattern of phytopathogenic plant protective mechanismhas been worked out. The microorganisms that create protective barriers at the root surface can control phytopathogenes rather effectively. The most common biocontrol agents are bacteria agents, which belong to the class of *Pseudomonas (Р. fluorescens, Р. chlororaphis, Р. corrugata, Р. putida*), as well as some species of *Serratia* (*S. marcescens)* and *Bacillus (В. cereus, В. subtilis).* Thesemicroorganisms belong to the group, called *PGPR* (*Plant Growth Promoting Rhizobacteria*). They protect the plants from pathogenic fungi infection (*Fusarium, Trichoderma, Verticillium*), employing various methods [144].

One of them is the phytopathogenic microorganisms growth inhibition by means of bacterial antibiotics. In particular, many *Pseudomonas* strains produce phenazines, for example, phenazine-1-carboximide (PCN) efficient against *Fusarium oxysporum* [145]. Bacterial mutations that result in PCN phenotype (that doesn’t contain phenazine), cause the lack of the biocontrol function. PCN synthesis genes transcription begins in the host rhizosphere with the participation of the root exudates. The synthesis of these antibiotics can be accompanied by the volatile antifungal metabolites secretion, cyanides, for example, participation of which in the phytopathogenes biocontrol was demonstrated during experiments with the mutual cultivation of *PGPR* and *Fusarium* [146].

The second method of phytopathogenes suppression by *PGPR*-bacteria implies the competition for nutrition sources. The siderophores from bacteria are rather effective means of this competition, for they have a higher affinity with the iron ions than fungi siderophores. The importance of siderofores from bacteria in the process of pathogenic biocontrol was proved with the use of genetically modified *PGPR* strains that have either a high capacity to synthesize these compounds or have completely lost it [147].

The outcomes of the competitive phytopathogenic exclusion are of the utmost efficiency, when bacteria have high potency of root surface colonization, but not colonize their inner tissues and only penetrate into outer layers of root cortical tissues in small numbers. The main environmental niches, which are occupied by *PGPR*, are the areas of the root exudates active emission, which may amount to 30 % of the plant photosynthesis. A mayor part of bacteria is located in the areas of root fibrillas development, as well as root elongation and epidermal cells junctions, where bacteria create microcolonies and biofilms.

The importance of the root colonization for the manifestation of the rhizobacteria protective properties is obvious, for the genes that encode bacterial adhesion factors (lipopolysaccharides, flagella), are vital for this type of protection. These genes inactivation triggers the loss in the phytoprotective functions of *PGPR*, while the increase in the genes activity (for example, when they are amplified or connected to the strong promotors), can contribute to their functions strengthening. *Fusarium’s* growth suppression may be related to the fact that *Р. chlororaphis* cells are being attached both to the root surface and pathogenic filaments [148].

 As a result, some *PGPR* strains carry out biocontrol procedures, acting as hyperparasites for the pathogenetic fungi or mycophagists. These functions may be associated with fact that bacteria generate enzymes that destruct pathogenic cell walls: Some *Serratia* and *Bacillus* strains produce extracellular chitinases, which inhibit the *Fusarium* development at various stages, including conidia penetration and filaments development. It is logical to assume that such protective symbiotes were derived from bacteria, which were the natural antagonists for phytopathogenic fungi.

A number of studies demonstrate that *PGPR* inoculation can be associated with the induced systemic resistance development(*ISR*), and that places the roots beyond the reach of the pathogenes. Initially, *ISR* was considered to be specific for non-pathogenic systems and substantially different from the *systemic acquired resistance* reaction that is typical for pathogenesis. Both reaction types are induced during pathogenic and non-pathogenic interactions, however, differ from each other in terms of endogenic elicitors. Typical *SAR* reaction is characterized by the salicylic acid and pathogen regulated proteins, acting as signals, while the *ISR* is based on the jasmonate and ethylene emission [149]. Systemic reactions of both types can get started by the signals that the plant receives from the PGPR cells, attached to the root surfaces or penetrated into outer tissues. Under the influence of some molecules produced by *PGPR* (lipopolysaccharides, glucans, flagella components, exzoenzymes, phytohormones, siderophores, protein-based effectors for the secretion systems of the type III), the host has the same protective responses as in the process of living bacteria inoculation.

 *PGPR* phytoprotective functions are performed under direct control of the host, which emits easily consumable nutrition and energy sources into the rhizosphere. *PGPR* cells, which colonize root elongation area, are the most active antibiotics producers. This is the area, where these bacteria have their peak values. The importance of trophic interactions in the process of pathogenic biocontrol is obvious, for among the specifically activated phytogenes *Р. fluorescens* rhizosphere has more catabolic processes genes.

Both rapid growth and PGRP genes expression, connected with the biocontrol, depend more on organic acids, emitted by roots than on sugars, and this makes protective associations similar with nitrogen-fixing associations of plants and *Azospirillum*. This similarity is confirmed by mutational analysis: *Р. Fluorescens* mutants, which have disorders in organic acids recycling enzymes (malate dehydrogenase) possess a drastically reduced biocontrol activity, though sugar recycling mutants (glucose-6-phosphate dehydrogenase) haven’t revealed such a decrease

High bacterial protective potency can be stipulated by the fact that it is regulated by the plants. It can be performed, for example, by root exudate composition change. If the plants, which suffered from pathogenic attacks inoculated *PGPR*, the number of acids in exudates increased. Besides, it is proved that the plants are able to manage *PGPR*, by emitting compounds, which imitate bacterial signals. They are the quorum sensing systems regulators that track root colonization and microorganisms’ antifungal activity [146].

Phytohormones synthesis is essential for revealing rhizobacteria biocontrol functions. For example, high biocontrol activity, revealed by *Pseudomonas* in the radish rhizosphere, can be linked to the synthesis of the indoleacetic acid from the root exudates tryptophane , the amount of which in radish is 30−100 times higher than in wheat or in tomatoes. Aminocyclopropane carboxylate deaminase also takes part in rhizobacteria protective properties expression. It catalyzes ACC catabolism (1-aminocyclopropane -1- carboxylate) — ethylene phytohormone predecessor. Exploration of the ability to recycle ACC as the nitrogen source showed that only several PGPR strains contain this enzyme. ACC deaminase genes transfer from *Erwinia cloacae* to *Р. fluorescens* is accompanied by a substantial increase in capability of recombinants to suppress pathogenic fungi*.* Almost all the plants keep various endophytic microorganisms in their tissues. These microorganisms generate protective substances that struggle vegetable-feeders or phytopathogenes. Ergot fungi is one of the most important protective endophyte types. They inhabit grass plants, including crops (wheat, rye, panic grass) and food plants (ryegrass, fescue). Deep interest in this fungi group can be stipulated by the mechanisms of symbiosis, which comprise a puzzle of mutualism and antagonism.

 **7.5 Genetic basis for microbial vegetative symbiosis**

Genetic basis for evolution of microbial-vegetative symbiosis is responsible for different functions: matrix processes, metabolism, membranes and surface structures formation, cell cycle. In addition to chromosome the «unitary» genome has the genetic elements that don’t depend on chromosomes: plasmids, episomes, transposones, prophages, regulating adjustive functions, including antibiotics’ synthesis, rare feed sources employment, heavy metals or xenobiotics resistance. However, these replicons aren’t obligatory for cell work and reproduction. Besides, symbionts chromosomes consist of the chromosomes that include a chain from one and a chain from the other symbiont. Plants bacterial symbiont research has revealed that many of them have much more complicated, compounded genomes (consisting of several replicons similar in size) that ensure the microorganisms existence in complex environmental systems «host-environment» (fig. 5).

Fig. 5. Genotype /phenotype correlation for free living organisms and free living symbiotic systems:

* + - For combination of products of action for genes partners;
		- If one of the gene partners generates a product which influences the other and causes the formation of product that is used by the first partner (fig. 6).

Fig 6. Gene/attribute correlation for free living organisms and free living symbiotic systems

Fig 5

|  |  |
| --- | --- |
| Cвободноживущий организм  | Free living organism |
| Симбиотическая система  | Symbiotic system |
| Внешняя среда | External environment |
| Экологические факторы | Environmental factors |
| Генотип  | Genotype |
| Фенотип | Phenotype |

Fig 6

|  |  |
| --- | --- |
| Cвободноживущий организм  | Free living organism |
| Симбиотическая система  | Symbiotic system |
| Гены | Genes |
| Продукты  | Products |
| Признаки  | Attributes  |
| Индивидуальные адаптации  | Individual adaptation  |
| Кооперативные адаптации  | Cooperative adaptations  |

Most interactions in ideal beanrhizobial system are performed according to the second type, and to the first type belongs the formation of peribacteroid membranes, which contain both bacteria and vegetable based proteins

A two-leveled genetic analysis has specified the previous notion that the symbiosis takes as a heredity unit not a gene (as for an individual organism), but at least two genes that belong to different organisms (fig 5 and 6). This unit can be considered as a functional equivalent of a gene, detected by a standard genetic analysis: couples of interrelated *Avr/R* genes in symbioses turn out to be functional equivalents for couples of *А/а* alleles in diploid organism [150]. The similarity of basic inheritance scheme for pathogenic system features with monogenic feature inheritance scheme, created by Mendeleev, indicates that symbiotic partners relationship can be described both in terms of intergenic and interallelic interactions. However, it should be emphasized that heredity unit in symbiotic system consists of nonhomologous genes, and this implies the principal difference between methods and results of the genetic analysis for the symbiosis and free living organisms.

It is demonstrated that rhizobacterial genomes are significantly larger than free living bacteria genomes, they range from 4300 to 7000 kbp. For species with high or moderate growth speed (*Rhizobium*, *Sinorhizobium*, *Mesorhizobium*) and can exceed 9000 kbp for species with low growth speed (*Bradyrhizobium*). Thus, the rhizobacterial genomes may amount to or exceed low eucaryotes in size (for yeast *Saccharomyces cerevisiae* the genome consists of 6600 kbp.). This is stipulated by the necessity to support genetic systems which operate on different life cycle stages (symbiotic and free living) *A*-proteobacteria wide range comparative analysis revealed that on the average plant symbionts have larger genomes (6,73±1,26 mln base pairs) than free living bacteria (4,34±0,99 mln base pairs). Rapid growing species genomes (Zuda) *Rhizobium* have *multiple plasmids* in addition to chromosomes. These plasmids can reach extremely large sizes. For the first time multicomponent genomes have been detected in lucerne rhizobacteria (*S. meliloti*): it was showed that almost all the strains contain two enormous plasmids (1100−1900 kpb), in addition to the chromosome (about 3600 kpb.), and sometimes 1−6 «moderate» plasmids with the size of 10−400 kpb as well. [151]. Not less complicated genomes were detected in agrobacteria — pathogenes, that cause tumors on plants. These bacteria are taxonomically close to the fast-growing rhizobacteria. It was proved that Zuda-plasmids determine the principal rhizobacteria symbiotic features: virulence, nitrogen-fixing activity and host specificity. Some *Sym*-plasmids are recommended to control adaptively significant features, important for survival in soils, for example, for complex and rare nutritious substrates catabolism, cell and bacteriocins surface components synthesis, as well as for acid resistance and growth speed. For the effective symbiosis development, (associated with active N2 fixation), other plasmids are also vital: loss of any of them results in a sharp increase in nodule formation speed and/or nitrogenase activity. A part of genome that is involved in the symbiosis accounts for 15−20% for rhizobacteria, but less than 1% for plants, demonstrating that microsymbionts genomes are more adjusted for symbiotic purposes than host pants genomes .

Extremely high flexibility is the all-round and symbiotically significant feature of rhizobacterial genomes. Adaptive role of symbiotic microbes’ high flexibility lies in regular transgenations that are the sources of the «raw material» for coevolution with the hosts. V. Heyman was the first to perform rhizobactera gene horizontal transfer. Model experiments, conducted for *Е. coli*, demonstrated that the mutations that reduce reproduction speed occur with the frequency of 10-4, per cell, while the mutations that increase it occur with the frequency of 4·10-9 per cell

The ability of the *Sym*-plasmids of wide range rhizobacteria to exist in the reсipients who are alien to the donor may derive from the fact that these bacteria got used to living in the nodules that belong to different hosts. This Sym-plasmids´ feature is critically important for symbiosis evolution, for wide range rhizobacteria (for example, *R. tropici*) can be the donors of the могут быть донорами sym-genes, transferred to different soil bacteria, and that causes new symbiotic nitrogen fixers

Some rhizobacteria have another peculiarity – their sym-genes form part of special genomic elements — «symbiotic islands» (SI). Having been transferred to the recipient strain the SI are usually integrated into phene-tRNA or val-tRNA, and that is typical for mobile genomic islands, encoding various bacterial properties (including pathogenic ones) [143]. Being deprived of their own replication system, the symbiotic islands can be moved between different strains *Mesorhizobium spp.* by Р 4. Intergrase. The symbiotic islands were also detected in some *Bradyrhizobium* strains that are able to deliver their sym-genes to soil bacteria, as well as to bacteria, linked to plants.

The symbiotic islands distinctive feature is the reduced quantity of GC (guanine cytosine) kb in comparison with other parts of the genome, and that is typical for DNA-elements that have undergone severe transfers in populations. Evolutionary implication of this transfer is that it can force free living organisms to transform into symbiotically active forms.

Transcriptomic and metabolomlic methods have proved that metabolic genes as well as different nutrition sources transport genes are the key symbiotic elements in the rhizobacterial genome [151]. Most symbiotic bacteria comprise both the capacity for genetically controlled biotrophic and necrotrophic interactions with host plants and saprophytic soil ecological niches exploitation. It should be noted that rhizobacteria are significantly inferior to enterobacteria (*Escherichia, Salmonella*) in terms of transfer severity. These enterobacteria have systems, indicated for high-frequent genes transfer (F- and F-factors, efficient transducing phages), and nevertheless, they exhibit strictly clonal population structure.

Under environmental conditions microorganisms are subject both to physical-chemical factors exposure and other creatures’ exposure (biological factors). The fact that microorganisms participate in biotic relations as a part of population, not as a single cell can be considered as their distinctive feature.

 The microorganism-higher organism symbiosis has its own peculiarities. Microbial cells may inhabit both surfaces and different cavities and tissues of higher animals and plants The residential microorganisms are constantly present in the higher animal or plant and are reproducing there, and the transient ones can come there from the environment Symbiotic relationship with the macroorganism is mainly based on metabolic products exchange and living space providing. Intravital excretas and mortified parts of the higher organisms are the main alimentation sources for the microorganisms. Macroorganism ensures rather stable conditions for microbial growth and frequently protects microbial population from externa exposures. When microorganisms create a symbiosis with a macroorganism, they contact with the host protective systems and with the other microorganisms-symbionts of this macroorganism. Macro-microorganisms symbioses may have different nature. For example, disease microorganisms, which join parasitic symbiosis with the macroorganism, do a substantial harm to it, causing infectious diseases and sometimes even death of the host organism. In the mutualistic symbiosis, on the contrary, microorganisms are vital for plants and animals. They provide them with some nutritive substances and vitamins and suppress pathogenes. None of natural macrooganisms can exist without «friendly» symbiotic microorganisms.

All-round pattern, which many microbial vegetative symbioses stick to, includes signaling microbial vegetative interaction, metabolite interchange, as well as the production of cell and tissue structures that are vital for the process of symbiosis. Signal exchange is the key process for all the symbioses. It controls cross regulation and coordinated partners gene expression [124].

Functionally and genetically integrated symbioses have emerged and evolved mainly as perception, transformation and storage systems and in number of cases — for inheritance of the signal information, received from the partners

The fact that plants strongly depend on these interactions results in that their genomes are filled with DNA sequences, encoding supposed receptors for the signals, received from microsymbionts

### 7.6. Soil actinomyces

In 1877 Bollinger, a pathologist, and Harz, a plant scientist, studied cow’s tumors (actinomycetic nodes) and discovered their agent, which was called *Actinomyces* , because its threads formed a ray This name has become a collective one for several close kinds.

In 1884 in Israel the first pure actinomycetic culture was cultivated (*Actinomyces* *israelii*). Hereinafter, many pathogenic forms were detected (in 1888 Nocard extracted the first representative of the kind Nocardia from the foot of the man, who had Madura disease), in 1890–1892 . Gospirini made a list of actinomyсetic kinds

In 1912–1916.the descriptions of non-pathogenic actinomyces that were extracted from standard natural substrates began to appear. In this period such scientists as S.A Vaksman, Krainsky and Rudolf Liske made a great contribution to actinomycetology. 1939 marked a new stage in the science development, when Krasilnikov got the original kind of the antibiotic mycetin, usually secreted by streptomyces. In 1945 г. Vaksman, Shatz and Bugi extracted streptomycin. Scientists paid much attention to actinomyces, but mainly were focused on the applied actinomycetology braches, related to getting and application of antibiotics.

Almost all the known actinomyces kinds were extracted from soil or found in it. The soil is the natural substrate, in which there is a great number of various actinomyces. The number of representatives of some actinomyces kinds (*Streptoverticillium*, *Micromonospora*) is significantly greater in floor than in soil. There is always a certain number of small microbial populations that are capable of increasing in numbers rapidly and join microbial community, if conditions change quickly or if any part of the trophic chain is missing [152].

Prokaryotic microorganisms succession in different soils is generally characterized by the prevalence of gram-negative bacteria at the initial stages and increase in actinomyces numbers at the latest ones. The actinomyces increase is observed, when the fungi biomass starts to decrease. Probably, the actinomyces frequently use the mortified fungi mycelium that contains chitin. However, until recently the mycelial prokaryotes distribution patterns in soils have been studied for kinds of *Streptomyces* and *Streptoverticillium* only. The notion of soil actinomycetic complex, which explains time-space correlation of certain taxons (kinds, species) of mycelial prokaryotic microorganisms helped to determine common distribution patterns for these microorganisms in environmental substrates, mainly in soil. Soil actinomycetic complex structure depends on the composition and quantity of typical kinds and species and species range size. Every biogeocenosis has its own soil actinomycetic complex .In the forest biogeocenosis there is one domimant kind of *Streptomyces* with the typical dominant species of a single section and class. Forest biogeocenosis soil actinomycetic species function in such circumstances that the principal environment forming factors for them are floor, small humus quantity and low pH value .Due to these conditions a mayor part of sorest soil (65%) is filled with the streptomyces that belong to the sort and class *Cinereus Achromogenes*. It was demonstrated that ecological niches of the explored actinomyces populations in sod-podzolic soils aren’t totally disconnected but overlap to a certain extent. In the forest biogeocenoses the so called oligosporic actinomyces (kinds *Actinomadura*, *Saccharomonospora*, *Microbispora*, *Saccharopolyspora*, *Termomonospora*, *Nocardia*) have been encountered in all the layers — the aboveground (shrub leaves , grass), ground (moss, floor layers L, F), soil (soil mineral horizons). The most favorable areas for oligosporic actinomyces in coniferous forests are shrub leaves, low floor layers (F) and upper soil horizon that are rich in plant remnants of a different decomposition degree, and there their quantity may amount to hundreds of thousands of colony-forming units (CFU) per 1 g. of substrate and the total share of actinomyces in the complex is 50%. The actinomyces represent a united branch of a trophic chain in any ecosystem, acting as microbe-reducers. The principal mycelial prokaryotes function is that they decompose such complex polymers as lignin, chitin cellulose and humus compounds. Actinomyces also accumulate biologically active substances in soil and maintain soil nitrogen balance.

One of the features of actinomyces as nitrogen fixer symbionts is that at the certain stage of their development they are able to form branched mycelium with the diameter of 0,4–1,5 µm, and that results in optimal living conditions [153]. They have a gram-positive cell wall structure and high quantity of GC couples in their DNA (60–75 %) The most favorable place for them is the soil, almost all the actinomyces kinds can be found there. Actinomyces usually account for a quarter of bacteria that grow in traditional environments, if their soil suspensions and 5–15 % prokaryotic biomass are planted. As a rule, their environmental role is to decompose complex stable substrates; they are supposed to participate in humus substances synthesis and decomposition. Can act as invertebrate animals and higher plants symbionts [154].

Mycelium differentiation is the amplification that occurs during actinomycetic colonies development. It mainly manifests in the division process. The mycelium is divided into two types primary (substrate) and secondary (air) mycelium. Air mycelium is thicker, it is hydrophobic, contains more DNA and enzymes, it has various structures on the cell surface (rod –like fibrils). The spore-forming species air mycelium has thin barriers, in case of non-spore generic species air mycelium has the thick ones (septas) The septa formation begins with cytoplasmic membrane embolying.

Vegetative cells of most of the species are divided by cross barriers, for *Geodermatophilus* and *Dermatophilus* — in orthogonally related directions, some actinomyces have cells with septas that go in opposite directions (*Micromonospora*, sporangia *Frankia* vesicules). Vesicules are *Frankia* encapsulated nitrogen-fixing compounds. The branching occurs according to budding mechanism [155].

When cell cytoplasm ages, it acquires uneven electron density, the ribosomes distinguishing stops, nucleoid border blurs, cell wall becomes thin and crumbly, a microcapsule appears. During autolysis in the cytoplasm appear large light areas, nucleoid decomposes, in the cell wall emerge openings, membrane structures fill the cell and destroy it.

Nocardial actinomyces seldom form spores and reproduce mainly by means of quickly decomposing mycelium. Actinomyces with long mycelial stages are different from each other in the way of spore formation.

Actinomyces (especially of a kind *Micromonospora*) are found in waters and bottom deposits, however, it is not clear, if they are constant inhabitants or come from soil. The study of actinomycetic complexes structure has enabled to establish principles of mycelial actinomycetic prokaryotes distribution in biogenocenoses of basic natural zones, as well as to demonstrate that the actinomycetic kinds, which are traditionally considered to be rare, in particular Мiсrотопоsроrа, Sассharотопоsроrа, Sассhаrоро1уsроrа etc. in certain circumstances can be equal to streptomyces in numbers, and sometimes dominate actinomycetic complex [156]. We should increase our knowledge about microbial diversity, and for this purpose we must describe the structure of actinomycetic communities in terrestrial ecosystems and establish the ecologic status of actinomycetic kinds that are being constantly extracted from soils and vegetative substrates.

 Great studies of actinomyces-plant symbioses have been conducted by now. The plants in question are *Hippophae*, *Elaeagnus*, *Myrica*. Oleaster family actinorhizal plants enter bacterial symbiotic relations with the soil actinomyces of *Frankia* kind. Actinomyces of *Frankia* kind can produce some phytohormones y siderophores. It is demonstrated that the above mentioned plants have a strong ability to interact with the rhizospheric actinomyces; which grow on roots and form nodules (actinorhiza). [157].

Bacterial symbiosis is one if the positive forms of higher plants and bacteria interactions. Microbial symbionts modify plant mineral elements, produce biologically active compounds, perform protective function, assimilate molecular nitrogen. With the participation of such plants the nitrogen accumulation volume may reach 150–300 кг per 1 ha per year

Nodular bacteria have an antagonistic impact on many pathogenic fungi. Nodular system on the roots of the oleaster plants family has the name of actinorhiza by analogy with mycorhiza Nowadays this type of metaspherm plants and nitrogen fixer actinomyces relationship is described for more than 200 species mainly for the tree ones. It is worth mentioning that in the symbiosis with the rhizobial bactera only bean family plants may be treated as macrosymbionts, and in case of actinomyces the range of macrosymbiont plant families is wider.

E.N Mishustin believes that actinomyces influence nitrogen soil balance [150]. Thus, *Frankia* representatives associate with non-leguminous plants (alder tree, sea buckthorn, etc.). The plant generates nodules at roots in response for actinomyces invasion. In such circumstances the actinomyces begin to fix air nitrogen rapidly. Nowadays there is strong evidence that *Frankia* actinomyces are able to fix nitrogen in vitro. The kind *Frankia*, to which belong the actinomyces that are able to form nitrogen-fixing nodules,was described on the basis of the actinomyces in vivo study. The first pure actinomycetic cuture was obtained only in 1975, under anaerobic conditions in the environment that was challenging for tissue culture by Lalonde method. The kind *Frankia* consists of the species with a strong mycelium, only a part of thread cells is involved in sporangia formation. Tumors can be either intercalary or terminal. When sporangia decompose, agile or fixed spores come out of them Air mycelium is absent. All the group members are chemoheterothrophic plants with severe food demands, aerobic organisms (mainly microaerophiles), mesophiles. Frankia nitrogen fixation process is realized through the special structures (vesicules) that have a size of 3-5 µm, intercalary located at hyphas terminal ends or at short mycelium sprouts, stringing like beads G.G Maistrenko argues that vesicules can be surrounded by a capsule layer thickness and density of which depends on age and state of the host plant. Generally, the mycelial configuration can be found in small nodules of plants and in single top cells of mature active nodules. The vesicules emerge in the plant nodule cells, when the nodules start to branch and prevail, when the nodules start to perform nitrogen-fixation actively.

L.V Kalakutsky [153] points out that *Frankia* are considerably superior to nodular bacteria on terms of fixed nitrogen quantity per nodule mass unit, and the actinomyces-plant symbiosis surpasses all the types of nitrogen-fixing symbioses in terms of energy saving .

Lately some interesting facts have been obtained that reveal a number of secrets, related to actinomyces-higher plants symbiosis. Without it active forestry hardly exists. Plant breeders cultivate the plants with certain properties that match to the most efficient *Frankia* strains. The development of the advanced methods of their cultivation and selection is a great merit of microbiologists. Taking into consideration their achievements, the novel methods of destroyed land have been developed, nitrogen biological fixation efficiency in agrosystems has been increased. It has become clear that the actinomyces deficiency in soil can be a insuperable obstacle for pioneer plants securing in sterile areas, as the actinomyces are indispensable for root system infection and nodules formation Meanwhile, you should only infect the plant material with pure *Frankia* cultures to boost the actinorhizal symbioses without substantial losses

Prospects of these methods have been undoubtfully proved by numerous experiments with the black alder, planted in artificial sand dunes. Nowadays the cultivation of various *Frankia* stains is becoming biotechnological production object. They can be widely used for the infecting of young plants, indicated for the recultivation of the destroyed territories, related to open-pit mineral resources operation. Plant material suppled with the actinorhizal germs in advance favorably compares with the other kinds of material in growth speed, biomass accumulation rate and nitrogen-fixation efficiency.

Soil is the best environment for actinomyces. They are typical for all the continents. In the wild the mutually profitable union of the frankia kinds and plants exists throughout the vegetation period. It proves the energy saving. Energy needs are satisfied by the phtosyntesis that occurs in the leaves of the macrosymbiont. Nitrogen accumulation may be stimulated by the light increase and addition of the superphosphate to the soil, molibdenium and cobalt are also essential.

The mycorhizal influence on the process of preservation and growth of the tree plants seedings has been repeatedly demonstrated, the stimulating effect of the root exudations on the fungi-symbionts was noted [157]. In the ecotrophic mycorrhiza the fungi symbionts provide more opportunities for plant-soil contact due the surface enlargening, biochemical activity increase and mushroom caps formation Besides, the ectomycorhiza protective function with regard to the phytopathogenes is proved. It manifests in root carbohydrates and other nutritive substances recycling, in the emission of antibiotics that maintain the rhizospheric population of other kinds of microorganisms. On the other hand, the microbial symbionts recycle and prepare mineral nutrition for the plants, produce biologically active compounds, perform protective function and assimilate molecular nitrogen.

In the wild all the plants make up a continuum of the associated with the roots microorganisms, that expands from the rhizospere to the rhizoplane and to the plant tissues, including epidermis, cortex, endoderma and root vascular system. Soil and plants interact with each other through rhizosphere and rhizospheric organisms during soil microbial regulation.

 The analysis of oleaster plants nitrogenase activity dynamics gives us the right to suppose that nitrogen fixation may be connected with oleaster plants biological features. It has been proved by now that in the beanrhizobial symbiosis, which is kindred to the actinorhizal one, host plant genes affect many symbiotic processes, determining the nodule-forming capacity influencing their quantity, as well as intracellular differentiation and nitrogen fixation efficiency In the south of the West Syberia when the oleaster plants are cultivated at plantations, they have the following development cycle: the vegetation starts in the first ten days of May, the sea-buckthorn blossoms in the middle-end of May, the moss blossoms in June. The oleaster plants study has revealed the connection of the vegetation with the root growth. The root formation and branching, in its turn is related to nodule development and Frankia infection. Sprout growth depends deeply on the root system development. Therefore, the nitrogen fixation process reaches its peak in this period Other relations of the oleaster plants nodular nitrogen fixation fluctuations that happen during vegetative development are in line with the infected tissue metastructure seasonal changes and the peculiarities of the endophyte functioning.

The actinomyces are vital for soil microbial complex, they account for a quarter of the total quantity of bacteria, grown in the commonly used nutritive media, when they are cultivated from dissolved soil suspensions The actinomycetic mycelium accounts for 5–15 % от of the total bacterial biomass in soil.

For a long time the number of emitted streptomyces has been the principal factor for actinomyces distribution in soils and related natural substrates. The development of the selective combined methods for micromonospores sphingosporangia and oligosporic actinomyces extraction has helped to establish certain patterns in the hierarchy of the actinomycetic complexes in biogeocenoses: the vertical stratification of the actinomycetic distribution in biogeocenoses, which is characterized by the continuity (for streptomyces) and discontinuity (for the other kinds). Various kinds of actinomyces participate in the process of organic substances decomposition step by step, occupying certain space and time position in the ecosystem, according to land existence adaptation, mycelium and spores, environmental strategies and the type of the relationship of this kind with the other kinds in the actinomycetic complex. In certain circumstances (soil type, succession stage) the actinomycetic kinds that are usually considered to be rare can be equal to streptomyces in the actinomycetic complex and sometimes exceed them.

It is demonstrated that the representatives of Мiсrотопоsроrаkind are the most frequently extracted from soils and vegetative substrates.

The number of micromonospores in mineral soil horizon is minor, it is inferior to streptomyces

The forest biogeocenoces are characterized by the sporangial actinomyces which are found in all the layers, they are comparable with streptomyces in numbers. The sporangia are known to endure acid environmental conditions.

In forest biogeocenoces the oligosporic actinomyces are minor, their share in the actimomycetic complex seldom amounts to 50%. The oligosporic actinomyces, slowly growing species as a rule, require additional alimentation sources. The numeric dominance of micromonosporic actinomyces over Streptomyces is one of the distinctive features of biogeocenoses in drained peat-bogs. In the actinomycetic complexes of these biogeocenoses the representatives of oligosporic actinomyces are commonly found.

Thus, the micromonospores and streptosporangia can’t be regarded as rare species in the forest area soils. The number of actinomyces of these kinds is in some cases comparable with the number of streptomyces and sometimes even exceeds it. The oligosporic actinomyces are rare for the forest area

In the arid area biogeocenoses micromonosporous actinomyces are constantly present in all the layers — aboveground, land and soil. In the steppe mat micronospores exceed the streptomyces, in the vegetative and soil layers of the steppe biomes the streptomyces and micromonospores are represented in equals shares. Streptosporangia are less common for the biogeocenotic actinomycetic complexes in the arid area

Since 1980s–1990s the scientists’ attention has been focused on actinomyces ecological functions study, their natural relationship with animals, plants and microorganisms There has been a systemic review of actinomycetic genome data collection [104, 105].

Structural trends for streptomycetic complex in ground ecosystems can be defined as follows

* The study of the soil streptomycetic complexes structure, pursuant to which the structure depends on typical kinds composition, dominants number and species range size, has enabled to find out the differences of these complexes in terms of basic bioclimatic zones soils. It has been identified that human impact results in creation of specific streptomycetic complexes that are significantly different from natural land ecosystem complexes. A streptomycetic complex. A streptomycetic complex is used to compile rating scale for increasing lime and mineral fertilizers doses effect on soil microbial system state and homeostasis preservation.
* The succession method of streptomycetic complexes studying has allowed to determine and evaluate ecological features of the streptomyces types that are constantly present in soil — adaptiveness, population ecological strategy, ecological niches overlapping — and to use these features for resolving issues of ecosystem microbial diagnostics.
* It has been identified that human impact results in creation of specific streptomycetic complexes that are significantly different from natural land ecosystem complexes. A streptomycetic complex. A streptomycetic complex is used to compile rating scale for increasing lime and mineral fertilizers doses effect on soil microbial system state and homeostasis preservation.

Thus, it has been established that apart from ubiquitous Streptomyces, which are found in all the explored soil types of the basic soil-climatic zones and are present in all the vertical structure layers of the basic biogeocenoses types, monosporous and sporangial actinomyces are also essential elements of the actinomycetic communities in the land ecosystems. The Мiсrотопоsроrа is the most common representative of these actinomyces kinds , their function in these areas is unknown .

Soils are the natural substrates which contain plenty of actinomyces. However, a mayor part of actinomycetic biomass is represented by spores which make colonies, if the population are controlled by seeding technique, mycelium aссounts only for 1–4 % of the biomass. It is located in the microzones with the high organic substance content.

Actinomyces are prevalent at the latest stages of the microbial succession, when exist conditions for remote substrates use. Atinomycetic microbial flora activation occurs, when such substances as starch, chitin, oil products etc. penetrate into the soil. At the same time due to the slow growth the actinomyces aren’t able to compete with non-mycelic bacteria for the easily reachable substances. It is possible that the secondary metabolites (melanoid enzymes, in particular) somehow participate in humus formation Actinomyces fulfil cenosis-forming function at the sites of primary soil formation, being associated with the seaweed in this case. Under laboratory conditions these associations have resulted in lichen-like thallome (actinolichen).

Actinomyces (kinds *Streptomyces*, *Streptosporangium*, *Micromonospora*, *Actinomadura*) constantly reside in the intestine of earthworms, termites and many other invertebrates. They destroy cellulose and other biopolymers and turn out to be their symbionts. Pathogenic forms, triggering actinomycosis in the human body, are located in oral cavity, intestine, in the respiratory ways, on skin, in dental deposit, in decaying teeth, at tonsils.

Most actinomyces are aerobial bacteria, facultative anaerobes are present only among the actinomyces with the short mycelial stage. In this case we can draw some analogies with the fungi, among which only non-mycelial yeast can exist in anaerobic conditions. The less effective anaerobic metabolism type is supposed to be successful, if the relative cell surface becomes larger and this can be obtained by mycelium fragmentation

Actinomyces are more resistant to drying than non-mycelial bacteria, that is why, they prevail in desert soils. Sclerotia produced by *Chainia* kind can be preserved at drying for quite a durable period of time. It is demonstrated that when aw=0,50, some spores sprout (*k. Streptomyces*, *Micromonospora*), however the emerged mycelium doesn’t branch. At aw=0,86 almost all the actinomyces spores sprout, some of them have a branching mycelium, the microcolonies are formed, at aw=0,95 the optimal results can be reached

In most cases the actinomyces are neutrophils however some kinds are acidophilic or alkalophilic. Acidoresistance is the actinomyces’ distinctive feature, owning to which their share in forest soil microbial complex is relatively big. It is noted that in acid media the vegetative phase is longer, while in the alkalic one the spore formation speeds up.

The actimomyces don’t require high organic carbon content in the environment, any of them are able to grow on the «starvation» agar. *Nocardia* members are able to perform chemosynthesis, oxygenating hydrogen, methane and methanol. Heterotrophic CO2. fixation is quite common among actinomyces

The ability to synthesize physiologically active substances antibiotics, colorants fragrant compounds (substances geosmin, argosmin, mucidon, 2-methyl-isoborneol). They are responsible for specific smell of soil and sometimes of water. The actinomyces are active producers of antibiotics they form up to the half of all the known kinds of them [155].

Actinomyces are the symbionts that are able to infect only the parenchymal cells of the root cortex. Similar to the bean family plants infection; the microorganism penetrates into roots through soil, through root fibrils that get crooked in the end. At the contamination site the root fibril walls are thickening and the hyphas that have penetrated into the cell are being covered with a thick case. As the hyphas are going through the root fibrils, the case is getting thinner and there appears a capsule around hyphas. It is considered to be created both by plant and actinomyces

From the root fibril the hyphas go to epidermis and root cortex, causing division and hypertrophy of the infected cells. Hyphas nodules fill the center of the plant cells, at the cell walls occur extension and division of the hyphas ends, then special structures, the so called vesicules, are formed. In the nodules emerge the substance that is similar to leghemoglobin of the bean family plants. When the vegetation ends, the vesicules lapse, but the plant cells still contain hyphas that contaminate new tissues in spring. As a rule, when we deal with the nonleguminous plants, the *Frankia* actinomyces have more nitrogen-fixation energy than leguminous plants nodular bacteria [156]

**7.7. Plant-** **actinomyces symbiosis (actinorhiza)**

Mycorhizal fungi (fron Greek. mykes — fungus, rhiza — root enter into symbiosis with higher plants. Mycorhiza is a very interesting and even unique natural occurrence: the organisms that have a totally different structure and life principles join and support each other during their life cycles. There is a good saying. "If there is no forest, there are no mushrooms". We deal with a special plant-mushroom community, and the names of mushrooms confirm that this community really exists (birch bolete, aspen mushroom). The discovery of the mycorhiza is the scientific rationale for the existence of such a community. This seems as follows: when a certain mycelium initially spreads in soils, at the certain stage of development it finds small rootlets that belong to a certain type of a plant, it covers them with a special invisible "clutch", creating a mushroom cap. This is beneficial for both organisms. The plant supplies a mycorhizal fungus with carbohydrates, carbon dioxide and oxygen, if necessary. The fungus, in its turn, provides plant with macro-and microelements and water. Besides, the mycorhizal fungus protects the plant from harmful microorganisms

Symbiotic fungi mycelium can exist in soil without mycorhiza for a certain period of time, but in this case it is unable to form fruit bodies. Thus, we can’t obtain fruit bodies from ceps, Russulaceae and amanitas — they all are mycorhiza formers and are incapable of bearing fruits without certain kind of tree. In its turn, the plant without the fungi symbionts grows and develops slowly, is subject to different diseases and can even die. For instance, pine seedings can become strong and high pines, only when their roots are covered with a certain type of fungi.

In the middle of the 1950s it was decided to make “green ring” around the city of Elista (Kalmukia) to protect the city from severe prairie winds. Acacia was chosen for this purpose. When it was planted for the first time — all the plants died, again and again — the result was the same. Then they had to resort to the scientists. Soil and wood remnants analysis showed that there were no acacia mycorhiza formers in soil. Next year new young plants were planted, they struck to root and started to grow, for the necessary fungi mycelium had been added to soil beforehand.

Orchid seeds are rather tiny and don’t contain any nutritive substances and therefore can sprout, only if they have a fungus-symbiont. The orchid sprouts should get food through symbionts for a long time. These symbionts destroy ready-made organic substances. Due to the mentioned above, the orchids initially have a lot of mycorhizal fungi that help them to absorb nutritive substances. When a given period of time passes, the orchids don’t need the symbiosis any more, and the fungus dies, but before that he manages to generate many ripe spores, which will be spread by water or wind, and then these fungi spores make a symbiosis with the new orchids sprouts.

The mentioned above examples demonstrate that the mycorhizal fungi are of utmost importance: they facilitate growth and successful development of tree, shrub and herb plants. Many micromycetes almost all the tubularids, all the Russulaceae and about 35% of gill fungi belong to the mycorhizal fungi. Therefore, everyone, who goes to the forest, shouldn’t destroy any fungi that are unknown to him, even if they are poisonous, because they are vital for the forest itself and its inhabitants. The fourth experiment showed that the shortened variants of SYMRK gene are enough for actinomyces, but not for nodular symbioses [157].

Like in the third experiment, here the Japanese lotus mutant kind was used. This kind neither generates actinomyces, nor enters plant symbiosis. "the gene of moderate length", borrowed from tomato and "shortened gene", borrowed from rice were transferred to plants. In both cases the mutant lotus restored the capacity for generating actinomyces, but still wasn’t able to enter plant symbiosis. Taking into consideration these and a number of other experiments; the scientists concluded that SYMRK protein is apparently vital for special intracellular structures formation — a kind of “symbiont-receivers” or "(pre-infection threads", which are further settled by symbiotic bacteria (and after that they are called "infection threads"). Similar „symbiont-receivers“ are formed in the root cells and before fungi symbionts acceptance in the process of АМ formation (these structures are called pre-penetration apparatus). Both structural similarity and similarity in the mechanisms of formation of these “symbiont-receivers” probably reflect the entity of the genetic scenario, responsible for all three types of the intracellular symbiosis: АМ, PS and АR [158].

Mutations that occur in some "common symbiotic genes" result in faults in "symbiont-receivers" structure (SYMRK, naturally, is not the only gene, required for their formation). It should be noted that "long" SYMRK protein is vital, but not sufficient for nodular symbioses formation. This is obvious, for not all the organisms can enter these symbioses, but only those that have a long variant. The plants that generate nodules apparently must have some other genetic pecularities as well, but they haven’t been identified yet.

The ability to form nodular symbioses (АR and PS) has been developed due to ancient АМ genetic scenario. The key evolutionary event is that the root cells have acquired the ability to react both on symbiotic fungi presence and nitrogen-fixing bacteria proximity by forming "symbiont-receivers ". It seems that this event has happened due to SYMRK restructuring, to be exact, due to addition of two new receptor domains to its intracellular part. Both of them could be borrowed from other proteins in the higher plants genome. Thus, nodular symbioses are striking examples of new function development by modifying of gene complex that used to fulfil other functions. Arbusculas, mycelium modifications in mycorrhiza formers fungi, which are similar to haustoria, are complex repeatedly and dichotomically branched hyphas that penetrate into root parenchymal cells. Within the cell they are surrounded by the cellular plasmalemma. The most active metabolite exchange between mycorhiza elements takes place in arbusculas, though they exist only for a few days (afterwards dissolve). The scientists believe that arbusculas are formed under the influence of plant cells protective reaction (ref. fig. 7).



Fig. 7. Morphological traits for various mycorrhiza types

|  |  |
| --- | --- |
| Эктомикориза  | Ectomycorhiza |
| Эктоэндомикориза  | Ecto-endomycorhiza |
| Сеть Гартинга  | Harting net  |
| Наружные гифы | External hyphas  |
| Клубочки  | Nodules  |
| Везикулы  | Vesicules |
| Мицелий  | Micelium |
| Споры  | Spores |
| Арбускулы  | Arbusculas |
| Арбускулярная микориза  | Arbuscular mycorhiza |
| Эрикоидная (вересковая) эндомикориза  | Ericoid (ericetal) mycorhiza |
| Эндомикориза орхидных  | Orchid endomycorhiza |

Plant symbiosis with the actinomyces of the kind *Frankia* — is the another example of a nodular symbiosis. The nitrogen-fixation for this type is less efficient than for rhizobial one. However, the latest data on the expression of some genes in actinorhizal plants allow to deduce that their nitrogen fixation process has much in common with the bean family. At least seven common genes are involved in these two kinds of symbiosis. These genes are called “common symbiosis genes”. For instance, to this group belong the genes that are engaged in the formation of “pre-infection threads”, in which further live symbiotic bacteria. It is probable that actinorhizal lectins (just like bean ones) initiate the symbiosis with *Frankia* [104, 105]. Thus, transgenic actinorhizal plants may become the first pattern non-leguminous plants to enter the symbiosis with the rhizobacteria and to be recognized by them (in particular, sea-buckthorn, the symbiosis of which is similar to parasponia and some species of the bean family [100].

Some cyanobacteria and actinomyces may enter symbiotic relationship with plants, in particular, *Frankia* species. *Frankia* can establish symbiotic relationship with more than 200 species of dicotyledonous arboreal plants from eight different families, for instance *Aims,* *Hippophae,* *Dryas*, etc. At penetrating into the plant some hyphas of *Frankia* evolve into morphologically unique structures, able to perform nitrogen-fixation. They are called vesicules. As a result, on the infected plant roots appear nitrogen-fixer nodules and there occur nitrogenase synthesis and nitrogen fixation. It is worth mentioning that *Frankia* bacteria are able to perform nitrogen fixation even in a free living state, i. е. without plant contact.

We have found out that the sea-buckthorn in question has not so large nodules in the shape of thick root networks (branched like corals), which mainly are located at the lateral roots in the top soil layer (5–20 cm). Microorganisms’examination showed that they penetrate into roots from the soil, through root fibrils that have crooked in the end. At the contamination site the root fibril walls are thickening and the hyphas that have penetrated into the cell are being covered with a thick case. As the hyphas are going through the root fibrils, the case is getting thinner and there appears a capsule around hyphas. It is considered to be created both by plant and actinomyces. From the root fibril the hyphas go to epidermis and root cortex, causing division and hypertrophy of the infected cells. Hyphas nodules fill the center of the plant cells, at the cell walls occur extension and division of the hyphas ends, then special structures, the so called vesicules, are formed with the size of 3–5 µm. The root nodules may be located at any place, they are of moderate size and mainly are situated at the lateral roots