PLENARY LECTURES

Medicinal Mushrooms: Ancient Traditions, Contemporary Knowledge, and Scientific Enquiries

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Medicinal mushrooms have an established history of use in traditional oriental therapies. Contemporary research has validated and documented much of the ancient knowledge. The interdisciplinary field of science that studies medicinal mushrooms has sprung up and has increasingly demonstrated the potent and unique properties of compounds extracted from a range of species in the last three decades. Currently, the field is being developed into a very fruitful area. Modern clinical practice in Japan, China, Korea, and other Asian countries rely on mushroom-derived preparations.

Ancient oriental medicine has stressed the importance of several mushroom species, mostly *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Ling Zhi or Reishi) and *Lentinus edodes* (Berk.) Singer (Shiitake). For instance, Ling Zhi was valued for both its medicinal and spiritual properties. It was considered a symbol of happy augury and good fortune, good health, longevity, and even life with the immortals. Many preparations from Ling Zhi helped thousands of patients through the centuries.

Mushrooms have also played an important role as a cure for ailments affecting the rural populations of Russia and other Slavic European countries. The most important species in these rural populations were *Inonotus obliquus* (Pers.: Fr.) Pilat (Chaga), *Fomitopsis officinalis* (Vill.: Fr.) Bond. et Singer, and *Fomes fomentarius* Fr.: Fr. These species were used in the treatment of gastrointestinal disorders, various forms of cancers, bronchial asthma, night sweats, etc. There is also a long history of traditional use of mushrooms as curatives in Mesoamerica (especially species of the genus *Psilocybe*).

Meanwhile, mushrooms comprise an extremely abundant and diverse world of fungi. The number of mushroom species on Earth is currently estimated at 140,000; yet, perhaps only 10% (approximately 14,000 named species) are known to science. Mushrooms are being evaluated for their nutritional value and acceptability as well as for their pharmacological properties. They make up a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, mushrooms present an unlimited source of polysaccharides and polysaccharide-protein complexes with anticancer and immunostimulating properties. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides in their fruit bodies, cultured mycelia, and cultured broth. The data on mushroom polysaccharides today include 660 species and intraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes.

Recently studied medicinal actions of mushrooms include antitumor, immuno-modulating, antioxidant, radical scavenging, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic effects.

Empirical approaches to discover anticancer drugs and cancer treatments have made limited progress in the past several decades in finding a cure for cancer. Investigating the anticancer action of mushroom substances comprises the majority of studies in medicinal mushroom science today. The expanded knowledge on the molecular basis of tumorigenesis and metastasis, together with the inherently vast structural diversity of natural compounds found in mushrooms, provided unique opportunities for discovering new drugs that rationally target the abnormal molecular and biochemical signals leading to cancer. Many mushroom preparations have shown clinically significant efficacy against human cancers: lentinan from *Lentinus edodes*, D-fraction from *Grifola frondosa*, schizophyllan from *Schizophyllum commune*, PSK (also called krestin), and PSP (polysaccharide peptide) from *Trametes versicolor,* befungin from *Inonotus obliquus,* etc. All of these preparations are chemically high-molecular-weight polysaccharides, namely, β-D-glucans. These glucans have biologi-

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cal properties that stimulate the immune system. All of these substances have been proven in clinical trials mostly in Japan, Russia, Korea, and China.

Mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various syngeneic tumors, and prevent tumor metastasis. Their activity is especially beneficial in clinics when used in conjunction with chemotherapy. Polysaccharides from mushrooms do not attack cancer cells directly, but produce antitumor effects by activating different immune responses in the host. The antitumor action of polysaccharides requires an intact T-cell component; activity is mediated through a thymus-dependent immune mechanism. Polysaccharides activate cytotoxic macrophages, monocytes, neutrophiles, natural killer cells, dendritic cells, and chemical messengers (cytokines such as interleukins, interferons, and colony stimulating factors) that trigger complement- and acute-phase responses. Also, polysaccharides can be considered as multicytokine inducers, able to induce gene expression of various immunomodulatory cytokines and cytokine receptors.

Chemical modification is often carried out to improve the antitumor activity of polysaccharides and their clinical qualities (mostly water solubility). The main procedures used for chemical improvement are: Smith degradation (oxydo-reducto-hydrolysis), formolysis, and carboxymethylation.

Polysaccharides from other less known, but promising mushroom species, also show positive results in treating cancers *in vitro* and *in vivo*. These species include *Agaricus brasiliensis* S.Wasser et al., *Phellinus linteus* (Berk. et W. Curt.) Teng, *Grifola frondosa* (Dicks.: Fr.) S.F. Gray, *Tremella mesenterica* Retz.: Fr., *Hypsizygus marmoreus* (Peck) Bigel., and *Flammulina velutipes* (W. Curt.: Fr.) P. Karst.

Mushrooms produce beneficial effects not only as drugs but also as dietary supplements (DS) or nutriceuticals. These are not strictly pharmaceutical products, but produce healthy effects through everyday use as part of a healthy diet. The market of DS from mushrooms is quickly growing and comprises a value of more than 15 billion US dollars today (representing 10% of the general market of dietary supplements, approximately 150 billion US dollars). Every year we accumulate new evidence for the beneficial effects from DS made from mushrooms. The daily use of mushrooms is also deeply rooted in ancient medicine of the Far East, mostly from China. Chinese doctors always stated that food and medicine should be considered one and the same.

One significant problem with DS is their safety. They do not go through several strict phases of laboratory and clinical evaluations as do pharmaceutical drugs. Furthermore, a major problem associated with mushroom-based DS is their wide variability and the current lack of standards for production and testing protocols necessary to guarantee product quality. The active ingredient components of the majority of present commercial mushroom products have not been indicated. There are some safety advantages of using mushroom-based dietary supplements, as opposed to herbal preparations. The advantages are the following. (1) The overwhelming majority of mushrooms used for production of DS are cultivated commercially (and not gathered in the wild); this provides proper identification as well as pure and unadulterated products. In many cases, it also means genetic uniformity. (2) Mushrooms are easily propagated vegetatively and thus keep to one clone. The mycelium can be stored for a long time, and the genetic and biochemical consistency may be checked after considerable time. (3) The main advantage, in my opinion, is that many mushrooms are capable of growing as mycelial biomass in submerged cultures.

The development of medicines and dietary supplements from mushroom polysaccharides is hampered by the fact that high-molecular-weight compounds are used. These compounds cannot be synthesized artificially, and their production, therefore, is restricted to extraction from fruit bodies or cultured mycelium. Such an approach imposes high-market prices. Today, science should concentrate on the beneficial medicinal effects of low-weight-molecular compounds produced by mushrooms, i.e., low-molecular-weight secondary metabolites targeting processes such as apoptosis, angiogenesis, metastasis, cell cycle regulation, and signal transduction cascades. Western pharmaceutical companies are more interested in compounds that can be relatively easily synthesized and produced for markets.

Medicinal Mushrooms as a Good Source of Dietary Supplements for HIV/AIDS Patients

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Human Immunodeficiency Virus (HIV) is the cause of AIDS (Acquired Immune Deficiency Syndrome). The virus attacks the human body's white blood cells, particularly the CD4 cells, leaving the body susceptible to opportunistic infections and damaging the intestinal lining. This makes it difficult for food to be absorbed into body, and causes many patients to loss weight and even to die simply from starvation. Since the first case was reported in 1981, AIDS has killed more than 25 million people and has become a global epidemic. It was estimated that in 2005, more than 40 million people worldwide have been infected with the virus that causes AIDS. Sub-Saharan Africa still bears the highest burden, with about 62% of the world's infected people, or 24.7 million people. However, the number of people living with HIV is growing – most notably in Asia and Eastern Europe.

Since the end of the 1960s, mushroom scientists using modern analytical techniques have confirmed traditional beliefs that medicinal mushrooms represent a valuable source of bioactive agents, "mushroom nutriceuticals, and dietary supplements", which exhibit many different medicinal properties. Of these, polysaccharides and polysaccharide-protein complexes, triterpenes, and lectins have been studied most extensively. Many medicinal mushroom polysaccharides and proteopolysaccharides exhibit antitumor and anticancer activities that appear to depend on associated immunomodulating effects. In addition, medicinal mushroom products have been demonstrated as having multiple functions and concerted effects on various diseases.

The UNDP/UNOPS Regional Project RAF/99/021 on 'Sustainable Development from Africa's Biodiversity' was initiated, in 2000, under which mushroom farming initiatives are the first priority of the project. Since its commencement, I have been invited as a resource person, to conduct several mushroom training courses/workshops/seminars. The main objectives of the mushroom farming initiatives are twofold: (1) Rural utilization of Africa's inedible crops, crop residues, and agro-industrial solid wastes as substrates for the cultivation of mushrooms as functional foods in protein-deficient communities, and (2) industrial development of potentially extractable mushroom products (e.g., nutriceuticals as dietary supplements) with health benefits such as those generated by the mushroom, *Ganoderma lucidum*. This is with a view to enhance people's health and fitness, and also to prevent and treat human disease conditions, e.g., HIV/AIDS patients.

Through intervention by various donor agencies and various governments in Africa, antiretroviral (ARV) drugs have been imported to a number of African countries, with a view to: helping to improve immunodefense systems of HIV/AIDS victims, reduce the multiplication of HIV in the body, and improve the general health of HIV/AIDS patients. Unfortunately, however, the cost of these drugs is beyond the reach of many affected people in most African countries. Additionally, ARVs are often offered too late, when the damage upon the affected HIV/AIDS victims is already almost beyond repair.

Medicinal mushrooms have been well-documented to be effective in enhancing the immuno-response. Using *Ganoderma* products for the treatment of HIV/AIDS patients is a complementary approach and is not to be considered as an alternative approach. Our preliminary clinical testing trials of *G. lucidum* on HIV/AIDS patients have generated very intriguing results in Tanzania and Zambia, in terms of: stimulating body weight increases of the treated patients; increases in CD4 counts, increases in hemoglobin levels; enhanced smoothness of the skin of the patients; and improvements in general body fitness. It is concluded that *Ganoderma* mushroom products have no adverse effects and no toxicity, even with high dosages. There are many positive benefits such as promoting appetite, improving digestion of food, and increasing the absorption of nutrients of HIV/AIDS patients. From these preliminary clinical trial results, I believe it is vital to fight HIV/AIDS on two fronts: (1) controlling HIV; and (2) boosting the immune system, and ensuring adequate nutrition for

those affected. I believe that medicinal mushrooms in general and the *Ganoderma* mushroom in particular are an important part of the second battle front. In conclusion, the immune-boosting attributes of medicinal mushrooms that have been highlighted, (e.g., *G. lucidum*) show great promise towards addressing the world's and especially the developing countries HIV/AIDS most devastating challenge.

Ostreolysin, a Lipid Raft-Binding Protein from the Edible Mushroom *Pleurotus ostreatus*

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Ostreolysin (Oly), a 15 kDa protein that can be found in large amounts in young fruiting bodies of the edible mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm., is a member of the aegerolysin protein family, a novel group of small acidic proteins found in bacteria, molds, mushrooms, and plants.

In the mushrooms *P. ostreatus* and *Agrocybe aegerita*, expression of proteins belonging to the aegerolysin family has been shown to be initiated specifically during the formation of primordia and fruiting bodies. Immunolocalization of Oly, visualized by epifluorescence, confirmed specific expression of the protein in the primordia and fruiting bodies but not in the vegetative mycelia. In growing and mature fruiting bodies Oly is concentrated in the lower part of the pileus, in particular in basidia and basidiospores, while in other parts only a few focal remains are observed. Confocal microscopy of immunolabelled sections shows that intracellular presence of Oly is located specifically along the inner edges of hyphae. Since the protein can be found preferentially in the rapidly growing primordia, and in the basidia and basidiospores of maturating fruiting bodies, it is suggested that it might play a role in the processes of fructification and/or sporulation.

Membrane activity is another distinctive feature of aegerolysin-like proteins. Oly successfully permeabilizes artificial and natural lipid membranes in nanomolar concentrations, using a colloid-osmotic mechanism and leading to the formation of pores with a diameter of 4 nm. Cytolytic and haemolytic effects that are a consequence of pore formation can also be responsible for the observed toxicity of this protein. Oly induces cardiorespiratory and lethal effects in rats, which may derive from the injury of endothelial cells, or from the dysfunction of the mechanism responsible for the acetylcholine endothelium-mediated relaxation response. In mice a lethal dose after intravenous application is 1170 μg/kg. On a molecular level, Oly recognizes and binds specifically to membrane domains enriched in cholesterol and sphingomyelin (or saturated phosphatidylcholine). These events, leading to pore formation and permeabilization of the membrane, suggest that a cholesterol-rich, liquid-ordered membrane phase, which is characteristic of lipid rafts, could be its possible binding site. This hypothesis is supported by the facts that (i) Oly can be found in isolated detergent-resistant membranes of both sphingomyelin/cholesterol (1/1, mol/mol) vesicles and Chinese Hamster Ovary (CHO) cells, and (ii) permeabilization of sphingolyelin/cholesterol vesicles by Oly appears only above 30 mol% cholesterol, the concentration at which this sterol induces the formation of a liquid-ordered phase.

Lipid rafts are transient, dynamic, and unstable membrane entities that are involved in several important biological functions such as exocytosis and endocytosis, signal transduction, pathogen entry, and attachment of various ligands. The increasing number of experimental evidence emphasizes the crucial biological roles of lipid rafts, thus increasing a need for new techniques and approaches to study these membrane microdomains. Fluorescently labelled cytolytic proteins that specifically interact with molecules enriched in lipid rafts, e.g., the G_{M1} ganglioside-binding cholera-toxin B subunit, sphingomyelin-binding lysenin, or cholesterol-specific perfringolysin O (θ-toxin), are gaining particular interest.

The properties of Oly described here make this protein and its engineered mutants – for example, fused with fluorescent proteins or labelled with fluorescent or spin probes – also a new tool in further studies of cholesterol-rich lipid phases and cholesterol-complexes in natural and artificial lipid membranes.

Osmophilic Genus *Wallemia* (Basidiomycota)—A Phylogenetic Maverick and Its Bioactive Compounds

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Wallemia is a genus of cosmopolitan osmophilic fungi, frequently involved in food spoilage of particularly sweet, salty, and dried foods and feeds. It can also be isolated from indoor and outdoor air. Members of the genus were occasionally observed in natural, non-domesticated environments such as sea salt and soil. Until recently, a single species, *W. sebi,* was recognized in the genus, encompassing a large number of synonymous names. The taxonomic situation has long remained ambiguous. On one hand, slight differences were observed between freshly isolated strains, which led to the description of numerous new taxa, but were subsequently judged not reliable to represent separate taxonomic entities.

The phylogenetic position of the genus was also a much debated issue. On the basis of osmophily, extremely rarely observed in Basidiomycota, and some other characteristics, *Wallemia* was placed among the Ascomycota. However, on the basis of septal pore structures, *Wallemia* was placed among the Basidiomycota, in the order now referred to as the Filobasidiales. This order contains two further osmophilic taxa, namely, the black yeast genera *Moniliella* and *Trichosporonoides*. Classification of *Wallemia* in the Filobasidiales also corresponded with the tubular parenthesome structure, which was considered similar to the structures known in some *Trichosporon* species.

Until our revision of the genus *Wallemia*, no molecular phylogeny has been presented to verify natural phylogenetic relatedness of *Wallemia.* Our analyses of morphological and physiological characteristics as well as of sequence data of the rDNA internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) including the 5.8S ribosomal DNA (5.8S rDNA), unraveled the complexity. Based on unique morphology, evolution, and xero-tolerance a new basidiomycetous class, Wallemiomycetes, covering an order Wallemiales, was proposed. Based on differences in conidial size, xero-tolerance, and sequence data of the rDNA internal transcribed spacer regions (ITS rDNA), at least three *Wallemia* species were segregated, identified as *W. ichthyophaga, W. sebi,* and *Torula epizoa* var. *muriae*, for which the combination *Wallemia muriae* was proposed. Since the three species differ in numerous nucleotides of the SSU and ITS rDNA, the existence of at least two cryptic genera not distuingishable by morphological characteristics was indicated.

So far, there were only a few reports on the production of secondary metabolites by *Wallemia*. Several strains of *W. sebi* have been reported to produce the highly toxic metabolites, walleminol and walleminon, comparable in toxicity to citrinin or penicillic acid. Because of the unraveled taxonomic situation, there were, so far, no reports on the production of either secondary metabolites or any other bioactive compound by the three, newly described species: *Wallemia ichthyophaga*, *W. muriae,* and *W. sebi.* Since most basidiomycetous fungi contain biologically active substances in their fruit bodies, cultured mycelia, and cultured broth, it was of interest to unravel the potential bioactive activity of the three *Wallemia* species.

Novel Antiviral Activity against Pox, SARS, Bird Flu, West Nile, and Other Viruses from Polypore Mushrooms Indigenous to the Old Growth Forests of the Pacific Northwest of North America

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In Traditional Chinese Medicine (TCM), the prevailing belief is that all mushrooms must be boiled in hot water to preserve their medicinal properties. Testing this hypothesis, the author prepared samples of fruit bodies from the Polyporaceae family and their derivative mycelia in both hot water and cold water for antiviral screening. Working with the U.S. Defense Department's BioShield BioDefense Program, an anti-biological warfare program coordinated by the National Institutes of the Health (NIH) and the United Army Institute of Infectious Diseases (USAMRIID), the author has submitted more than 300 samples of mushroom species for antiviral activity against orthopoxes (including *Vaccinia,* cowpox, and *Variola major* (smallpox)), West Nile, SARS, and flu viruses, including H5N1, a bird flu virus.

The BioShield program has developed screening programs at several testing laboratories specializing in particular sets of viruses. A Selectivity Index (SI) scale has been developed for determining antiviral activity. This index is the ratio of the CC50/EC50: the Cytotoxicity Concentration that human cells can tolerate before 50% succumb to the toxicity of the sample versus the Effective Concentration that will kill 50% of viruses. The CC50 is also known as "load-tolerance". Additionally, a subsequent test compares the CC90/EC90, to confirm the SI ratios at higher cell tolerances and their corresponding virus-kill rates. The higher the SI ratio, the more target specific the antiviral activity while proportionately being less toxic to the living cells. Samples with high antiviral activity testing >10 on the Selectivity Index are chosen by USAMRIID for further testing, potentially leading to mammal studies. In these *in vitro* tests, several of the author's cultures isolated from polypore species endemic to the old growth forests of the Pacific Northwest of North America exhibited SI scores exceeding 10. A combination of samples showed SI's of >50, reinforcing the approach that a plurality of species was more effective than any one species.

Samples were prepared by boiling fruit bodies in hot water for six hours versus room temperature water/ethanol preparations of live mycelium extracted over two to six weeks. Samples from mycelium were immersed into ~ 30% ETOH/water, causing heavy molecular weight polysaccharides to precipitate. Some sets of samples were freeze-dried while other sets of liquid samples were centrifuged, pressed through 2 μm cell-free filters, and an aliquot-supernatant was prepared for *in vitro* cell assay testing. Replicates were submitted, unbeknownst to the receiving laboratories, to verify activity.

Highly active, antiviral activity was discovered against several viruses from the non-heat extracted mycelial samples, species-specifically. Surprisingly, none of the hot water extracted boiled fruit bodies, nor hot water boiled mycelial samples showed any significant antiviral activity. In contrast, many of the room temperature and cold-water extracted samples had significant activity against poxviruses, SARS, West Nile, and Flu A and B viruses, including H5N1. Fractionation of these active samples also has revealed other unexpected results. Additional antimicrobial activity was also discovered against *Mycobacterium tuberculosis,* the cause of tuberculosis, a disease of increasing concern due to drug resistance.

The author demonstrated that not only many of the non-hot water mycelial samples showed strong antiviral activity, but their immumoenhancing, anti-oxidative, and aromatase- and 5-alpha-reductase-inhibiting properties were preserved. Given these results, clearly the premise that all mushrooms must be boiled in hot water to extract their medicinal properties is not true.

Production of Intra- and Extracellular Polysaccharides of *Grifola frondosa* by Solid State and Submerged Cultivation

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In the present work, culinary-medicinal mushroom *Grifola frondosa* biomass and its pharmaceutically active polysaccharides were produced by: (1) solid-state cultivation in polyethylene bags, (2) solid-state cultivation in a horizontal stirred tank bioreactor, and (3) submerged cultivation in a liquid medium in a 10-L bioreactor. A Slovenian isolate of *G. frondosa* (GF3, Fungal bank of the Biotechnical Faculty, Department of Wood Science and Technology, University of Ljubljana, Slovenia) was used in all experiments.

In farming *G. frondosa* mycelium in polyethylene bags, 527.5 g of fresh biomass was produced on 1000.0 g of substrate (yield: 0.53 g of fresh biomass per 1 g of substrate). 2.86 g of dry intracellular polysaccharides (5.4 mg/g of fresh biomass) and 3.67 g (7.0 mg/g of fresh biomass) of dry extracellular polysaccharides were isolated from the mycelium. Crude polysaccharides were further separated by ion-exchange, gel, and affinity chromatography. Four fractions of pure intracellular β-D-glucans were isolated (total mass: 47.2 mg; 89.6 μg/g of fresh biomass) and four fractions of pure extracellular β-D-glucans were isolated (total mass: 127.2 mg; 241.1 μg/g of fresh biomass).

Solid-state experiments were carried out in a horizontal stirred tank bioreactor. Optimal cultivation conditions were temperature, 30°C; air flow, 5 L/min; periodical mixing after 14 days of cultivation, 1 minute once a week, 80 rpm. The experiments showed the importance of the substrate moisture content. Optimal moisture content for biomass growth and polysaccharide production was 60%–80%, the critical lowest moisture content point was 45%, where the production of biomass and polysaccharides stopped. Fungal growth was controlled also by electron microscopy using Field-Emission Scanning Electron Microscope Karl Zeiss Supra 35 VP.

Submerged cultivation of *G. frondosa* mycelium was performed in Erlenmayer flasks and in a 10-L mixing bioreactor. The liquid substrate consisted of glucose as a source of carbon, yeast extract, and polypeptone as sources of nitrogen. Optimal growth conditions in a bioreactor were as follows: temperature 28°C, air flow 5 L/min, mixing speed 220 rpm. Mycelium was grown in the form of pellets from which intracellular polysaccharides were isolated. After 30 days of cultivation in Erlenmayer flasks, 1.8 g of fresh biomass (36 g/L) or 0.9 g of dry biomass (18 g/L), was obtained. The yield of total polysaccharides was 3.2 g/g dry biomass or 116.4 mg/L of liquid medium. After 60 days of cultivation in a 10-L bioreactor, the amount of isolated fresh biomass was 307.7 g (30.8 g/L), which corresponded to 167 g of dry biomass (16.7g/L), respectively. Extracellular polysaccharides (3.64 g) were isolated from the mycelium (0.037 g/g of dry biomass) and 1.29 g of dry intracellular polysaccharides (0.013 g/g of dry biomass), respectively. Isolated polysaccharides were separated by ion-exchange chromatography, gel filtration, and affinity chromatography. Five fractions of extracellular β-D-glucans were obtained, with a total mass of 6.7 mg (40.1 μg/g dry biomass or 4.0 μg/L liquid substrate) and 2 fractions of intracellular β-D-glucans with a total mass of of 4.9 mg (29.3 μg/g dry biomass or 0.5 μg/L liquid substrate).

Immunostimulatory effects of isolates were tested on the induction of cytokines (TNF-α, IFN-γ) synthesis in primary cultures of human mononuclear cells (PBMC) isolated from a buffy coat. The TNF-α inducing activity is comparable to romurtide, which has been used as a supporting therapy in cancer patients treated with radiotherapy and/or chemotherapy.

The above experimental results confirmed that *G. frondosa* biomass can be successfully produced in a bioreactor by solid-state or submerged cultivation on artificial substrates. Both cultivation methods were suitable for the production of fungal polysaccharides, including β-D-glucans, which are the most important

active compounds of *G. frondosa*. Compared to submerged cultivation, solid state cultivation seems to have some advantages: higher biomass yield, lesser susceptibility to bacterial infections, and a potential for using agricultural wastes as effective lignocellulosic substrates for the production of *G. frondosa* biomass.

An Overview of the Traditions on Edible and Medicinal Mushrooms in Mexico

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Mexico has a high diversity of fungi due to its complex territory, which has a great variation of weather and, consequently, complex vegetation. This vegetation is formed from tropical forests in the lowlands in both the Pacific and Gulf of Mexico coasts, until coniferous forests in the high mountains, up to 4,000 m in altitude. Between these tropical and coniferous regions, at 1000–1800 m in altitude, there is a temperate humid zone, covered by cloud subtropical forests, which is the richest in biodiversity. In contrast, the north of the country is covered by xerophytic vegetation, forming true deserts in the NW. Besides the complex orography and weather of the country, its peculiar geographical situation is important. Mexico is situated between two biogeographic zones, the Neotropical in South America, and the Boreal in North America. Both regions make contact in Mexico with a mix of species of both zones, and also favor the existence of endemic species. However, this rich biodiversity of Mexico is still poorly studied. There are probably more than 20,000 species of fungi in the country, but only 6% is already known, mainly in temperate regions. The tropical mycobiota is still poorly known. Nevertheless, there are, in Mexico, great traditions in the use of mushrooms as medicine, as food, and in religious ceremonies; all of these uses were in place before the Spanish conquered the country in the 16th century. Unfortunately, these traditions have influenced the strong evolution of modern civilization. In Mexico, there are more than 200 common species of edible fungi, around 40 species of medicinal fungi, and more than 50 species of hallucinogenic mushrooms identified with more than 2,000 common names, both in indigenous dialects or in Spanish. This high number of common indigenous names is a consequence of the many traditional cultures that still survive through the country. In this lecture, the diversity of fungi used in Mexico, mainly in traditional medicine, will be discussed.

Some edible fungi, commonly sold in the popular markets, are also used in traditional medicine, e.g., *Clitocybe gibba, Lactarius indigo, Langermannia gigantea, Lycoperdon perlatum, Pleurotus djamor,* and *Ustilago maydis*. Several polypores are also used in traditional medicine such as *Pogonomyces hydnoides*, *Pycnoporus sanguineus,* and *Trametes versicolor*. There are more than 40 types of illnesses that people combat with fungi, among them: constipation, conjunctivitis, diabetes, diarrhea, dysentery, epilepsy, grains on the skin, and pneumonia. Several lichens species of genera *Dictyonema, Pseudevernia, Pseudoparmelia, Ramalina, Usnea,* and others are pectoral or used to combat ulcers in the mouth or for digestive problems. Among the hallucinogenic mushrooms belonging to the genus *Psilocybe*, some species are used to fight toothaches, as well as their use in religious ceremonies. Concerning the hallucinogenic species of *Psilocybe,* it is interesting to observe that 90% of these mushrooms grow in cloud subtropical forests situated between the tropical and boreal boundary. Other fungi are used due to traditional beliefs by indigenous people, such as *Geastrum saccatum* to combat the evil eye. In this way, the report by Heim and Wasson (Arch Mus Nat d'Hist Nat, 1967, Ser 7, 9) on some lycoperdaceous mushrooms used by Mixe Indians to hear voices is not true, as discussed by Ott et al. (Bol Soc Mex Mic, 1975, 9:67–76). All the species reported by Heim and Wasson used by the Indians to hear voices, such as *Lycoperdon candidum* and *Vascellum pretense,* are edible mushrooms, without any nervous effects. *Lycoperdon perlatum* in other indigenous cultures is used against wasp stings, which the author used himself with excellent results.

Medicinal Mushrooms and Drug Discovery: Identification of Potent Inhibitors of Thrombin, of Pancreatic Lipase, and Novel Antibiotic and Antigenotoxic Compounds

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Medicinal mushrooms have been used as natural medicines or dietary supplements for thousands of years. The most known and traditionally used mushrooms that exhibit some biological activity are the following: tinder polypore (*Fomes fomentarius*), quinine conk (*Polyporus officinalis*), fly agaric (*Amanita muscaria*), honey mushroom (*Armillaria mellea*), reishi (*Ganoderma lucidum*), maitake (*Grifola frondosa*), shiitake (*Lentinus edodes*), and oyster mushroom (*Pleurotus ostreatus*).

In order to find some novel physiological effects of mushrooms, we decided to perform a screening of more than 300 different species grown in Central Europe.

In the first part of our study we searched for the inhibitors of pancreatic lipase in methanol-water extracts from the fruiting bodies of 92 different species of mushrooms belonging to higher Basidiomycetes. The highest inhibitory activity was found in *Lyophyllum connatum.* In a similar study, a total of 120 methanol and dichloromethane extracts from 60 species of wood-damaging fungi and 50 methanol/water extracts from macrofungi, were screened for inhibition of pancreatic lipase using the chromogenic substrate p-nitrophenyl-palmitate. Of the extracts screened, those form *Laetiporus sulphureus, Tylopilus felleus,* and *Hygrocybe conica* exhibited the highest lipase inhibitory activities of 83%, 96%, and 97%, respectively.

Additionally, inhibitory activities of mushroom extracts on thrombin and trypsin were measured. Thrombin is the key serine protease of the coagulation cascade and therefore a suitable target for the inhibition of blood coagulation. The inhibitory activities of extracts from 95 Basisiomycetes species have been determined. The majority of samples inhibited trypsin and thrombin with various potencies, however, some extracts showed no activity against one or both of the enzymes. An aqueous extract of *Gleophylum odoratum* exhibited high inhibitory activity on both thrombin and trypsin (72% and 60%, respectively), while extracts of *Clitocybe gibba, Amanita virosa, Chantarellus lutescens, Suillus tridentinus, Hypoloma fasciculare*, and *Lactarius badiosanguineus* considerably inhibited thrombin (49, 48, 36, 34, 32, and 31%, respectively).

In an attempt to find some mushrooms with potential antibiotic properties, a *Vibrio fischeri* bioluminiscence test was developed. From 89 species of mushrooms and 12 species of endophytic fungi, extracts from *Truncatella hartigii* and *Amanita virosa* were found to have antibacterial activity.

In summary, several novel compounds or extracts were found that possess some promising pharmacological effects. These findings confirm the well known prediction that medicinal mushrooms represent an almost unlimited source of starting compunds for further discovery of new drugs.

Medicinal Mushrooms—Their History, Present Use, and the Possibility of Becoming Relevant in Germany

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Officially, in Germany we do not have medicinal mushrooms. We only have useful mushrooms and some of them also have a medicinal effect – in other words – healing properties. If we speak about medicinal mushrooms, the pharmaceutical industry in my country and its lobby would intervene until the German authorities produced an appropriate regulation to classify mushrooms as medicine. This would result in a long-lasting and extremely expensive process of registration before *Ganoderma lucidum* or *Grifola frondosa,* for instance, could be used. This should be avoided. Therefore, we deal with mushrooms which count only as foodstuffs, e.g., asparagus, tomato, lemon, apple and others, which all have specific healing properties.

No doubt East Asia, especially China, is leading in the cultivation and use of medicinal mushrooms. However, Europe, especially Germany, has also had a long tradition in this field. Already antique scholars reported on medicinal applications of *Laricifomes officinalis*. Also, later in the Middle Ages, we have reliable reports on the use of mushrooms in herb preparations for healing purposes. In "Codex Manuscriptus Medicinalis", published in 795 A.D in Germany and also in the writings of Hildegard von Bingen, the outstanding German natural scientist in the 12th century, the use of mushrooms, mainly these of Agaricum as part of herb mixtures, is well documented. The following species at least were used in those days: *Fomes fomentarius, Laricifomes officinals* (Agaricum), *Langermannia gigantea* (Giant Puff Ball), *Auricularia auricula-judae* (Jew´s Ear), *Amanita muscaria* (Fly agaric), *Phallus impudicus* (Stinkhorn), *Boletus satanas,* and *Armillaria mellea* (Honey Fungus).

In modern times the book "The Healing Power of Fungi – Healthy with Mycotherapy", published in 1997, set up a guidelines toward the use, sale, and manufacturing of medicinal mushroom products. Nowadays, in Germany, several small companies have gone into business dealing with this subject.

For the future we expect a constantly increasing demand for powder and extracts of so-called medicinal mushrooms. Although these products are only used as food supplements, consumers like them, medical doctors and practitioners of alternative medicine recommend them, and in the meantime, all involved have learned a lot about their very satisfying therapeutical effects. I believe using mushroom powder and extracts for maintaining good health and also curing different disorders has been firmly established in Germany.

The question is how we can handle the status of these mushrooms and the products made from them. Can we establish nutriceuticals as a new class of products, or can we use them alternatively as food supplements? Maybe, in the future, some of them can also be registered as medicine. It is a very exciting development. However, it is, unfortunately, not likely that a constantly increasing use of medicinal mushroom by German consumers could help to establish relevant medicinal mushroom farms in Germany or, generally, in Europe. The tremendous advantages of Chinese suppliers, especially by offering low prices on the one hand, and providing good-quality material on the other hand, cannot be beaten, at least in the next two decades. Noteworthy is the economic exchange with China has a high priority, also from a political point of view in most industrialized countries. Therefore, it seems to be very clear: we are going to buy and use Chinese fungal material as a source for nutriceutical production in Germany.