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The Fungal Kingdom:

MINI-PREDATORS AND MAXI-HEALERS

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At the top is a mycophilic fungus in the chaga culture and micromycete fungi from samples of atmospheric aerosols. Photo from the author's archive

*On the right is a macromycete fungus known as red-belted conk (*Fomitopsis pinicola*). Public Domain*

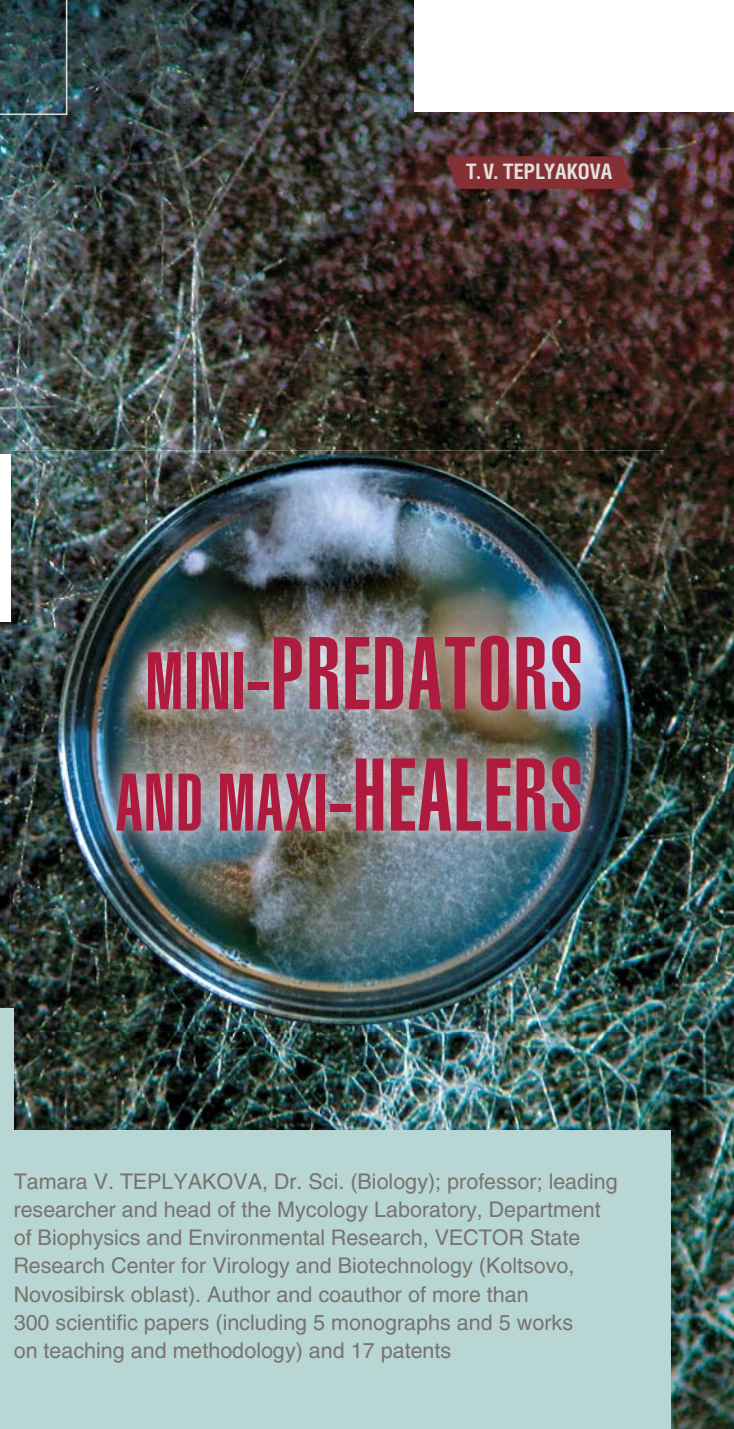
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A noose-equipped hunter, only visible under a microscope, that waits in ambush for prey many times larger than itself; a strange black nodule on a birch-tree trunk, which helps with cancer and coronavirus infection; flocks of appetizing orange caps on autumn stumps... All these creatures, so wonderful and so dissimilar, are fungi, i.e., ubiquitous and not well understood organisms, which were singled out from the kingdom of plants into a separate kingdom of living nature only half a century ago. Officially classified as microorganisms, fungi (along with bacteria and viruses) have long become an object of research at the VECTOR State Research Center of Virology and Biotechnology (Koltsovo, Novosibirsk oblast). Here, scientists develop fungi-based biopreparations for plant protection and methods for cultivating edible and medicinal fungi; they also study the antiparasitic, antiviral, and antitumor properties of various fungi. For over two decades, Tamara V. Teplyakova, the author of this article, has been in charge of this research. Since 2002, she has been heading unrelentingly the mycology laboratory, which has assembled a unique collection of fungal strains isolated into pure culture from natural habitats

Everyone is familiar with such representatives of the fungal kingdom as edible mushrooms, primarily capped ones (*pileate fungi*), which you can see in a supermarket or in the forest. However, these organisms stand out for a striking diversity of their appearances and sizes as well as their properties and biospheric functions.

Humans value fungi not only for their gastronomic qualities. Fungi have long been used in baking and in cheese- and wine-making; these organisms play a crucial role in traditional medicine. Nowadays, fungi help to combat agricultural pests and serve as raw materials for the production of preventive and medicinal products for restoring the immune system and treating infectious and oncological diseases.

A layman would hardly understand all the subtleties in the classification of fungal diversity. For convenience, mycologists themselves divided all fungi regardless of their systematic affiliation into two groups based on their size: *micro-* and *macromycetes* (the latter include all edible mushrooms).



Results of a successful hunt: a soil nematode caught in the trapping ring of the fungus *Dactylariopsis brochopaga* (bottom) and a nematode caught in the sticky loops of the fungus *Arthrobotrys compacta* (right). Light microscopy



Fungi go hunting

The micromycetes include an ecological group that relates evolutionarily to the common inhabitants of the soils, i.e., *nematodes* (roundworms). These fungi, which can formally be classified as parasites, behave as actual predators.

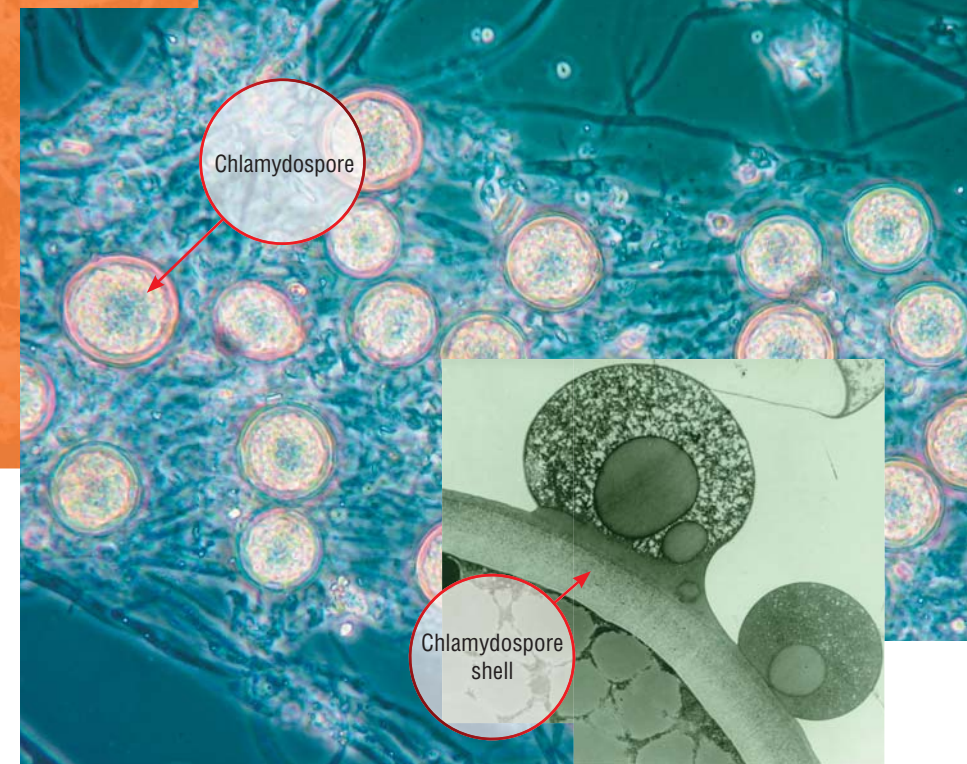
The great variety of free-living species of nematodes includes those living at the expense of other organisms. These parasites act as causative agents of dangerous helminthiasis of plants and animals. Penetrating the roots of cucumbers, tomatoes, etc., they cause the formation of “tumors,” thus disrupting the metabolism. The affected plants wither; they easily succumb to diseases and die.

The enemies of nematodes – *predatory fungi* – occur almost worldwide. This means that in nature they play an important ecological role by utilizing a huge mass of nematodes. They also help improve the mineral nutrition of plants by converting phosphorus into a soluble state; i.e., the presence of predatory fungi ensures that phosphates enter the roots directly.

The predation mechanism of microscopic hyphomycete fungi, regardless of the trap type (sticky loops, contracting rings, sticky heads, etc.), includes the release of attractive and toxic substances; the penetration of fungal hyphae into the body of a paralyzed victim; the release of enzymes and antibiotics to utilize the victim; and the prevention of development of competing microorganisms

Most of the micromycetes are only visible through a microscope. However, when they grow on a suitable substrate, they become visible to the naked eye as they form the well-known “molds.” But these fungi, like the ones commonly used in cooking and medicine, never grow large fruiting bodies.

The most important life form for predatory fungi is the chlamydospore, i.e., a cell at a dormant stage, protected by a dense membrane. On the right are chlamydospores of *Duddingtonia flagras*. Light and electron microscopy



The *mycelium* (vegetative body) of predatory fungi is essentially a finest web of *hyphae* threads, with a diameter that is orders of magnitude smaller than the nematode body. However, the hyphae carry various devices, also filled with “chemical weapons,” to ensure that the fungi will catch their vigorously moving victims.

Studies of predatory fungi in search of strains holding promise as producers of a biopreparation against nematodes commenced at the Siberian Research Institute of Agronomy and Agriculture Chemicalization back in 1971.

The then exiting scarce and contradictory experimental data on predatory fungi’s nematophagous efficiency cast doubt on the possibility of their successful application in biological protection. Scientists had to investigate many aspects of fungi’s vital activity, including features of behavior and relations with other microorganisms in the culture and soil; to learn more about the very mechanism of predation; to substantiate the methods of selection and stabilization of effective strains.

An important step in this direction was the discovery by Siberian mycologists that the crucial (in some cases, the main) life form of predatory fungi is the *chlamydospore*, i.e., a large, thick-shelled cellular formation, which, as it was previously believed, develops only under unfavorable conditions (Teplyakova, 1999). It turned out that chlamydospores help predatory fungi not only survive in the soil but also spread effectively,

maintaining a stable population size. Thus, anti-nematode biopreparations are best based on strains capable of forming in the soil and in culture a large number of chlamydospores, whose protective shells are too hard for ticks, amoebae, and other representatives of the soil fauna and can withstand prolonged drying and exposure to other unfavorable environmental factors.

By searching in natural populations, scientists were able to select effective strains of predatory fungi with high nematophagous activity. At the top of the list stands *Arthrobotrys oligospora* 3062D, which reduces the number of nematodes in the soil by 86%. In 1990, this strain was used to develop the Nematophagin-BL bioproduct as well as the corresponding production technologies, including those using deep culture mycelium (Teplyakova, 1999). Later, the list was supplemented by the strain *A. oligospora* F-1303, capable of infecting the eggs of the *potato nematode* (Teplyakova, 2019). In 2022, the Mikopro company, working under a license agreement with VECTOR, registered the Nematophagin-Mikopro biopreparation, which was based on this patented strain.

Today, predatory fungi-based preparations are produced by two companies (Mikopro and Trading House BIONIKA) located in the science city of Koltsovo. The results suggest that if the user strictly follows the application technology instructions, predatory fungi not only continue to exist after their introduction into the soil but also increase in number and, hence, in efficiency.

But the scope of application of predatory fungi goes beyond plant cultivation. These fungi do not reproduce in the body of warm-blooded animals, are not toxic, do not cause an infectious process, and are quickly eliminated from the body. These properties, together with the wide range of biologically active substances released by the fungi, suggest a potential for the development of antiparasitic drugs for veterinary and medical purposes.

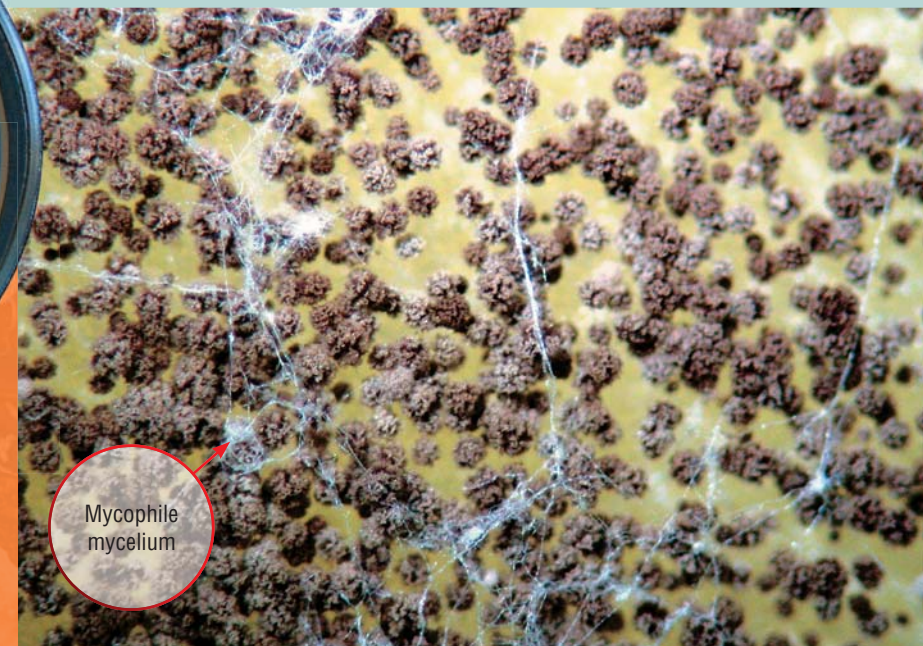
Thus, laboratory studies confirmed the nematophagous activity of the *Duddingtonia flagras* F-882 strain against roundworms that cause *strongyloidiasis* in sheep, marals, and horses. In addition, experiments showed that fungal chlamydospores do not lose their viability when passing through the digestive tract; i.e., predatory fungi continue working in excrements, thus improving the environment.

Different forms of predatory fungi-based biopreparations were tested on agricultural crops to demonstrate a considerable increase in the yields: by 2 kg or more per 1 m² for greenhouse cucumber and even by the factor of 1.5–2 for potato varieties susceptible to infection. The proportion of large berries in strawberries increased by 5–17%. The biopreparations also had a stimulating effect on the growth and development of other plants, from flower seedlings to pine saplings

Plants that grow on the soil treated with dry and liquid forms of the predatory fungi-based biopreparation noticeably outpace control samples in growth and development



Fungal colonies from atmospheric air samples (top). On the right is the mycelium of a mycophilic fungus ("white web") on the surface of a colony of the predatory fungus *Aspergillus*



As far as humans are concerned, it is highly important to obtain an effective and nontoxic drug against *opisthorchiasis*, which is caused by *flatworms* (not by roundworms) of the *Opisthorchis* genus. Together with the Institute of Cytology and Genetics (Novosibirsk), Siberian Branch, Russian Academy of Sciences, researchers at VECTOR found that metabolites of predatory fungi of the *Arthrobotrys* and *Duddingtonia* genera are able to attack liver flukes at the early (egg or larva) stages of their development.

However, the therapeutic potential of predatory fungi is not limited to their antihelminthic properties. In experiments, extracts from the *D. flagrans* deep culture biomass were able to neutralize *herpes* and *smallpox viruses*; direct feeding of laboratory mice with a grain culture of this fungus enabled a sharp reduction in mortality from the highly pathogenic *bird flu*, which is also dangerous for humans.

Moreover, the mycelium extract of this mushroom inhibited the development of experimental oncological models of *cervical cancer* and increased the life expectancy of experimental animals. These data suggest that the unique creatures – predatory fungi – have many more surprises in stock for their researchers.

Against tumors

The class of higher *basidiomycetes*, such as capped mushrooms and active wood destroyers, includes over 15,000 species. More than a hundred of them are used in traditional medicine in China, Korea, Japan, and other countries of Southeast Asia.

European healers did not attach such great importance to the medicinal properties of fungi, although about 2,000 years ago, the ancient Roman scholar Dioscorides described the medical use of the *agaricon* (*Laricifomes officinalis*). On the North American continent, Indian shamans used this fungus to treat many ailments, believing that it had supernatural powers. The agaricon was also popular in Russia, where healers used it as a hemostatic agent and as a treatment for tuberculosis. In 1879, the export of dry fruiting bodies of this fungus to Europe was about 8 tons!

Some of the first scientific publications on fungi-based medicinal compounds appeared in the late 1960s in Japan as a "trace" of the nuclear explosions in Hiroshima and Nagasaki. These works presented the results obtained by studying the antitumor activity of aqueous extracts from the fruiting bodies of wood-destroying fungi (*reishi*, *shiitake*, *chaga*, etc.), which were conducted on laboratory animals with grafted human tumors.

The studies revealed the anticancer activity of polysaccharides, i.e., high-molecular organic compounds consisting of monomer *monosaccharides*. These substances, which are isolated from natural raw materials, usually represent a mixture of molecules with different degrees of polymerization. Sometimes polysaccharides are named after the source from which they were first isolated; hence the names of the first fungi-sourced antitumor polysaccharides, e.g., *lentinan* from shiitake (*Lentinus edodes*) and *schizophyllan* from common *schizophyllum* (*Schizophyllum commune*) (Wasser, 2002).

Polysaccharides of basidiomycetes and their protein complexes are now considered a new type of antitumor compounds. Unlike conventional chemotherapy, these

FUNGAL RAIN

Microscopic fungi get from the soil into the atmospheric air and then spread in different directions, which explains why fungi grow so vigorously after a heavy rainfall. Many of these fungi pose a threat to agricultural crops and can damage the health of animals and humans.

Researchers at VECTOR took air samples in the course of biological monitoring of atmospheric aerosol in the south of West Siberia in 2008–2014. They analyzed the samples to find representatives of 19 genera of fungi, not counting unidentified ones. High-altitude air samples contained mostly pigmented forms; i.e., the presence of pigments such as melanin in the cell membranes of fungi helps protect them from adverse external factors, primarily ultraviolet radiation (Safatov, Teplyakova, Belan *et al.*, 2009; Vorob'eva, Teplyakova, Safatov *et al.*, 2014).

The dominant species in the "fungal aerosol" were those of the genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria*, which are potentially dangerous to humans; also present were phytopathogenic species, which cause dangerous diseases in agricultural crops.

The composition of atmospheric aerosol in Siberia is mostly affected by sources located in Central Asia and Northwestern Kazakhstan. It is believed that the mass growth of *brown rust* on spring wheat in West Siberia is due to the introduction of the pathogen from winter crops in the southern regions

of the European Russia. Currently, the aggressive Uganda-99 race of *stem rust* is spreading around the world as it migrates in the direction of prevailing winds. This rust stands out for its ability to virtually eradicate wheat crops. Scientists believe that in the near future, the pathogen may reach Central Asia, from where it may spread into West Siberia.

The microscopic analysis of a fungal colony grown from atmospheric air samples revealed frequent cases of parasitism of one fungus species on another. Such *mycophilic* fungi occur widely in different climatic zones and in all habitats. They play an important role in natural ecosystems, facilitating the decomposition and mineralization of fungal remains and limiting the population size of other fungi.

Mycophiles are natural enemies of phytopathogenic fungi, so the former find use in biological plant protection. For instance, fungi of the genus *Trichoderma* are a well-known producer of biopreparations. On the other hand, mycophiles pose a serious threat to cultivated edible mushrooms, such as champignons, because they can noticeably reduce the yield. These parasitic fungi can also play a negative role in other situations related to fungi cultivation: in the maintaining of fungal strain collections, when colonies are transferred to new nutrient media; in the production of commercial mycelium; in the cultivation of strains that serve as producers of biologically active substances



The fungus *Ganoderma lucidum* is also known under the Japanese name *reishi*. It has antirheumatic, antitumor, and immunomodulatory properties. It is grown in culture for medical purposes.
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Shiitake (*Lentinus edodes*) has surpassed, in terms of world production, such traditional cultivated fungi as champignons and oyster mushrooms. Shiitake is grown successfully on farms; a specialist can even cultivate it at home in the country or even in the city.
Photo by D. Minakov

substances are not toxic, and their action is based on enhancing immunity.

Thus, *in vitro* and animal experiments showed that fungal extracts and polysaccharides stimulate immune cells to produce noticeably more cytokines, i.e., small information molecules that play an important role in the actualization of innate and adaptive immunity. They also induce the synthesis of the *tumor necrosis factor* and increase the phagocytic activity of human *neutrophils*, i.e., the largest group of *leukocytes*, or protective blood cells. It is assumed that polysaccharides, in particular β -D-glucans, are able to bind to specific receptors on the surface of lymphocytes, which contributes to an increased activity of a number of immune cells (*macrophages*, *T-lymphocytes*, *killer cells*).

These biologically active substances can be extracted not only from fungal fruiting bodies but also from fungal mycelium during its cultivation and even from the liquid in which fungi were grown. In this sense, fungi are an accessible and inexhaustible source of polysaccharides with antitumor and immunostimulating properties.

One should also bear in mind that different strains of the same species can produce polysaccharides with different properties. For example, the *krestin* proteoglycan was obtained in Japan from the CM 101 strain of *Trametes versicolor*, and the polysaccharide peptide PSP was obtained in China from the Cov 1 strain of the same species. Both of these compounds have the same polysaccharide component associated with different protein molecules.

Fungi were also found to contain other carbohydrate – protein compounds with anticancer activity, e.g., *glycoproteins*, whose carbohydrate component has an irregular structure. Thus, the *Enoki mushroom* (*Flammulina velutipes*) produces EA6 anticancer glycoproteins and *bacteriostatic proflavin* (Wasser, 1999). In those provinces of Japan where this mushroom was traditionally grown and eaten, the risk of cancer was much lower than the national average (Ikekawa, 2005). A report published by the Point Institute (2013) mentioned evidence of a direct antitumor effect of fungal extracts.

Nowadays, pharmaceutical companies from different countries produce a number of antitumor drugs from fungi to be used as an additional therapy with the main treatment (surgical operations, chemotherapy, and radiotherapy). Such

a combined use of chemotherapy drugs and medicinal fungal extracts was proven to considerably increase the effectiveness of traditional therapy.

Against viruses

When it was ascertained that aqueous extracts, polysaccharides, and other compounds from higher fungi show antitumor activity, mentions began to appear in the literature about antiviral activity of the same preparations. It turned out that the lentinan polysaccharide from shiitake showed antiviral activity against several viruses, including the *encephalitis virus*, the *influenza A virus*, and even the *human immunodeficiency virus* (Tochikura, 1988).

Therefore, VECTOR started the works on isolating higher fungi into pure culture in view of two circumstances. Firstly, the forest ecosystems of West Siberia are rich in medicinal fungi species, as evidenced by papers published by mycologists from the Central Siberian Botanical Garden. Secondly, VECTOR, which possessed a representative collection of human pathogenic viruses, was able to provide all the conditions for working with these dangerous agents. This was sufficient to hope for success of the studies on antiviral activity of various fungal metabolites. A targeted selection was made to prioritize the species already known for their antitumor properties.

Thus, starting in 2008, VECTOR began to form a collection of pure cultures of Siberian higher fungi, which today includes 132 strains of 60 basidiomycete species, which were first isolated from natural habitats in the south of West Siberia.



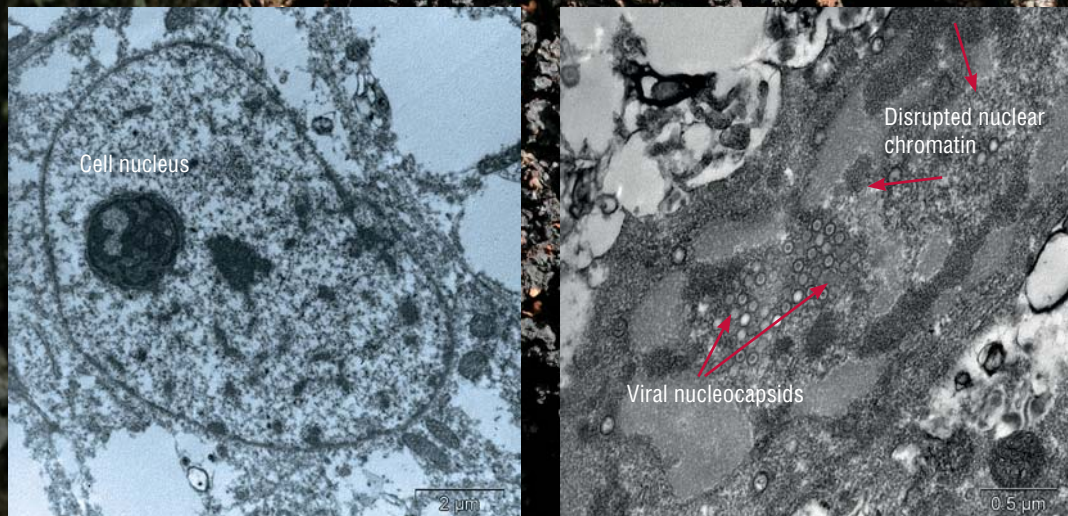
In experiments, aqueous extracts of the fungus *Trametes versicolor* showed activity against herpes virus type 2, HIV 1, and influenza viruses.
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Most of these species have long been used in folk medicine, and some of them are edible.

The latter include various types of *oyster mushrooms*, *shaggy-mane mushroom*, *sulfur-shelf mushroom*, *Enoki mushroom*, etc. The inedible medicinal fungi include various types of tinder fungi (Teplyakova and Kosogova, 2014).

Based on the results of screening the antiviral activity of more than half a thousand samples (aqueous extracts and individual compounds) of Siberian fungi, the most promising natural strains were selected with activity against *HIV 1*, *herpes simplex virus type 2*, *West Nile*, influenza of various subtypes, and *orthopoxviruses* (*smallpox* and *vaccinia* viruses).

The widest spectrum of antiviral activity was demonstrated by samples from *chaga* (a sterile nodule of *Inonotus obliquus*), which suppressed all the viruses under study. The therapeutic effect of chaga may be due to a very wide spectrum of biologically active substances that emerge in the close interaction between the birch tree and the fungus. These substances include not only high-molecular water-soluble pigments *melanins* (up to 30%), which form a chromogenic polyphenoloxycarbonic complex with antitumor activity, but also *pterins* with a cytostatic



Antiviral activity of an aqueous chaga extract was tested on the *Vero* cell culture infected with the herpes virus. The cells that were pretreated with the extract did not get infected (left) while the control cells showed evidence of damage to the structure of the cell nucleus and presence of numerous viral nucleocapsids (right). Photo by E. Ryabchikova. Photo of the chaga: © CC BY-SA 2.0/ Björn S.

effect; steroid substances, including *inotodiol*, which exhibits antiplastic activity; polysaccharides, organic acids, free phenols, flavonoids, etc. Chaga also contains many microelements, primarily manganese, which serves as an enzyme activator.

The six chaga strains in VECTOR's collection differ in their characteristics and biomass productivity. By calibrating nutrient media and cultivation conditions for the F 1244 strain, we were able to optimize the biomass yield, which, when grown in a fermenter, can reach 22.3 g/l in terms of dry matter. Based on this strain, a regulation was developed for obtaining melanin with a wide range of antiviral activity (Teplyakova and Kosogova, 2015; Anan'ko *et al.*, 2015).

The chaga's antiviral properties passed the test during the recent coronavirus pandemic. In 2020, a group of VECTOR's virologists, led by Oleg V. Pyankov (Cand. Sci. (Biology)), studied the antiviral activity of aqueous extracts of pharmacy-sold chaga against the SARS-CoV 2 coronavirus. The experimenters first applied aqueous chaga extracts (or melanin obtained from the fungus) in different concentrations to a monolayer of culture cells and then, after some time, added a pathogenic strain of coronavirus. When prepared properly, concentrated chaga extracts

showed high inhibitory activity against the pathogen. The results gave grounds for obtaining the patent "SARS-CoV 2 coronavirus replication inhibitor based on an aqueous extract of *Inonotus obliquus*."

The results obtained by the Siberian researchers were confirmed by scientists from Egypt and Saudi Arabia, who assessed the potential interaction of chaga components with the receptor-binding domain of the SARS-CoV 2 surface glycoprotein. The scientists believe that chaga can serve as an effective natural antiviral agent to complement the existing drugs against SARS-CoV 2.

Further adding to the eulogy of chaga, we can say that in experiments, it also demonstrated high antibiotic activity against some bacteria and yeast. The aqueous chaga extract and melanin samples suppressed the growth of yeast strains in culture, including the highly pathogenic strain *Candida sp. Ft 5*, isolated from a deceased patient with generalized candidal infection.

If we add to this the well-known pronounced protective effect and low toxicity shown by extracts and compounds from this fungus, then we can confidently speak about prospects for the development of complex chaga-based medicinal and prophylactic preparations with antibacterial and antifungal, as well as antiviral, properties.

Over the past few decades, scientists have verified and provided documental proof for a large corpus of ancient knowledge about medicinal fungi. Of particular importance is the discovery of new species and strains of fungi with unique properties and the introduction of these finds into culture for medical purposes.

Of great scientific and practical value for the development of therapeutic and prophylactic drugs are the strains of basidiomycete fungi from VECTOR's collection, which were isolated from natural habitats in West Siberia. This work matters also in connection with the international Convention on Biological Diversity (CBD), and it should be continued with an emphasis on the search for and study of new strains that can be successfully applied in biotechnology.

The data obtained by Siberian scientists indicate the possibility of developing and obtaining effective antiviral and antitumor drugs from fungal mycelium biomass. The biologically active components of fungal biomass (melanin, polysaccharides, proteins, etc.) are low-toxic, which makes this area of research and development extremely promising. Further research, including clinical trials, should be aimed at assessing the effectiveness of fungal drugs in the prevention and treatment of specific diseases.

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Chaga is a sterile form of the phytopathogenic fungus *Inonotus obliquus*. The photo shows a culture of the fungus on the surface of a liquid medium

This publication uses photos from the author's archive

