Medicinal Mushrooms against Influenza Viruses

Tamara V. Teplyakova,[a,*] Tatyana N. Ilyicheva,[a] Tatyana A. Kosogova,[a] & Solomon P. Wasser[b]

[a]State Research Center of Virology and Biotechnology Vector, Koltsovo 630559, Novosibirsk Region, Russia; [b]Department of Evolutionary and Environmental Biology and Institute of Evolution, Faculty of Natural Sciences, University of Haifa, Mount Carmel, Haifa 31905, Israel

*Address all correspondence to: Tamara V. Teplyakova, State Research Center of Virology and Biotechnology Vector, Koltsovo 630559, Novosibirsk Region, Russia, E-mail: teplyakova@vector.nsc.ru

ABSTRACT: This review provides results obtained by scientists from different countries on the antiviral activity of medicinal mushrooms against influenza viruses that can cause pandemics. Currently, the search for antiviral compounds is relevant in connection with the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Medicinal mushrooms contain biologically active compounds (polysaccharides, proteins, terpenes, melanins, etc.) that exhibit an antiviral effect. The authors present the work carried out at the State Research Center of Virology and Biotechnology Vector in Russia, whose mission is to protect the population from biological threats. The research center possesses a collection of numerous pathogenic viruses, which allowed screening of water extracts, polysaccharides, and melanins from fruit bodies and fungal cultures. The results of investigations on different subtypes of influenza virus are presented, and special attention is paid to *Inonotus obliquus* (chaga mushroom). Compounds produced from this mushroom are characterized by the widest range of antiviral activity. Comparative data are presented on the antiviral activity of melanin from natural *I. obliquus* and submerged biomass of an effective strain isolated in culture against the pandemic strain of influenza virus A/California/07/09 (H1N1 pdm09).

KEY WORDS: *Inonotus obliquus*, influenza viruses, SARS-CoV-2 coronavirus, antiviral activity, polysaccharides, melanin, biotechnology, medicinal mushrooms

ABBREVIATIONS: AIDS, acquired immunodeficiency syndrome; **CoV**, coronavirus; **COVID-19**, coronavirus disease 2019; **H1N1 pdm09**, pandemic strain of influenza virus A/California/07/09; **HIV**, human immunodeficiency virus; **HSV**, herpes simplex virus; **IC_{50}**, half-maximal inhibitory concentration; **MDCK**, Madin-Darby canine kidney; **MERS**, Middle East respiratory syndrome; **PSK**, krestin; **PSP**, polysaccharide peptide; **SARS**, severe acute respiratory syndrome; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2; **SRC VB**, State Research Center of Virology and Biotechnology

I. INTRODUCTION

Viruses causing influenza are characterized by high genetic variability, which leads to the appearance of mutants resistant to antiviral drugs. This review provides information on pandemics caused by respiratory viruses in the 21st century. Characteristics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which leads to coronavirus disease 2019 (COVID-19) and severe infections of the human respiratory tract, are also described. Due to the high variability of SARS-CoV-2, it is still difficult to judge the effectiveness of drugs that were previously used against influenza viruses (e.g., oseltamivir, zinamivir).

Mushrooms, many of which are edible, contain a wide range of different biologically active compounds. Many of these compounds exhibit antiviral activity and have an immunomodulatory effect. Particularly relevant now is the search for antiviral compounds in connection with the COVID-19 pandemic caused by SARS-CoV-2.

This review presents the research completed thus far on the antiviral activity of mushrooms. We pay attention to the research carried out at the State Research Center of Virology and Biotechnology (SRC VB) Vector in Russia, where a collection of strains of higher Basidiomycetes and Ascomycetes mushrooms, newly isolated from habitats in Siberia, were established for the first time. It was possible to screen water extracts, polysaccharides, and melanins from fruit bodies and fungal cultures against pathogenic viruses from the SRC VB Vector collection.

The half-maximal inhibitory concentration (IC_{50}; inhibiting pandemic virus reproduction by 50%) of melanin obtained from a submerged culture of *Inonotus obliquus* (Ach. ex Pers.) Pilát strain F-1244 (Hymenochaetaceae, Agaricomycetes) was four times lower than that of melanin from natural *I. obliquus* (10 μg/mL and 40 μg/mL, respectively).

Electron microscopy examination has shown that melanin disrupts replication of the pandemic strain of influenza A/California/07/09 (H1N1 pdm09) virus in Madin-Darby canine kidney (MDCK) cell culture.

I. obliquus grows very slowly in nature, and its reserves are depleted. Therefore, a biotechnological method for obtaining *I. obliquus* biomass based on effective strains will facilitate the production of drugs against influenza viruses, including pandemic strains.

II. PANDEMICS CAUSED BY RESPIRATORY VIRUSES

The 21st century has seen two pandemics caused by respiratory viruses. These are the 2009 H1N1 flu pandemic and the ongoing COVID-19 pandemic caused by SARS-CoV-2.

Influenza viruses belong to the family Orthomyxoviridae comprising seven genera. Representatives of four genera—alpha-, beta-, gamma-, and delta influenza viruses, corresponding to the former genera A, B, C, and D—cause flu-like diseases. The family contains RNA viruses with a negative genome, which is represented by single-stranded segments of RNA of negative polarity. Transcription and replication of the genome occurs in the nucleus of an infected cell.[1,2]

Influenza A viruses are most widespread in nature. Besides humans, they infect birds, pigs, horses, marine mammals, bats, and some other animals. Influenza A viruses are divided into subtypes based on the properties of two surface proteins: hemagglutinin (H) and neuraminidase (N). There exist 18 different subtypes of hemagglutinin and 11 different subtypes of neuraminidase: H1–H18 and N1–N11, respectively.[1,2]

The 2009 pandemic was caused by the influenza A/H1N1 pdm0 virus. Molecular genetic analysis revealed that the pandemic influenza virus was the result of genetic recombination of human and animal viruses, which may have occurred in pigs, although there is no evidence of direct pig-to-human transmission of this virus. However, it is highly likely that pigs were a "mixing vessel" for the generation of new human influenza A viruses.[3]

In December 2019, several medical institutions in the Chinese city of Wuhan (Hubei Province) reported patients with pneumonia.[4] Clinical manifestations resembled symptoms of severe acute respiratory syndrome (SARS), a disease that appeared in 2002 in a neighboring region, Guangdong Province, caused by SARS-CoV coronavirus.[5] On January 7, 2020, a new strain of coronavirus was isolated. It was named SARS-CoV-2 due to its similarity to SARS-CoV in structure. Both pathogens belong to the family Coronaviridae. All known coronaviruses are divided into four genera, including α-, β-, γ-, and δ-CoV. Representatives of the first two genera can infect mammals, while γ- and δ-CoV infect mainly birds. Six coronaviruses were previously known to infect humans; they cause mild respiratory symptoms similar to the common cold. Two β-coronaviruses, SARS-CoV and MERS-CoV, lead to severe and potentially fatal infections of the human respiratory tract. The new SARS-CoV-2 coronavirus caused the COVID-19 pandemic.

The symptoms of influenza and COVID-19 are similar, including fever, chills, cough, sore throat, myalgia, headache, fatigue, and sometimes diarrhea, nausea, and vomiting. As a result, it is very difficult to differentiate these diseases only by symptoms. COVID-19 is often accompanied by sputum formation and shortness of breath, but not in all cases and not in all patients. In addition, these symptoms are also found in influenza. Therefore, the diagnosis must be based on the results of laboratory tests, mainly PCR data on the presence of virus RNA in nasal and pharyngeal smears. Compared with other acute respiratory viral infections, more characteristic symptoms of COVID-19 likely are loss of smell (full or partial) and impaired taste (anosmia, hyposmia, and dysgeusia).[6]

There are highly effective drugs against influenza viruses, such as oseltamivir (Tamiflu; Genentech, South San Francisco, CA) and zanamivir (Relenza; GlaxoSmithKline, Research Triangle Park, NC). Unfortunately, drug-resistant mutants appear over time due to the high variability of influenza virus. Regarding coronavirus, it is not yet clear which drugs are effective in treating COVID-19. Some medical providers use a combination of lopinavir and ritonavir, which is effective in treating human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), SARS, and MERS; chloroquine, which is used to treat malaria and autoimmune diseases; or ribavirin, which is being tested in treating SARS and MERS.[7]

III. THERAPEUTIC POTENTIAL OF MEDICINAL MUSHROOMS

More than 200 medicinal functions are thought to be produced by medicinal mushrooms and fungi,[8–10] including antitumor, immunomodulating, antioxidant, radical scavenging, cardiovascular, cholesterol-lowering, antiviral, antibacterial, antiparasitic, antifungal, detoxification, hepatoprotective, antidiabetic, antiobesity, neuroprotective, neuroregenerative, and other effects. Substances derived from medicinal mushrooms can also be used as pain relievers or analgesics. The best implementation of medicinal mushroom drugs and medicinal mushroom dietary supplements has been in preventing immune disorders and maintaining a good quality of life, especially in immunodeficient and immunodepressed patients, individuals undergoing chemotherapy or radiotherapy, and patients with different types of cancers or other illnesses, including chronic bloodborne viral infections from hepatitis (B, C, and D), anemia, HIV/AIDS, herpes simplex virus (HSV), Epstein Bar virus, influenza viruses A and B, H5N1,[11] COVID-19,[12] West Nile virus, chronic fatigue syndrome, chronic gastritis and gastric ulcers caused by *Helicobacter pylori*, and dementia (especially Alzheimer's disease).

New classes of drugs were developed from medicinal mushrooms called "mushroom pharmaceuticals or mushroom drugs" or biological response modifiers (like krestin [PSK] and polysaccharide peptide [PSP]) from *Trametes versicolor*, lentinan isolated from *Lentinus edodes*, schizophyllan (sonifilan, sizofiran) from *Schizophyllum commune*, befungin from *I. obliquus*, D-fraction from *Grifola frondosa*, polysaccharide fraction from *Ganoderma lucidum*, active hexose correlated compound, and others.[8–10]

Many, if not all, mushrooms contain biologically active compounds in fruit bodies, cultured mycelium, and cultured broth. Medicinal mushrooms present an unlimited source of polysaccharides (especially β-glucans) and polysaccharide-protein complexes with anticancer and immunomodulating properties and different types of low-molecular-weight compounds (triterpenes, lectins, steroids, phenols, polyphenols, lactones, statins, alkaloids, and antibiotics).[8–10,13–15]

Special attention is paid to mushroom polysaccharides. The data on mushroom polysaccharides and different secondary metabolites are summarized for > 700 species. Numerous bioactive polysaccharides or polysaccharide-protein complexes described from medicinal mushrooms appear to enhance innate and cell-mediated immune responses, and exhibit antitumor activities in animals and humans. Particularly, and most importantly for modern medicine, are polysaccharides and low-molecular-weight secondary metabolites with antitumor and immunostimulating properties.

Medicinal mushrooms represent an unlimited source of polysaccharides with antitumor properties and immunomodulatory properties. The first drugs derived from mushrooms were polysaccharides: krestin, lentinan, schizophyllan, ganoderan, grifolan, and pleuran.[16–20]

Several mushroom compounds have proceeded through Phase I, II, III, and IV clinical trials and are used extensively and successfully in Asia to treat various cancers and other diseases. Clinical studies on the effects of various medicinal mushroom preparations on humans were published in > 1000 papers and reports. Approximately 300 clinical studies were conducted only on *G. lucidum* and some other species of genus *Ganoderma.* The largest number of clinical trials were performed mainly using *G. lucidum*, *L. edodes*, *G. frondosa*, *T. versicolor*, *Sch. commune*, *Phellinus linteus*, and *Agaricus brasiliensis* (= *A. blazei* sensu Heinem.) for treatment of cancers and oncoimmunological and immunological diseases and in immune-adjuvant therapy. Fruiting bodies of mushrooms and/or their biomass from submerged cultivated mycelia, different types of extracts, rare spores (from *G. lucidum*), and pure β-glucans, proteoglucan (PSK), or PSP have been used in clinical trials for cancer treatment.[20] In many cases, mushrooms were used as adjuvant treatment with conventional chemo- or radiotherapy in different kinds of cancer.[21–24]

However, no clinical trials have been performed to confirm antiviral studies, which should be done before using these compounds for prevention and treatment of CoVs in the future, especially regarding SARS-CoV-2.[25] It is anticipated that not only antitumor properties but also antiviral activity will be found in medicinal mushrooms in the future.[26–29]

IV. ANTIVIRAL PROPERTIES OF MEDICINAL MUSHROOMS

Much information on biologically active substances of medicinal mushrooms possessing antiviral activity has been accumulated. Fungi-derived compounds producing an inhibitory effect on influenza virus are known. A protein substance, which prevents the replication of influenza A virus, was isolated from the aqueous extract of the fungus *Cortinarius caperatus* (= *Rozites caperatus*).[30]

Analysis of the antiviral activity of *Ganoderma pfeifferi* extract against influenza A virus and HSV-1 revealed that triterpenoids such as ganodermadiol, lucidodiol, and aplanoxinic acid G were the main antiviral components of the extract.[31]

The substances hispidin and hispolon, which have an isoprenoid nature and are found in the ethanol extract of the fungus *I. hispidus*, showed antiviral activity against influenza A and B viruses. Both fruit body extracts and mycelial extracts exhibited antiviral activity.[32,33]

Animal studies have shown that α-glucans isolated from *L. edodes* can increase the body's resistance to pathogens as shown in experiments with influenza virus.[34,35] Sterols and triterpenes with antiviral activity were isolated from the fungus *G. pfeifferi* and other species of the genus *Ganoderma*. *In vitro*, they showed activity against influenza A virus in MDCK cells at concentrations > 0.22, 0.22, and 0.19 mmol/L, respectively (IC_{50}). Ganodermadiol, lucidadiol and applanoxidic acid have shown antiviral activity against both influenza type A virus and HSV-1.[36,37] Interest in the results on antiviral activity of fungi, especially in connection with the latest pandemic, continues to grow, as evidenced by new publications.[38] The authors believe that genetic methods should be applied to strains to improve them and increase their production in order to produce compounds with antiviral activity.

The SRC VB Vector of Rospotrebnadzor created a scientific collection comprising 132 strains of 60 species of basidial fungi isolated for the first time from natural habitats of Southwestern Siberia. To guarantee the use of true producers of Basidiomycetes in biotechnology, a standard operating procedure titled "Control of Basidial Fungi Cultures for the Presence of Mycophiles at Different Stages (Storage, Seeding, Biomass Cultivation)" was developed.

Water extracts, total polysaccharides, and melanins from fungi isolated for the first time in Siberia were screened for several pathogenic viruses available in the collection of SRC VB Vector of Rospotrebnadzor.

It was shown that water extracts from fruit bodies of *Phallus impudicus* suppressed the replication of influenza virus H5N1 in cells by a mean ± SD of 5.20 ± 1.50 lg and 4.45 ± 1.60 lg of that from cultured mycelium.[39]

Among other fungi, the most active against influenza viruses were samples from fruit bodies of artist's bracket *G. applanatum* (neutralization index for subtype H5N1 = 5.00 ± 0.15 lg), sulphur polypore *Laetiporus sulphureus* (H5N1 = 5.00 ± 1.67 lg and H3N2 = 6.16 ± 0.14 lg), sclerotia of *I. obliquus* (neutralization index for subtype H5N1 = 4.7 ± 1.2 lg), and *Pleurotus pulmonarius* (H5N1 = 6.06 ± 0.18 lg and H3N2 =

5.73 ± 0.14 lg).[40–43] Antiviral activity of extracts of the same species, such as *L. sulphureus*, *P. pulmonarius*, *G. applanatum*, and *I. obliquus*, was shown for the pandemic influenza virus on MDCK cell culture and Balb/c mice. All mushroom extracts examined had low toxicity to MDCK cell culture and laboratory animals. Extracts of Basidiomycetes were found to inhibit the reproduction of the pandemic influenza A virus/Moscow/226/2009 (H1N1)v in MDCK cell culture by 2.6–3.2 lg, which was comparable in these experiments to the effect of the reference drug, Tamiflu (suppression of virus reproduction was 2.9 lg). Extracts from *G. applanatum*, *L. sulphureus*, and *I. obliquus* were tested against the pandemic influenza virus A/Moscow/226/2009 (H1N1)v on MDCK cell culture and then on laboratory mice. Four days after infection, the virus titer in the lung homogenates of control mice was 3.83 lg. In the lungs of mice treated with extracts of *I. obliquus* and *L. sulphureus*, the virus titer was 1.83 and 2.00 lg, respectively. In the lungs of mice treated with Tamiflu, the virus titer was 1.67 lg.[44]

An aqueous extract from mycelium of *Fomitopsis officinalis* was active against different subtypes of influenza virus (H5N1 IN = 3.00 ± 0.11 lg and H3N2 IN = 1.50 ± 0.25 lg). The antiviral activity of *Daedaleopsis confragosa* 2266 against H5N1 and H3N2 subtypes was demonstrated.[40,45,46] Later, two strains of *D. confragosa* were studied on the H1N1 pdm09 strain. They showed the advantages of the new strain of *D. confragosa* F-1368 such as antiviral activity and the presence of antitumor properties as well.[47]

The results of screening some samples from West Siberian mushroom cultures against influenza viruses and other viruses are summarized in reviews and monographs.[11,29,48–51]

Due to the current global situation with coronavirus, studies of biologically active compounds from fungi that are most effective against influenza viruses will be particularly relevant.

V. ANTIVIRAL PROPERTIES OF *I. OBLIQUUS*

The main biologically active substances of *I. obliquus* are water-soluble, intensely colored chromogens formed from a complex of chemically active phenolic aldehydes, polyphenols, oxyphenol carboxylic acids, and their quinones. Humin-like substances are also isolated from the chromogenic complex. All compounds are genetically related to oxyaromatic precursors of biosynthesis of birch bark tannins and birch wood lignin.[52–55]

In other countries, various compounds of *I. obliquus* are also actively studied for anticancer, antioxidant, antiallergic, anti-inflammatory, immunomodulatory, and antimicrobial activities.[56–58]

More than 4000 health products are produced from *I. obliquus* globally, and the scale of commercial exploitation of natural raw materials of *I. obliquus* is enormous.[59] Natural resources of this valuable medicinal mushroom are being depleted. Therefore, the production of bioactive compounds based on cultured strains of *I. obliquus* is one of the main tasks of medicinal mushroom biotechnology.

As noted earlier, according to the results of research launched by the SRC VB Vector in 2008, water extracts and melanins of *I. obliquus* exhibited the widest range of antiviral activity by suppressing the reproduction of all the studied viruses in cell cultures, including influenza virus, West Nile virus, HIV-1, HSV-2, variola virus, and vaccinia virus.[50,60]

The results obtained by other scientists demonstrate the effectiveness of *I. obliquus* extracts against hepatitis C virus, herpes virus, and HIV.[61–64] The range of viral pathogens on which water extracts, polysaccharides, and other components of *I. obliquus* are studied is constantly expanding.[65,66]

Melanins are of great interest for pharmaceutics. Melanins is a collective name for a group of high-molecular-weight black and brown pigmented, polymeric compounds formed during the oxidative polymerization of phenols, mainly pyrocatechin and tyrosine.[67] Melanins are found in animals, plants, bacteria, and fungi, giving them a dark color. They are involved in DNA repair and in the processes of functioning of the respiratory chain as an electron acceptor; they present a modulator of important systems of cellular metabolism such as photo- and radioprotection, neutralize the products of lipid peroxidation, and participate in neurotransmitter processes in numerous pathological disorders of the functional structures of neurons.[68] Among tinder mushrooms, *I. obliquus* contains the greatest amount of melanin, up to 30% of weight. This is evidenced by literature data and our own research results.[29,69]

Table 1 shows the antiviral activity of water extract and melanin derived from natural *I. obliquus* and melanin from a submerged culture of the *I. obliquus* strain F-1244 against various viruses.[70]

Table 2 presents data on antiviral activity of melanin derived from the *I. obliquus* strain F-1244 and natural raw materials against the H1N1 pdm09 strain on MDCK cells.[70] The study was performed within the framework of the state task (SRC VB Vector of Rospotrebnadzor). Table 2 shows that *I. obliquus* strain F-1244 produces melanin, which exhibits a higher antiviral effect than melanin from natural raw materials of *I. obliquus*. It was shown that the IC_{50} (inhibiting virus reproduction by 50%) of melanin obtained from a submerged culture of *I. obliquus* strain F-1244 was four times lower than that of natural *I. obliquus* melanin (10 µg/mL and 40 µg/mL, respectively). The highest therapeutic index equal to 160 was shown for melanin of cultured *I. obliquus*, which was 2.5 times higher than for melanins from natural *I. obliquus*. Therefore, it is important to search for effective strains of *I. obliquus* for obtaining melanin by biotechnological methods.

Electron microscopy studies conducted by us previously showed that there were no signs of reproduction of HSV-2 in the nucleus after treating Vero cells with *I. obliquus* extract, which contains melanin, whereas numerous nucleocapsids of herpes virus in the cell nucleus and disturbed structure of the nucleus were observed in the control.[71]

To conduct a similar study on influenza virus, melanin obtained from a submerged *I. obliquus* culture was used. MDCK cells were grown in culture vials, and the antiviral effect against H1N1 pdm09 was estimated. The results obtained on a series of sections demonstrated the influence of melanin on the morphogenesis of H1N1 pdm09 virus in MDCK cell culture. Virus reproduction decreased under the influence of melanin. In the experimental sample, in the presence of melanin, mainly globular small viral particles were observed in the intercellular space (Fig. 1). In control cells (without melanin), filamentous forms of the virus prevailed on the cell surface (Fig. 2), which correlates with a higher cytopathic effect of influenza

TABLE 1: Manifestation of antiviral activity of water extract and melanin of *Inonotus obliquus*

Sample	HSV-2	WNV	HIV-1	Influenza virus, subtypes			Orthopoxviruses	
				H5N1	H3N2	H1N1	VARV	VACV
Water extract from natural raw materials of chaga	●	●	●	●	●	●	●	●
Melanin from submerged culture of chaga strain F-1244	●	●	●	NP	NP	●	NP	NP
Melanin from natural chaga material	●	●	●	●	●	●	NP	●

NP, studies not performed; WNV, West Nile virus; VACV, vaccinia virus; VARV, variola virus.

TABLE 2: Antiviral activity of melanin from naturally occurring *Inonotus obliquus* and a cultured strain against the pandemic A/California/07/09 virus strain (H1N1 pdm09)

Sample	Toxic dose (TC_{50}), µg/mL	Effective dose (IC_{50}), µg/mL	Therapeutic index (IS)
Melanin from *I. obliquus* F-1244 culture	1600 ± 188	10.0 ± 2.0	160 ± 30
Melanin from natural raw materials of *I. obliquus*	2500 ± 240	40.0 ± 4.4	62.5 ± 21

Values are presented as the mean ± SD.

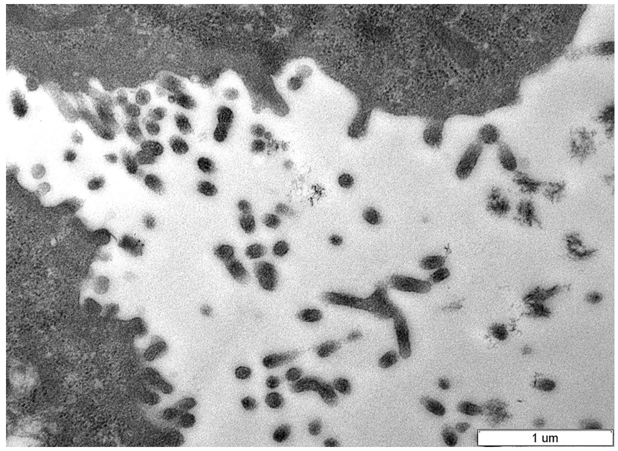

FIG. 1: The effect of melanin on influenza virus. In the intercellular space, mainly spherical, small viral particles were observed. The reproduction of the influenza virus was reduced.

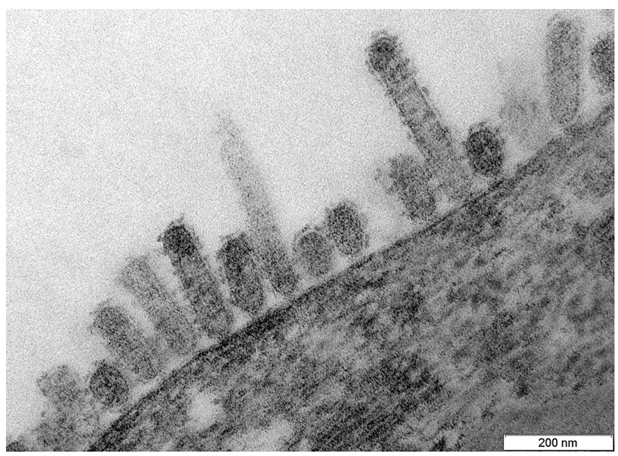

FIG. 2: Influenza virus A/California/07/09 (H1N1 pdm09). Control. In control cells (without melanin), filamentous forms of the virus prevailed on the cell surface, and this correlated with a higher cytopathic effect of the influenza virus.

virus. The most likely mechanism of antiviral activity may be the direct interaction of melanin with virions in the intercellular space.[72]

VI. DISCUSSION AND CONCLUSIONS

The search for new antiviral drugs in connection with new influenza viruses that threaten the population and lead to pandemics are currently very relevant. The number of new publications on this topic continues to increase.[73]

Based on the data presented, it can be observed that many species of medicinal mushroom have antiviral activity, including that against influenza viruses. The development of preventive and medicinal products based on polysaccharides and their complexes with proteins as well as terpenes and melanins is necessary to protect the population from new influenza pandemics, since the development of vaccines requires time. Many medicinal compounds can be derived from fruit bodies of cultured mushrooms. However, promising species such as *I. obliquus* should be protected from predatory extermination, since mainly natural raw materials are used to produce many drugs. At the same time, the production of melanin based on strains isolated in culture demonstrates its higher antiviral effect against pandemic H1N1 pdm09 in MDCK cell culture. The highest therapeutic index (equal to 160) was shown for melanins of cultured *I. obliquus*, which was 2.5 times higher than for melanins from natural *I. obliquus*. It is advisable to use genetic methods for strains producing medicinal compounds in order to increase the productivity of active substances and to improve drugs. Thus, the search for new effective strains of medicinal mushrooms that produce antiviral compounds and obtaining them by biotechnological methods is a promising and relevant direction for the development of preventive and curative medicinal products against influenza viruses, including SARS-CoV-2.

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