ENDOPHYTIC FUNGI OF MEDICINAL PLANTS OF UZBEKISTAN AS PRODUCERS OF ANTIBIOTIC COMPOUNDS

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Abstract.

At present, all over the world, the problem of increasing the antibiotic resistance of pathogenic microorganisms is very urgent. More and more complex mechanisms of antibiotic resistance of microorganisms are being deciphered, which creates prerequisites for the search for new antibiotic substances from natural sources. One such poorly studied source is endophytic fungi synthesizing biologically active compounds of various structures and biological effects. The potential for use in medicine, pharmaceutical and agricultural practice of fungal endophytic metabolites is very rich and has not yet been fully studied. The endemicity of plants in some regions of Uzbekistan, combined with a sharply continental climate, determines the unique biodiversity of endophytic fungi of medicinal plants. For the first time, researchers (prof. T.G Gulyamova at al.) picked out more than 200 strains of endophytic fungi from 30 medicinal plants growing in various ecological niches on the territory of the republic, and studied their antimicrobial and anticarcinogenic potential.

Keywords: endophytic fungi, plant endemicity, isolation, identification, extracts of secondary metabolites, producers of biologically active substances, interaction with the plant host, antibacterial, antifungal and cytotoxic activity, vincristine, vinblastine.

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Relevance. Antibiotics are the greatest discovery in human history. A. Fleming managed to discover the "miracle substance - penicillin" from which the enormous "Biography" of antibiotics began. The strain of the fungus *Penicillium notatum* produced a substance that killed staphylococcus, streptococcus, diphtheria bacillus and anthrax bacillus, but did not harm humans and animals [31, 32, 34]. Thus, penicillin and later discovered other antibiotics became the most powerful weapon against bacterial pathogens.

However, to date, many of the antibiotics have lost their power due to uncontrolled use, which has increased the resistance to them of more disease-causing bacteria. For example, *Staphylococcus aureus,* the dangerous hospital microbe MRSA, or ESBL bacteria that synthesize beta-lactamases, are immune to several classes of active substances. In this regard, the World Health Organization (WHO) called for the search for alternative antibacterial agents [29].

Endophytic microorganisms, in particular endophytic fungi, have become one of the natural sources of such alternative antibacterial agents. As you know, the concept of the ecological niche of an organism includes its habitat, communication in the community, as well as a set of environmental conditions necessary for its existence and reproduction. The term "endophyte" is translated as "inside the plant." Spatial niches of endophytic microorganisms include any organs and tissues inside any plant [23, 13, 20, 6].

Since the mid-80s of the twentieth century, in biological science, an independent field was formed - symbiology, which deals with the study of symbiosis, in which a separate direction was further distinguished - symbiogenetics, which considers the genetic, evolutionary and ecological aspects of the formation of over organismic systems that unite interacting organisms into a single whole, such as microbial-plant systems. This system is presented by endophyte - host plant that has been scrupulously studied by scientists all over the world in recent years.

Adaptation to environmental conditions through symbiotic interactions with microorganisms is one of the fundamental properties of higher plants. [28]. Each microorganism associated with a plant, being an evolutionarily verified component of a complex plant-microbial system, has a significant impact on the biological structure and functioning of the entire system.

Microorganisms that exist inside a plant, including the aboveground (stems, leaves, flowers, fruits, seeds) and underground (roots) parts that have a beneficial effect on its development, are called endophytic. They use the internal environment of the plant

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(endosphere) as a unique ecological niche that protects them from changes in the external environment, which has been formed as a result of hundreds of millions of years of joint evolution. A feature of endophytic microorganisms is that they are not specific to the host plant and do not lead to the formation of anatomical structures such as nodules and galls, which distinguishes them from symbiotic and some pathogenic microorganisms. Endophytes can be passed on in generations from ancestors to descendants, being an integral part of the endosphere of a plant organism.

Endophytic microorganisms include only those microorganisms that are able to colonize the internal tissues of a plant without causing its diseases and without negatively affecting its development. Endophytes have a symbiotic or mutualistic relationship with plants.

There are more than 320,000 plant species in the world, and each of them can be a host for one or more species of endophytic microorganisms. However, until recently, only a few of them in several dozen plant species have been sufficiently fully studied.

Scientists pay close attention to endophytic fungi that colonize the same parts in the plant as pathogens, and therefore are a promising agent for combating phytopathogens, as a "biocontrol" agent. Endophytic fungi are able to stimulate plant growth, improve their nutrition, induce systemic ISR-type resistance, reduce plant diseases caused by pathogenic fungi and bacteria, nematodes and insects, and increase the productivity of agricultural crops. They are able to improve the phosphorus nutrition of plants, produce vitamins, IAA (indoleacetic acid), siderophores, and also have properties for improving plant development, such as regulation of osmotic pressure and stomatal function, modification of the development of the root system and control of nitrogen nutrition [5, 26, 33].

Endophytic fungi are recognized as one of the richest sources of bioactive compounds identical and/or similar to host plant metabolites. It is believed that endophytes are a potential source of new antibiotics and other biologically active substances not only for agriculture, but also for medicine, pharmaceuticals, biotechnology [24, 30]. In this regard, the production of bioactive compounds from endophytic fungi of medicinal plants is a new alternative direction.

To date, a significant number of works have been published on various aspects of the biology of endophytic fungi, both fundamental and applied [7, 8]. Endophytic fungi are widespread in nature and are found in all regions of the world. The ability to colonize is explained by active processes of sporulation, as well as the release of biologically active substances. These substances are high-molecular complex compounds that perform

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catalytic, biotic, abiotic and other functions of the vital activity of organisms.

Endophytes are of particular interest that synthesize biologically active substances (antibiotic, anticancinogenic (paclitaxel, camptothecin, deoxypodophyllotoxin, hypericin), immunostimulating, antiviral, antiparasitic), which can be used to treat various human diseases. According to recent studies, there is every reason to believe that the possibilities of endophytic fungi as producers of biologically active substances are far from being exhausted [9, 10, 21]. Nevertheless, endophytic fungi are still underutilized as sources of biologically active substances.

Studies of the “secondary metabolites” of endophytes of medicinal plants, based on the hypothesis that they determine the medicinal properties of plants, may open up new possibilities for “biochemical” pharmacology. Therefore, if an organism has the possibility of biosynthesis of any substance, then it is necessary for this organism in any conditions that are also “normal” for it, evolutionarily conditioned. Otherwise, the biochemical synthesis of such a substance would not be supported by this organism during the evolution of the biosphere [11, 22].

The search for effective antibiotic compounds for the creation of new medical preparations is most urgent in connection with the rapidly growing rate of antibiotic resistance of pathogens of human infectious diseases. One strategy for controlling microbial pathogens is to find natural products with new scaffolds that have an increased chance of having new binding modes [[14], [15], [16]].

Secondary metabolites with antimicrobial action are more often isolated from endophytic fungi than from unicellular bacteria and actinomycetes. For example, colletotric acid, a secondary metabolite of the endophytic fungus *Colletotrichum gloeosporioides*, isolated from *Artemisia mongolica*, has antimicrobial activity against bacteria. Another culture, *Colletotrichum sp.*, also isolated from *Artemisia annua* annual wormwood, synthesizes biologically active metabolites, which showed an antimicrobial spectrum of activity similar to *A.annua*, a traditional Chinese herb well known for its ability to synthesize artemisinin [27]. The culture was isolated from ground wheat fungus *Phomopsis sp.*, which turned out to be a producer of phomopsychalasin, a substance with three cytochalasin and macrolide rings. This drug had antimicrobial activity. The culture of the fungus *Phomopsis longicolla S1B4*, isolated from the mint *Dicerandra frutescens*, forms antibiotic substances of the dicerandrol group. The results of studies of antibiotic activity showed that diacylfomoxanthone B is more active than other compounds of this group. In addition, dicerandrol A showed significant activity against seven other strains of

X. oryzae compared to the control (2,4-diacetylphloroglucinol) and antimicrobial activity against gram-positive bacteria (*S. aureus, B. subtilis*) and fungi (*Candida albicans*) [17]. The culture of the endophytic fungus *Periconia sp.*, isolated from *Piper longum L.*, synthesizes a biologically active substance of alkaloid nature, piperine, which had high antibiotic activity against the pathogens of tuberculosis *Mycobacterium tuberculosis* [25].

Fungi of the genus *Alternaria* can play an important regulatory role in the formation of phyllosphere biocenoses, and are also promising producers of antimicrobial, herbicidal, insecticidal, antiviral, and antitumor compounds with potentially new mechanisms of action. Currently, about 300 metabolites isolated from fungi of this genus are known, and their active search continues. Many species of this genus have not yet been studied for their biological activity [19].

Thus, endophytes represent a rich and far from fully studied source of new natural biologically active compounds with various structures and various biological effects. Currently, great prospects are opening up for the search, isolation and study of new species of endophytic fungi in unique ecological niches and systems of various countries. Classical studies of the biodiversity of endophytic fungi are based on the characterization of isolates obtained from the internal tissues of plants after surface sterilization.

In order to isolate endophytic fungi with biological activity, the staff of the laboratory "Biochemistry and biotechnology of physiologically active compounds" of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan (BBPAC IMB AS RUz), under the leadership of Professor, Doctor of Biological Sciences T.G. Gulyamova, conducted research on the mycoflora of local species of medicinal plants, kindly provided by Professor K.Sh. Tojibaev (Institute for the conservation of the gene pool of flora and fauna of the Academy of Sciences of the Republic of Uzbekistan).

The endemicity of plants in some regions of Uzbekistan, combined with a sharply continental climate, determines the unique biodiversity of endophytic fungi. More than 200 endophytic fungi were isolated from the bulbs, tubers, roots, stems, leaves and inflorescences of 30 plants growing on the territory of the republic (southwestern Kyzylkum (foothills of the Kulzhuntog mountain), Pap adyrs of the Kurama ridge (Uygursay), the foothills of the Chatkal and Nurata reserves). It should be noted that the endophytic population varied from plant to plant and from species to species. Within the same species, there were changes not only depending on the region, but also on the climatic conditions of one region.

The southwestern part of the Kyzyl Kum territory belongs to subtropical deserts. There is not a single surface watercourse throughout the territory. The soils are gray-brown, sandy, and there are salt marshes. The climate is sharply continental with an annual rainfall of 100-200 mm. Kulzhuntog is a mountain island ridge in Kyzyl Kum.

Pap adyrs of the Kurama Ridge are inaccessible and therefore poorly studied. Belonging to the territory of the Western Tien Shan, this site includes the flora of the Turan deserts, the mountain and foothill flora of the Pamir-Alai. Many species found here are listed in the Red Book.

Nurata Reserve, being part of the Nuratau-Kyzylkum Biosphere Reserve, is rich in more than 800 plant species, 29 of which are rare, unique, and not found anywhere in the world. Recently, 28 new species have been identified. The Nuratau Ridge is the place where in ancient times (about 400 million years ago) the first terrestrial plants of the planet - psilophytes - appeared. This area invariably remains always land, while throughout the history of the geological development of Central Asia, its overwhelming part was the sea.

In the Chatkal Reserve, which received the status of a UNESCO as "biosphere reserve", currently more than 200 species of medicinal plants grow, of which 84 species are included in the Red Book of Uzbekistan.

Based on the results of the isolation of endophytic fungi, their systemic distribution and quantitative content in various parts of plants were established. The largest amount of endophytes was isolated from leaves - 33%, from stems and roots - 26%, as well as 21% of isolates were obtained, respectively. The endophytic microflora included representatives of various taxonomic groups. Most of it belonged to the class *Hyphomycetes* - 38 species assigned to 17 genera. *Perenomycetes* class is represented by 2 species and 2 genera, *Zygomycetes* class - only 1 species and 1 genus.

The *Hyphomycetes* class formed the basis of endophytic microflora, including representatives of such widespread genera of fungi as *Aspergillus*, *Penicillium, Acremonium, Alternaria, Fusarium*. Of the above genera, 4-5 species were found in the plants under study, the remaining genera are represented by 1-2 species, such as *Sclerotium, Monilia, Gliomastix, Ulocladium, Torula, Cladosporium*. The most frequently isolated species was *A. terreus*, found in all studied plants. Among the genera *Aspergillus, Penicillium, Acremonium, Alternaria, Fusarium*, about 10% of the strains were found, the species identification of which is not possible by classical methods.

The antibacterial activity of the extracts of the isolated strains was investigated by the well-diffusion method against Gram "-" (*Escherichia coli, Pseudomonas aeruginosa*) and Gram "+" (*Staphylococcus aureus, Bacillus subtilis*) of opportunistic test cultures stored in the Collection of Industrial Microbiological Strains of the Institute of

Microbiology of AS RUz. As a result of the conducted studies, it was found that 30% of the isolated endophytic fungi had antibacterial properties. At the same time, 21% showed activity only against 1 pathogen, 8% - against two and only 1% - against three pathogens simultaneously. The antifungal activity of both culture liquid and total extracts of endophytic fungi isolated to phytopathogens of the genus *Fusarium* was also studied. It was found that in 20% of isolates, the culture fluid inhibits the growth of *Fusarium gillosum* and *Fusarium vasinfectum* [4].

It is known that many antibiotic substances also have anticarcinogenic properties. For example, secondary metabolites of endophytic fungi: diterpenoidtaxol, known in the literature as paclitaxel, alkaloid camptothecin ($C_{20}H_{16}N_2O_4$), ergoflavin ($C_{30}H_{26}O_{14}$), belonging to the class of ergochromes, secalonic acid D ($C_{32}H_{30}O_{14}$), erotoxin induce apoptosis of leukemic cells [[12], [18]].

Vinca alkaloids are also widely known, which have antitumor activity, on the basis of which the drugs Vinblastine and Vincristine are produced, which are used in the complex therapy of acute leukemia, with lymphogranulomatosis and hematosarcomas. The genus *Allium*, which includes garlic (*Allium sativum*), leeks (*A. porrum*), onions (*A. cepa*), chives (*A. schoenoprasum*), have anticarcinogenic, and antimicrobial properties. There are few studies on endophytes from plants of the *Allium* family, however, these studies are of undoubted interest, since several rare wild-growing species of this plant live on the territory of Uzbekistan [2].

Mutually with the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, studies of the cytotoxic activity of some extracts of endophytic fungi isolated by the staff of the BBPAC laboratory from the genera *Vinca* and *Allium*, verified cultures of cancer cells of cervical carcinoma (HeLa), larynx (Hep-2) and mammary glands (HBL-100) obtained from the bank of cell cultures of the Institute of Cytology of the Russian Academy of Sciences. As a result of the studies, it was found that 55% of the investigated extracts in low concentrations of 1-10 μg/ml have high cytotoxic activity. At the same time, in comparison with the anticancer drug "Cisplatin" (India), they exhibit low cytotoxic activity in the culture of normal cells of hepatocytes [3]. TLC and HPLC methods also showed the presence of compounds that coincide in mobility and release time with drugs of antitumor action cytocristin and cytoblastin (the active substance is vinkristine and vinblastine, respectively) in extracts of 5 strains of the studied endophytic fungi isolated from plants of the genus *Vinca* [[1]].

Thus, the totality of data indicates the diversity of endophytic fungi of medicinal plants in Uzbekistan and their high biotechnological potential. The isolated strains have the ability to synthesize a wide range of biologically active metabolites with undoubted prospects for use in industry, agriculture, medicine, pharmaceuticals for the development of environmentally friendly biological products with antibacterial, antifungal and anticancer properties.

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