GENERAL LECTURES

Antihyperlipidemic Effect of *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm. in HIV: Results of a Pilot Proof-of-Principle Clinical Trial

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Antiretroviral treatment regimens in HIV patients commonly include low-dose ritonavir with another protease inhibitor (PI). The addition of ritonavir to some PIs "boosts" their blood concentration and improves the efficacy of the regimen. However, use of PIs, especially ritonavir, causes significant lipid elevations in many patients, including both increases in triglycerides as well as cholesterol. Standard treatments for hypercholesterolemia include the HMG CoA reductase inhibitors, or "statins." Because many PIs and statins share a common metabolic pathway that uses the CYP3A4 enzyme system, coadministration of ritonavir with most statins increases statin levels significantly. This increases the likelihood for adverse effects, including elevated liver function tests and muscle breakdown, which, if left untreated, may progress to renal failure.

A safe and effective alternative to statins for treatment of hyperlipidemia in patients on ritonavir-containing antiretroviral regimens would be of value. *Pleurotus ostreatus* has been shown in animal models to decrease lipid levels, a finding that has been supported by preliminary data in a small study in humans. Our pilot study was designed (1) to determine whether there are detectable lipid-lowering effects of *P. ostreatus,* specifically in patients with HIV and hyperlipidemia, who are taking ritonavir in combination with another PI; (2) to assess whether the concomitant administration of daily *P. ostreatus* and such regimens in this population is safe; and (3) to investigate the mechanism of action by which *P. ostreatus* may exert an antihyperlipidemic effect.

Pleurotus ostreatus was cloned and maintained *in vitro* at the spawn laboratories before being expanded into edible mushrooms. Clusters of young mushrooms were harvested and flash-frozen. We designed a single-arm, open-label study of 8 weeks' duration with a target enrollment of 20 subjects. Study participants (patients) with ritonavir-induced elevated LDL cholesterol levels (>160 mg/dL) were eligible to enroll. After screening and obtaining informed consent, patients were admitted to the inpatient General Clinical Research Center (GCRC) at San Francisco General Hospital for a one-night stay to obtain pretreatment PI blood levels. Patients were given their first mushroom dose in the GCRC and then received packets of freeze-dried *P. ostreatus* (15 gm/day) to be administered orally each day for the 8-week trial period. Patients were followed with lipid levels drawn every 2 weeks to assess efficacy. Safety assessments include a pharmacokinetic substudy to determine if *P. ostreatus* alters the hepatic metabolism of the PI, self-reported incidence of muscle aches, and measurement of liver and muscle

enzymes. HMG CoA reductase inhibition activity after *P. ostreatus* ingestion will be measured.

Seven of the 20 patients have completed the trial to date with no safety issues observed. The goal is to fully enroll the study by the end of summer 2005. Efficacy and safety data will be available at the time of presentation.

ACKNOWLEDGMENTS

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Selenium Enrichment of *Grifola frondosa (Dicks.:Fr.)* S.F.Gray (Maitake) Mushrooms

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Grifola frondosa (Dicks.:Fr.) S.F.Gray (Maitake) is a popular culinary mushroom that is well known to contain unique β-glucans, which have potent immune-modulating and antitumor properties. However, they generally contain low levels of important micronutrient minerals, such as selenium. Selenium has recently generated great interest in nutritional and medical research, because it serves as a cofactor for the enzyme glutathione peroxidase, which is involved in quenching free radicals. Thus, the objective of this study was to determine if *G. frondosa* can be enriched with selenium by addition of sodium selenite to the growth substance, as was done previously with *Agaricus bisporus* (J.Lge) Imbach. Two separate crops were grown using a sawdust-based synthetic medium with various levels of selenium added by addition of appropriate amounts of sodium selenite prior to pasteurization and inoculation. Basidiomes were harvested from the treated substrates, weighed for yield determination, freeze-dried for solids determination, and than analyzed for selenium content using graphite-furnace atomic absorption spectrophotometry. Yield was not significantly affected by addition of selenium to the substrate, but basidiomes were harvested 5–7 days earlier when selenium was added. No selenium (<0.5 μg/g) was detected in the untreated (control) substrate and the basidiomes harvested from it. Selenium increased in the basidiomes in direct response to levels added to the substrate. Treated substrates contained 0.6, 2.4, and 7.2 μg/g (d.w.) selenium and basidiomes harvested from them contained 0.6, 2.2, and 9.3 μg/g (d.w.), respectively. These results indicate that *G. frondosa* can be predictably enriched with selenium in a manner similar to *Agaricus bisporus* to become an excellent nutritional source of selenium. Also, production of Maitake extracts or powders for nutraceutical or medicinal purpose that can contain significant levels of selenium in addition to β-glucans would be possible.

Mushroom Poisoning in North America

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Americans remain a very mycophobic people, although in the last 30 years popular mushroom identification field guides, a journal for amateurs (*Mushroom: The Journal*), and more than 64 mushroom clubs have led to a growing number of mushroom enthusiasts. In the Northwest, commercial mushroom picking has become economically important, with many pickers out gathering in the wild, sometimes with little or no knowledge of mushroom identification or mushroom ecology. All of this occurs with relatively few cases of mushroom poisoning, although a parent finding a child with half a mushroom dangling from his mouth will inevitably lead to panic.

In order to assist doctors, who are often unfamiliar with mushroom poisoning, and to better inform the public, the North American Mycological Association (NAMA) maintains a continent-wide group of identifiers who stand ready to assist doctors and poison centers in identifying mushrooms suspected of causing poisoning. NAMA provides educational materials on mushroom identification and information on diagnosis and treatment of mushroom poisoning and promotes the reporting of mushroom poisoning and the treatment used.

The challenge we face is to get a more complete reporting of poisonous incidences in order to understand the frequency and severity of mushroom poisoning in North America. New patient confidentiality rules also inhibit reporting of poisonings, even though the reports do not involve identifying individuals involved.

What is clear is that the majority of cases reported to poison centers involve ingestion of mushrooms by children where there are no adverse symptoms. Cases involving symptoms typically involve gastro-intestinal distress. The distress is at times severe and may leave the individual in a weakened state for a week or more. The main threat of death from mushroom ingestion is from highly toxic species by young children and by individuals not otherwise in good health. Adults in good health normally survive ingestion of even the deadliest mushrooms provided they get prompt, good medical treatment and provided that they have not consumed a huge amount of the toxic species.

A major challenge in North America is to educate recent immigrants who may make mushrooms a significant part of their diet and may pick toxic species that resemble edible species from their home country.

This presentation will focus on characteristics of mushrooms in North America that cause substantial numbers of poisoning, with an emphasis on cases in the past 4 years.

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Chemopreventive Properties of Mushrooms Against Breast Cancer and Prostate Cancer

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Previous research from our laboratory has found mushrooms, including white button mushrooms, containing phytochemicals that can suppress aromatase/estrogen biosynthesis. Aromatase is the enzyme that converts androgen to estrogen. An abnormal expression of aromatase in breast tissue is considered to be a risk factor for breast cancer. In our laboratory, we have found that of the seven vegetable extracts tested, mushroom extract was the most effective in inhibiting the activity of human placental aromatase activity. Cell culture experiments were performed to further evaluate the anti-aromatase and anti-breast-cancer activity of mushrooms. Our laboratory has prepared one breast cancer cell line, MCF7aro. This cell line is ER positive/aromatase positive and demonstrates increased cell proliferation in the presence of testosterone. The addition of mushroom extract decreased the advantage gained by the addition of testosterone to a similar level as seen with 4-OHA, a known aromatase inhibitor. Furthermore, mushroom extract was found not to affect the proliferation of MCF-10A, a noncancer cell line. These findings suggest that the inhibitory effect of white button mushroom extract is through a specific anti-aromatase action, not a cytotoxic effect.

To better understand the cancer protective effects of mushrooms, our laboratory decided to characterize the anti-aromatase chemicals and to investigate the *in vivo* action of mushroom extract. Three sets of animal experiments have been conducted, and the results suggest that the oral intake of mushroom extracts might slow down MCF-7aro-derived tumor growth in nude mice. Histological examination of the tumors revealed that the levels of apoptosis between tumors from the control and mushroom-extract–fed animals were similar, once again indicating that the tumor-suppressing effect of mushroom extract is not through a cytotoxic effect. These results significantly indicate that these phytochemicals in mushroom are orally active and maintain their activity after ingestion. Preliminary studies from this laboratory have found more than one chemical in mushrooms that can inhibit aromatase, and some of them may be fatty acid derivatives. The exact nature of the active chemicals is not yet determined.

A series of *in vitro* and *in vivo* experiments have also been carried out to demonstrate that white button mushrooms can be a chemopreventing agent against prostate cancer. A 20% methanol extract of white button mushrooms has been found to contain phytochemicals that suppress steroid 5α-reductase and aromatase. Steroid 5α-reductase converts testosterone to dihydrotestosterone (DHT) and has been shown to play an important role in the development of prostate cancer and benign prostate hyperplasia. The use of steroid 5α-reductase inhibitors has been found to decrease the incidence of prostate cancer. Cell culture experiments involving cells treated with mushroom extract for 10 days have been carried out. Through these experiments, we have revealed that white button mushroom extract has the ability to

suppress the growth of hormone-resistant prostate cancer cells such as PC-3 and DU145 as well as hormone-dependent LNCaP cells in a dose-dependent manner. The mushroom extract was found not to affect the proliferation of normal prostate epithelial cells.

We have also carried out *in vivo* chemoprevention studies using prostate cancer cell-implanted male athymic nude mouse models. There were two groups of mice gavaged with two different concentrations of mushroom extract and also a pair-fed control group that was gavaged with water. From this *in vivo* study, we demonstrated that our mushroom extract decreased tumor size in a dose-dependent manner. Therefore, our findings on white button mushrooms indicate that the intake of mushrooms could reduce the incidence of breast cancer and prostate cancer. An effective chemopreventive agent should not significantly alter quality of life and is ideally inexpensive, safe, and well tolerated. This prevention method should be readily available and affordable to the general population, including underserved populations.

While it is exciting to find that mushroom extract contains anti-aromatase and anti-5α-reductase chemicals, it is reasonable to think that mushroom chemicals also affect other cellular pathways. As the first step, we have performed gene expression microarray analysis on MCF-7aro tumors from the mushroom-fed animals and those from the control animals. It was our goal to identify additional gene targets whose expression could also be modulated by the mushroom diet. We have performed the analysis using Affymetrix Human Genome U133A among three biological replicates—i.e., RNAs were isolated tumors from three individual animals and analyzed separately. The intensities of the probe signals on GeneChips were statistically normalized following a background noise subtraction and were analyzed using algorithms in R/Bioconductor. We compared more than 22,000 gene expressions using two-sample *t* tests and applied the Benjamini–Yekutieli *p*-value correction to control the false discovery rate. Using a minimum fold change criteria of 1.2 and an adjusted *p*-value cutoff of 0.05, we identified 515 genes that were upregulated and 1805 genes that were downregulated in tumors from mushroom-fed mice versus those from control mice. The results indicate that a higher percentage of signal transduction genes are upregulated, and a higher percentage of genes involved in DNA processing and transcription/translation are downregulated in tumors from mushroom-fed mice versus those from control mice. A careful evaluation of the results from microarray analysis will yield novel insights into the mechanisms as well as important signal-transduction pathways regulated by the phytochemicals in white button mushrooms.

Recently, we extended our studies on other medicinal mushrooms. *Ganoderma lucidum* (W.Curt.: Fr.)Lloyd and several strains of *Fomitopsis officinalis* (Vill.) Bond. et Singer have been found to contain chemicals that can suppress aromatase and steroid 5α-reductase.

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Lignocellulose and White-Rot Basidiomycetes: Some Strategies for Their Potential Utilization

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Lignocellulose composes more than 60% of plant biomass produced on earth. In addition, municipal and agro-industrial waste generation is fast increasing and causing pollution and many other environmental problems. The agro-industrial waste is a potentially vast source of energy, substrate for mushroom productions, biofertilizers, animal feed, enzymes, and other biochemical products. The most efficient decomposers of lignocellulose are the white-rot fungi because of the capability of these fungi to synthesize the relevant hydrolytic (cellulases and hemicellulases) and oxidative (ligninolytic) extracellular enzymes required to degrade the major components of the substrate—i.e., cellulose, hemicellulose, and lignin—into low-molecular-weight compounds that can be assimilated in fungi nutrition.

Recently, extensive research on these fungi has been conducted with the aim of isolating new organisms with tremendous secretion of ligninolytic enzymes as well as enzymes with properties important for their industrial application in the bioremediation of polluted, hazardous xenobiotic soils and industrial waters; biobleaching and biopulping; the textile, dye, and food industries; biotransformation of pharmaceutical and other intermediates; biosensor construction; cosmetics; medicine; analytic biochemistry. All these biotechnological applications require huge amounts of enzymes. However, their application is hindered because the enzymes that are presently being investigated are still expensive because of the low yield and high cost in production and isolation. Overexpression of laccase and peroxidases in heterologous systems has not been achieved yet, and they still have to be obtained from natural sources. Therefore, the search for powerful producers of enzymes and the development of alternative technologies for enzyme production are mandatory. Consequently, an overview of various approaches will be presented aiming to stimulate the production and yield of target enzymes as well as the analysis of the studies proving that the regulation of lignocellulolytic enzymes synthesis appears to be subject to the complex effects of nutritional, environmental, temporal, and genetic factors. Understanding of physiological mechanisms regulating enzyme synthesis in lignocellulose bioconversion could be useful for improving the technological process of edible and medicinal mushroom production.

Special attention will be paid to edible and medicinal mushrooms of the genus *Pleurotus* and *Lentinus edodes* (Berk.) Singer, which serve as the basis for various biotechnological and environmental applications. Some of these applications are traditional and are practiced throughout the world, and some need further research and development. Cumulative evidence indicates that the selection of an appropriate cultivation technique and plant residue adequate for fungus growth and target enzyme synthesis plays an important role in the development of an efficient biotechnology. Cultivation of *Pleurotus* strains and *L. edodes* on wheat straw, tree leaves, and cotton wastes as growth substrates showed clear physiological and biochemical changes taking place during plant raw material colonization and

fructification. The vegetative growth of these edible mushrooms is characterized by the comparatively low activity of polysaccharases and high activity of ligninolytic enzymes. During the period of fruiting body formation, when mushroom growth demands a large quantity of plastic materials and energy, the secretion of cellulases and xylanase sharply increases to accelerate polysaccharide hydrolysis. In contrast, ligninolytic enzyme production appears to be, to some extent, repressed in preventing the expenses of constructive materials and energy.

In addition, the investigation of the hydrolytic and oxidative enzyme production during *Pleurotus* spp. and *L. edodes* cultivation is mandatory, because after harvesting the fruit bodies the residual spent substrate may become a cheap source of lignocellulolytic enzymes for several biotechnological applications, and it may be used as a bioremediation agent.

The Changing Scientific Names of Edible and Medicinal Mushrooms

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Scientific names in use for edible and medicinal mushrooms can change as a result of new research on relationships and species limits, issues relating to the international rules controlling the publication of scientific names, or even incorrect identifications. Sadly, not all mycologists explore or utilize all the options now available under the internationally agreed rules (*The International Code of Botanical Nomenclature*) to avoid making name changes before publishing them. Progress in the development of the rules to these ends is considered in the light of actions and discussions at the International Botanical Congress in Vienna in July 2005. Issues of changing names are a problem across the whole of biology, and various ways of limiting changes have been proposed and debated—for example, by establishing protected lists of names and requiring the registration of newly published names. Mycologists have also recently launched MycoBank, a freely available database containing descriptions and illustrations and assigning each species of fungus a unique reference number (to be allocated before hard-copy). Initiatives are being taken by the Global Biodiversity Information Facilty (GBIF), a body established by the Convention on Biological Diversity, to develop and support schemes to improve, harmonize, and simplify approaches to scientific names across all groups of organisms. The International Society for Mushroom Science (ISMS) and the International Commission on the Taxonomy of Fungi (ICTF), as well as regional and national mycological societies, should be involved in and contribute to the important debates and actions now underway or being planned. Nevertheless, much will always depend on the integrity, responsibility, and actions of individual mycologists.

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Medicinal Value of Turkey Tail Fungus *Trametes versicolor* (L.:Fr.) Pilát (Aphyllophoromycetideae)

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Trametes versicolor, formerly *Coriolus versicolor*, is a common fan-shaped polypore fungus of dead and dying trees throughout the world, which is an important part of forest ecology as a primary decomposer of hardwood. The fungus is characterized among the white-rot basidiomycetes. *T. versicolor* produces a laccase used to detoxify xenobiotics such as polychlorinated biphenyls, dyes, and a variety of synthetic polymers and as a pulp biobleach for making paper.

Turkey tail is arguably the best-researched medicinal mushroom, with a number of controlled clinical trials demonstrating increased long-term survivability in patients with gastrointestinal cancers, and to a lesser degree other cancers, with oral application of concentrated extracts of the fruit bodies and mycelium, particularly PSK and PSP. These commercially produced extracts are often prescribed along with chemotherapy for treating cancer in Japan and are paid for by national health care.

PSK and PSP have demonstrated a wide range of immunological effects *in vitro* and *in vivo,* particularly reticuloendothelial system activation, cytokine modulation (IFN-γ production, IL-2 production), enhancement of dendritic cell viability, T-cell maturation, natural killer cell activity, antibody production, and antitumor and anticancer effects. The extracts can inhibit carcinogenesis and tumor cell growth by activating cancer cell apoptosis.

Current clinical use in North America, Europe, and Asia of products containing turkey tail extracts and their use as health food supplements has rapidly increased in the last few years, with many types of products becoming available. They are often recommended as supportive treatment for their supposed immunorestorative effects in patients with chronic conditions such as various cancers and viral syndromes such as hepatitis C and for its hepatoprotective effects.

Since 1990, a number of controlled clinical trials have been performed in Japan with PSK as a supportive treatment in hospitals and clinics for treating patients with a variety of cancers, particularly colorectal and stomach cancer, but also breast and lung cancers. PSK is given orally, always along with various chemotherapeutic regimes. In several of these multicentric trials ($n = 103–462$), the 15-year survival rate was increased by 10–15%, and side effects of chemotherapy, such as nausea and anorexia, were reduced when PSK was given along with chemotherapy. Improvements in immune functions such as blood leukocyte and neutrophil counts, serum IgG and IgM, and body-mass index were seen in patients receiving turkey tail extracts with chemotherapy vs. chemotherapy alone.

Fundamental questions about the effectiveness of turkey tail extracts for life prolongation and mitigation of side effects from chemo- and radiotherapy in cancer patients, as well as its effectiveness as an immunorestorative and antiviral treatment in patients with chronic viral syndromes, remain to be fully answered. The dose, the solvents used for extraction, the source of starting material, the question of whether fruiting bodies or more easily

obtained mycelium cultures should be used, and the duration of treatment have only been partially clarified. Because ubiquitous cell wall components of fungi (including yeasts) and bacteria, (1→3)-β-D-glucans, are thought to be among the most important active constituents, a careful review of the large body of existing literature on the cellular mechanisms of effects on the immune system with oral exposure of these compounds has to be carefully considered.

Cancer Risk Reduction by Intake of Mushrooms and Clinical Studies on EEM

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An epidemiological survey was carried out concerning farmers producing *Flammulina velutipes* (W.Curt.:Fr.) Singer "Enokitake" from 1972 to 1986 in Nagano prefecture, Japan. Cancer death rates of the farmers were lower than those of the average rate of total Nagano prefecture. Intake of edible mushrooms was suggested as being effective for risk reduction of cancer.

This was an ecological study. Next, a case-control study was made to elucidate the relationship between risk reduction of cancer and intake of edible mushrooms on stomach cancer in the same prefecture from 1998 to 2002. The odds ratio (OR) of subjects who were practically not ingesting mushrooms at all was 1.00; the OR of those ingesting *Hypsizygus marmoreus* (Peck) H.E.Bigelow (Bunashimeji) or *Pholiota nameko* (T.Ito) S.Ito et S.Imai (Nameko) more than once a week was 0.57 and 0.56, respectively. Whereas the OR of subjects taking mushrooms less than once a week was 1.00, the OR of those taking *Flammulina velutipes* more than three times a week was 0.66, and the OR of those taking *Lentinus edodes* (Berk.) Singer "Shiitake" more than three times a week was 0.95. It can be concluded that the intake of *Hypsizygus marmoreus, Flammulina velutipes,* and *Pholiota nameko* has the possibility of decreasing stomach cancer incidence.

Studies on antitumor activities of mushrooms were carried out in the National Cancer Center, Japan. Many antitumor polysaccharides and protein-bounded polysaccharides were isolated from mushrooms.

Based on these studies, extracts were made from *Hypsizygus marmoreus* and *Flammulina velutipes,* and a preparation called "EEM" (extracts of edible mushrooms) was supplied. Clinical studies of EEM were performed to investigate the effectiveness for cancer patients. Positive effects of EEM were studied on the cachexia of advanced cancer patients in comparison with MPA (methylacetoxyprogestrone). EEM revealed better results than MPA in clinical response, performance status (PS), and quality of life (QOL). Another clinical study was made with a combination therapy of EEM and cancer chemotherapy agents for advanced cancer patients. The clinical response rate, PS, and QOL of the patients treated with combination therapy (EEM and anticancer drugs) were better than those of the patients treated with anticancer drugs alone. The positive effects of EEM were also investigated for a precancerous lesion on the esophageal mucosa. After the patients of grade II of atypical hyperplasia on the esophageal mucosa were administered EEM tablets for 6 months, a positive effect of EEM was found.

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Protecting Intellectual Property Assets of Mushroom Genetic Resources for Invention and Innovation

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The concept of intellectual property (IP) allows people to own creativity and innovations in the same way as physical property. An IP asset is any codified knowledge, innovation, or practice of actual or potential economic value, associated with fundamental research, analysis, and manipulation of biological systems, biological prospecting, industrial applications, or commercial use. Mushroom genetic resources are currently being utilized and exploited by the pharmaceutical, cosmetic, agricultural, food, enzyme, chemical, and waste-treatment industries. Nevertheless, the role of IP assets in today's knowledge-driven enterprises is frequently overlooked, despite their potential as sources of both monetary value and financial gain. IP rights are often under-managed or under-leveraged. The challenge is how to create, protect, and extract value from IP assets for invention and innovation.

In the valuation of IP assets, it is important to differentiate between "invention" and "innovation." Because an invention may not involve the commercialization of new ideas, it is obvious that not all inventions result in innovation. Innovation is an interactive process of effectively creating, managing, and leveraging an invention and successfully bringing new products to market. Therefore, IP assets can be used effectively to sustain mushroom production and business development.

The IP assets of mushroom genetic resources are divided into three basic yet interdependent components: (1) intellectual material, (2) intellectual capital, and (3) intellectual property. Intellectual material provides both tacit and codified knowledge of mushroom genetic materials including intangible content (*genetic information*), implemented know-how (*product/process innovation*), and knowledge databases (*bioinformatics*). Intellectual capital encompasses various kinds of knowledge and innovative ideas that are mainly driven by, and derived from, individuals or groups of scientists and technologists. Intellectual assets with legal protection become intellectual property. An IP right is thus a legal right granted or registered under the relevant national or regional law by the relevant IP office. Codified knowledge and know-how form a body of interactive IP assets that relates directly to product/process innovations and codified ideas of their creators and users.

It is often not possible to protect IP assets and gain IP rights unless they have been applied for and granted or registered through an IP system. IP assets that can be protected by formal legislation are patents, trademarks, copyrights, and plant breeders' rights. Know-how and trade secrets are forms of IP that are not protected by formal legislation but have national and international value. As with any property, IP assets possess specific dollar value and can be bought and sold.

One of the methods of gaining the value of mushroom genetic resources through IP is with a material transfer agreement (MTA). An MTA is a contract generally utilized when any proprietary

material and/or information is exchanged with "trade secret" protection as embodied, for example, in various state laws in the United States. It defines the rights of the provider and the recipient with respect to the mushroom materials and any replicates or derivatives. A replicate is any mushroom material that represents a substantially unmodified copy of the original material and includes material produced by the growth of cells or amplification of material. A derivative is material created from the original material that is substantially modified to have new properties. Derivatives include recombinant DNA clones made using a vector. However, know–how and trade secrets are, in certain cases, protected contractually by the application of certain legal concepts and statutes, such as customary laws and practices. Signed contracts such as MTAs are the most powerful tools in trade secret and customary laws in which registration is not required.

Today, the new challenge for mushroom scientists and technologists is to realize that IP should no longer be considered as an inert legal title, but as an economical asset that can realize the potential of knowledge and creativity. PI assets are tools that maximize the value of special knowledge, either by providing data or information or by delivering expertise and augmentation to mushroom scientists and technologists. Concerns about access to the IP assets of mushroom genetic materials have recently surfaced because of the reluctance of IP owners to allow their use for research that may lead to commercial applications. IP holders need assurance that access will be controlled. A number of bilateral and multilateral initiatives have been implemented to protect IP assets among the proposed users. All agreements are negotiated in a manner that is coherent and mutually supportive of national and international laws, local customs, rules, and regulations and implemented through collaborative action by governments, appropriate organizations and professional societies, field collectors and their sponsors, and curators and users of mushroom genetic resources.

Although the Convention on Biological Diversity (CBD) establishes principles of access to, and benefit sharing of, biological materials, it does not establish any specific mechanisms for such activities. Several factors influence the design of mechanisms for access and benefit sharing. Two are of particular importance: (1) how clearly the target for access and sharing can be defined, and (2) whether the transaction costs associated with more precise targeting outweigh the benefits to be shared. At the same time, expectations of contingent returns must be tempered by the real probabilities of discovery of new applications. Different contractual arrangements have been designed for different resources and different uses. Negotiation of legal and policy tools for formulating and obtaining *mutually agreed terms* following *prior informed consent* can include many of the following: (1) access agreement, (2) confidentiality agreement, (3) material transfer agreement, (4) research agreement, (5) intellectual property protection agreement, (6) co-development agreement, (7) technology licensing agreement, (8) distributorship agreement, (9) commercial agreement, and (10) benefit-sharing agreement.

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Anti-MRSA Compounds from Hericium erinaceus (Bull.:Fr.) Pers.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently one of the most prevalent pathogens in nosocomial infections. Because hospital-acquired MRSA strains exhibit resistance to many antibiotics and are transmitted from patient to patient via transiently colonized hands of hospital personnel, MRSA infections pose a serious problem for hospitalized patients.

We found that extracts of the fruiting bodies and the mycelia of *Hericium erinaceus* (Bull.:Fr.) Pers. exhibited anti-MRSA activity. Therefore, we tried to isolate the anti-MRSA compounds from the fungus.

Isolation of the active compounds was guided by anti-MRSA activity. The fungus was cultivated by shaking at 30˚C for 4 weeks. The culture was centrifuged, and the resulting residue was extracted with 85% ethanol, and the extract, after evaporating the solvent, was partitioned between chloroform and water and then ethyl acetate and water. Repeated silica gel chromatography and HPLC of the chloroform-soluble and ethyl-acetate–soluble parts gave five active compounds. The fruiting bodies of the fungus were also extracted with 85% ethanol, and the extract was concentrated and fractionated by solvent partitions between chloroform and water, and then ethyl acetate and water. Repeated silica gel chromatography and HPLC of the chloroform-soluble part gave an anti-MRSA compound.

As a result, erinacines A (1) and B (2) were isolated as anti-MRSA compounds from the mycelia

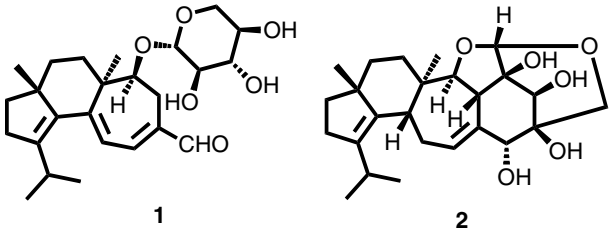

FIGURE 11. Structures of erinacine A (1) and erinacine B (2).

(Figure 1). These compounds have already been isolated as nerve growth factor (NGF) stimulators (Kawagishi et al., 1994, 1996). Determination of the structures and estimation of detailed biological activity of the other active compounds are now in progress.

In addition, a clinical test of this mushroom was done in a hospital in Japan, and MRSA in some patients disappeared when they were given this mushroom to eat.

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Hepatoprotective Effects of Waxy Brown Rice Cultured with *Agrocybe cylindracea* (DC.) Gillet

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The hepatoprotective effects of functional rice, produced by cultured *Agrocybe cylindracea* (Agaricomycetideae) on waxy brown rice, were investigated. After oral administration of the methanol extracts of functional rices for 14 days, rats were treated with carbon tetrachloride to induce hepatotoxicity. The enzyme activities were determined, and biochemical analyses of serum were carried out in order to examine the hepatoprotective effects exerted by the samples. A histological study on liver tissue using electron microsope was also conducted.

The body weights and internal organs weights of the rats were measured. The body weight of the group treated with only CCl_4 increased less than those of the groups fed with sample extracts prior to CCl_4 treatments. The weight of internal organs among the treatment groups did not show substantial difference except that the hepatic hypertrophy was observed in the group treated only with CCl_4. On the other hand, the COCIII group and the CIC groups administered with the methanol extracts from the waxy brown rice and cultured with *A. cylindracea*, respectively, showed an organ weight similar to that of the normal group.

The activity of serums AST and ALT of rats increased highly in the group treated with CCl_4 only. The COC and CIC groups showed a strong suppression on the AST and ALT augmentation almost to the level of normal rats without CCl_4 treatment. In particular, the CICII and CICIII groups were able to maintain both the AST and ALT activity in a manner similar to the normal group without CCl_4 treatment, and was statistically not significant.

The activity of serums ALP, LDH, and Y-GPT was significantly increased by carbon tetracholoride treatment, but methanol extracts of brown rice, cultured with *A. cylindracea,* significantly decreased the activity of those emzymes, which was supposed to be increased by the carbon tetrachloride treatment. CICII and CICIII groups especially demonstrated a strong protective effect by suppressing the increase of these enzymes.

The control group treated only with CCl_4 showed a severe decrease in albumin level, total protein, and HDL-cholesterol content in comparison with the normal group. The COC group and the CIC group considerably alleviated the decrease of those components. The content of total cholesterol, triglyceride in liver was increased by administration of CCl_4. However, the COC and CIC groups did not show the increases and kept them at almost the same level as the normal rats without CCl_4 treatment.

The content of lipid peroxide in the CCl_4-treated group significantly increased in comparison with the normal group, but the COCIII and CIC groups had a tendency to maintain lipid peroxide content at a much lower level.

The liver tissue of rats was observed by transmission electron microscope. The injury range of cells was shown to be widest in the control group treated with CCl_4 only. In groups administered with the extracts from waxy brown rice cultured with *Coprinus*

comatus (Müll.:Fr.) S.F.Gray and *C. cinereus* (Schaeff.: Fr.) S.F.Gray prior to CCl_4 treatment, diminished lipid degeneration and infiltration of local inflammation caused by CCl_4 were alleviated considerably. In particular, the CICIII group showed almost the same integrity as normal rats without CCl_4 treatment.

Medicinal Mushroom Substances as Cancer Molecular Therapy

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Medicinal mushrooms possess a variety of health promoting qualities as well as being a potential source of a variety of pharmaceuticals for some diseases, including cancer. Although most of the attention for their anticancer activity revolved around the activity of high-molecular-weight polysaccharides with no clear mechanism of action, our focus is on low-molecular-weight mushroom substances with a well-defined mechanism of action.

A neoplasm is an abnormal mass or colony of cells produced by a relatively autonomous new growth of tissue arising from the clonal expansion of a single cell that has undergone neoplastic transformation, which is usually accompanied by the loss of some specialized functions and the acquisition of new biological properties mediated by alteration in the expression or function of specific molecular targets. Our research interest is on mushroom substances that specifically modulate molecular targets implicated in carcinogenesis, especially in chronic myelogenous luekemia (CML) and prostate cancer (PCa).

CML is a malignancy of pluripotent hematopoietic cells characterized by distinctive cytogenetic abnormality resulting in the creation of a p210 Bcr-Abl fusion protein with increased tyrosine kinase activity.

Imatinib is a potent inhibitor of Bcr-Abl and is used as standard therapy for CML. Unfortunately, clinical efficacy continuously decreases with the advancement of the disease. Secondary resistance is mostly due to the acquisition of point mutations in Bcr-Abl, which argues for the need of developing alternative inhibitors of Bcr-Abl.

Medicinal mushrooms exhibiting selective anti-CML activity were selected. Active mushroom substances induced apoptosis and erythroid differentiation in CML cells and caused a reduction in Bcr-Abl levels. Focusing on mushroom #540 and #514, we show that medicinal mushroom substances were effective in inhibiting auto-phosphorylation from wild types as well as from imatinib-resistance mutants of Bcr-Abl.

PCa is the second leading cause of death in Western men. Primary PCa is hormone dependent and is manageable by hormonal therapy. However, it rapidly develops into hormone-refractory tumors due to the accumulation of mutations in the androgen receptor (AR) or to the acquisition of alternative cellular pathways that support proliferation and inhibit apoptosis of PCa in androgen-independent mechanisms.

Whereas PCa is very common in Western countries, its levels are very low in several countries in Asia. Several reports linked Eastern diets and cancer occurrence, especially for PCa. Of special interest is the implication of several mushrooms in the prevention of PCa in Asia.

We evaluate the ability of mushroom substances extracted from our collection of mushroom strains to interfere selectively with the activity of AR, the leading molecular target implicated in the development and maintenance of hormone-refractory PCa. Data showing anti-prostate cancer activity and the ability to modulate AR and other molecular targets will be presented.

Molecular Systematics of *Ganoderma:* What Is Reishi?

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Ganoderma is a very distinctive genus of white-rot polypore fungi that is primarily characterized by the formation of a double-walled, generally echinulate basidiospore. Most *Ganoderma* species are very variable macromorphologically and lack micromorphological distinctiveness. As a consequence, earlier taxonomic studies in the genus have created many synonymous names and have resulted in largely ambiguous species delimitation and identification systems, making species identification in the genus virtually impossible. *Ganoderma* strains used in oriental folk medicine refer to Reishi and have traditionally been labeled *Ganoderma lucidum* (W.Curt.: Fr.) P. Karst. in the scientific literature. However, there is now accumulative evidence that most species reported as *G. lucidum* in most of the pharmacological and phytopathological studies were wrongly identified.

G. lucidum was first described as *Boletus lucidus* by William Curtis in 1781 from a filbert plant (= *Corylus avellana*) in London, UK. Curtis's original collection (i.e., the type specimen) has been lost. The actual type is represented in a color plate, which is in agreement with the International Code of Botanical Nomenclature. However, the typified plate is of no use for providing cultural characteristics and genetic information that would be useful for distinguishing *G. lucidum* from the other British laccate *Ganoderma*.

Over the years, at least 166 laccate *Ganoderma* species have been described worldwide, of which at least 48 names were, at some point, considered to be synonyms of others. There is nevertheless a strong consensus about the true identity of *G. lucidum* among contemporary European mycologists. Molecular phylogenetic studies indicate that *Ganoderma* is a young genus in agreement with earlier morphological evidence as pointed out by the Norwegian mycologist Leif Ryvarden. DNA studies have shown that the *G. lucidum* species complex is composed of several species that can be difficult to distinguish from one another. These species include the European *G. valesiacum* Boud.; *G. ahamdii* Stey., described from Pakistan; North American *G. tsugae* Murrill and *G.oregonense* Murrill; and other taxa variously labeled *G. resinaceum* Boud., *G. oerstedii* Fr., and *G. praelongum* Murrill.

Recent genetic and biogeographic studies have indicated that most *Ganoderma* species are geographically restricted. *G. lucidum* is probably restricted to western parts of Europe, although its distribution range can possibly also include parts of Siberia and of north western regions of China. Based on molecular phylogenetic evidence, it appears that most collections labeled *G. lucidum* in North America do in fact best correspond to the taxon labeled *G. resinaceum* in Europe, whereas *G. tsugae* in North America is genetically very close to the "true" *G. lucidum* from Europe.

This leads to the question of what is the Oriental Reishi? A nonexhaustive molecular survey of taxa labeled *G. lucidum* in Asia, including strains com-

mercially cultivated for the production of health tablets or teas, shows that this name has been largely misapplied and encompasses many distinct species.

Incorrect taxonomic identification of *Ganoderma* strains hampers comprehensive strategies for drug discovery as well as for monitoring and managing diseases caused by *Ganoderma* in woody crops and forest ecosystems. Both molecular phylogenies and morphological evidence indicate that Africa probably harbors the highest genetic and taxonomic diversity in the genus.

Valuing Medicinal Fungi in Forest Management

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Numerous species of medicinal fungi evolved in forests, and humanity has long harvested these fungi from their native arboreal habitats. Many of these species are now being cultivated; however, some are still harvested from forests. For instance, nutraceutical ectomycorrhizal mushrooms are difficult to cultivate. *Inonotus obliquus* (Ach. ex Pers.) Pilát (Chaga) does not produce the betulin-containing sclerotia known as chaga without infecting live birch trees, and among customers that value natural products, wild-harvested *Ganoderma* species can command premium prices. Lastly, cultivated species can be improved by isolating new cultivars from wild strains to replace senescing mycelia, to obtain strains that have better medicinal properties, or to select clones with superior growth characteristics in cultivation.

Scientists and foresters have a broad appreciation of the innumerable functional roles that fungi play in sustaining healthy, productive, resilient, and diverse forest ecosystems, but quantifying the value of these functions is often difficult because the roles that fungi play are so fundamental and all-encompassing that forests would not even exist without fungi. Focusing more specifically on the monetary value of medicinal fungal species in forests is also difficult because such fungi are expensive and time-consuming to study *in situ*, the products that humans derive from fungi are often harvested sporadically or opportunistically, and much potentially useful information is proprietary. Fungi are too often overlooked in forest management plans because their value is harder to quantify than amenities such as trees or water and because some foresters have a cultural bias that fungi are unimportant.

The goal of this study is to examine how the value of selected forest fungi might be quantified so that foresters can better justify management plans that sustain their wild populations in forested ecosystems.

We begin by examining the relative value of several edible forest fungi and timber in scenarios in which forests are managed for the production of both. Assumptions about how to compare these annually (mushrooms) and periodically (timber) harvested forest products are made and explained, along with

how these comparisons vary by mushroom species, forest type, and forest management plans.

Next, we discuss how the assumptions of these comparisons might differ for medicinal fungi that are harvested from forests, rather than cultivated. Differences include mode of nutrition, distribution and abundance on the landscape, periods between sporocarp harvest, inoculation methods, and forest management goals. Examples will include chaga harvesting in boreal birch forests and *Ganoderma* spp. harvesting in the temperate rain forests of western North America.

We conclude by considering the value of genetic diversity among and within wild populations of medicinal fungi. Advances in pharmacology, fungal biotechnology, genetic analyses, and genetic manipulation might ameliorate some of the need for genetic diversity in wild populations, but these technologies could still pose unknown perils to human or ecosystem health.

Regardless of the actual risks, the public is likely to remain fearful of genetically modified fungi, as evidenced by substantial markets for organically grown mushrooms and derivative products. We explore similar efforts to quantify the value of genetic diversity in other biotic resources and how these approaches could apply to populations of medicinal fungi in forests. Such information will likely improve the ability of foresters and policy makers to ensure the fungal populations are adequately considered in forest management plans and that this resource remains available to the medicinal mushroom industry.

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The Development of the Antiviral Drug RC 28 from *Rozites caperata* (Pers.:Fr.) P.Karst. (Agaricomycetideae)

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Many mushrooms are reported to have antiviral activity against viruses that cause human disease. The active substances described include polysaccharides, conjugated polysaccharides, proteins, peptides, lignins, triterpenes, phenoloic derivates, and nucleic acid bases. RC 28 is an antiviral protein of MW 28 kD prepared from aqueous extracts of the mushroom *Rozites caperata* with novel antiviral activity. It is active exclusively against enveloped viruses including human Herpes viruses HSV-1 and HSV-2, *Cytomegalovirus, Varicella zoster,* Respiratory syncytial virus, and Influenza Virus type A. It is not active against the non-enveloped viruses Adenovirus type 6, Coxsackie viruses B5 and B6, and several strains of ECHO viruses. Our present objectives are to obtain the complete amino acid sequence of RC 28, determine its antiviral mechanism, and identify its cellular targets. I will present some initial studies with these objectives in mind.

The antiviral effects of RC 28 were studied in Buffalo Green Monkey kidney cells (BGMK) and Hep-2 cells infected with the KOS strain of HSV-1 virus. The synthesis of viral proteins in BGMK cells was studied using Western blots of infected cell extracts from 4–12% PAGE gels stained with antibodies to HSV-1. Effects of RC 28 on the early functions of the HSV-1 immediate early protein, ICPO, and disaggregation of PML nuclear bodies was studied in Hep-2 cells stained with anti-ICPO antibodies and anti-PML antibodies. Localization and colocalization studies of RC 28 with cellular organelles were studied in Hep-2 cells stained with antibodies to RC 28, lysosomes, proteasomes, and ICPO. Cellular targets were studied using the Invitrogen Yeast ProtoArray Slide Kit.

RC 28 interfered with the synthesis of viral structural proteins even when the drug was added as late as 13 hours after infection. RC 28 prevented the translocation of the HSV-1 immediate early protein, ICPO from the nucleus to the cytoplasm, and ICPO directed disaggregation of PML nuclear bodies. RC 28 localized exclusively in the cytoplasm but not in the nucleus of Hep-2 cells, and RC 28 did not colocalize with nuclear ICPO, cytoplasmic proteasomes, or lysosomes.

Presently, the drugs of choice for the treatment of Herpes virus infections are those that interfere with viral DNA replication—i.e., Acyclovir and second generation derivatives. RC 28 has unique and novel antiviral activities because it interferes with both early and late viral functions. It interferes with the functional activities of the immediate early protein ICPO and prevents the synthesis of late structural proteins even when RC 28 is added after viral DNA replication. Because of its unique antiviral activity, RC 28 may prevent infection by HSV-1 and HSV-2 Herpes viruses if the drug is applied within the first few hours following contact. It is to be hoped that the elucidation of the antiviral mechanism of RC 28 and the identification of its cellular targets will lead to the discovery of second generation RC 28-like drugs with even greater effectiveness, specificity, and usefulness for the treatment of Herpes virus infections.

The Ice Man's Fungi: Facts and Mysteries

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The discovery of a Neolithic corpse in 1991 in an Alpine glacial field, near the Austrian–Italian border, attracted worldwide attention. The finding's circumstances and the recovery of the mummy proved to be quite chaotic: it took five days for the corpse and most of the artifacts found with it to be transferred to a lab of forensic medicine in Innsbruck, the capital of Tyrol. During this time (September 19–24, 1991) the Neolithic origin of the corpse was unknown, and at least 22 different persons came into contact with it (Egg and Spindler, 1993). Many of the artifacts, some damaged by the visitors, were carelessly thrown into a garbage bag and brought to Vent, the next mountain village. Therefore, the exact original position of these artefacts (including fungal objects) could not be reconstructed.

Today, we know that the real age of the so-called "Ice Man" ranges, according to nine independent radiocarbon measurements, between 3350 and 3100 BC (Prinoth-Fornwagner and Niklaus, 1995). Among the numerous items of the Ice Man's equipment were three fungal objects: two different shaped, polypore-like fungal fragments, each mounted separately on a leather thong; and a mysterious "black matter," filling up the major part of his "girdle bag." The black matter, which was first thought to be resin representing part of a prehistoric repair kit (Lippert and Spindler 1991; Egg and Spindler 1993), was later shown to be tinder material prepared from the true tinder bracket *Fomes fomentarius* (L.: Fr.) Fr. (Sauter and Stachelberger 1992; Pöder et al., 1995; Peintner et al., 1998). The two whitish, polypore-like objects—one shaped more or less like a Scots pine cone, the other more spheroidal—were identified as fruitbody fragments of the polypore *Piptoporus betulinus* (Bull.: Fr.) P.Karst. (Pöder et al., 1992; Peintner et al., 1998).

So far, this represents the only case in which mushrooms were obviously part of a prehistoric person's equipment; it fired the imagination not only of the public and the media but also of scientists. Due to a general fever of excitement, facts have often been mixed up with fictions.

Thus, for instance, our first publication on the identity of one of the polypore-like fragments (Pöder et al., 1992) has prompted a flurry of controversial discussions both in newspapers and scientific journals (e.g., Nieszery, 1992; Chapela and Lizon, 1993; Denman, 1993; Grant, 1993). Our "educated guess" was—and still is—that the "razor strop fungus" (*P. betulinus*) does not provide a good tinder, and, therefore, might have served some purpose other than making fire. Referring to biologically active compounds produced by *P. betulinus* and its special host—it grows exclusively on birch, which is regarded as the tree of life and fertility in many European and Siberian myths (e.g., Heeger, 1936; Wasson, 1968)—we indicated a possible medical–spiritual use. At this time (the existence of tinder material in the Ice Man's girdle bag was still unknown) the reaction of the public, archaeologists, and some biologists to our assumption was clearly negative. Mycological facts and ethnomycological hints could not argue archaeologists out of their

standard hypothesis: ancient humans used polypores as tinder and for nothing else.

Such discussions stopped after the discovery of classic tinder material prepared from the tinder bracket *Fomes fomentarius,* which filled up the major part of the Ice Man's girdle bag (Pöder, 1993, Pöder et al., 1995). Others, obviously confusing "spiritual" with "spirituous," complained that the Ice Man was not a drug dealer. Referring to biologically active components produced by polypores, some authors (e.g., Sauter and Stachelberger, 1992; Capasso, 1998) mistook *Piptoporus betulinus* for *Laricifomes officinalis*. The latter contains agaric acid (2-hydroxy-nonadecan-tricarbonic acid) and has been used as a purgative or as medicine against pulmonary diseases right up to the 20th century. This mushroom was already known to the ancient Greeks for its medical properties and played an important spiritual as well as medical role in many societies worldwide (Buller, 1914; Blanchette et al., 1992; Blanchette, 1997; Peintner and Pöder, 2000).

Thus, presumptions such as "the Ice Man was aware of his intestinal parasites and fought them with measured doses of *Piptoporus betulinus*," which contains agaricine (Capasso, 1998) is simply wrong (Pöder and Peintner, 1999). In our studies, agaricine was not detected in the two fungal fruit body fragments of the Ice Man or in recent material of *P. betulinus* (Pöder et al., 1992; Pöder, 1993). The pharmacologically active substances of *P. betulinus* are ergosta-7,22-dien-3-β-ol, fungisterol, ergosterol, tumulosic acid, and a group of triterpenes. Among the latter, polyporenic acid A, B, and C were separated (Cross et al., 1940). Recently, the finding of a new antibiotic produced by *Piptoporus betulinus*, called piptamine (a tertiary amine) was published by Schlegel et al. (2000). It is active against a series of Gram-positive bacteria and fungi including yeasts. Unfortunately, piptamin is a homonym of an alkaloid isolated from plants (*Piptanthus nanus*, *Ormosia nobilis;* Fabaceae) more than 40 years ago (Wilson, 1965).

Although no indications could be found regarding a prehistorical use of *P. betulinus*, what can be finally said about the significance of the Ice Man's fungi?

Concerning the Black Matter, its interpretation as classical fire-starting tinder seems well confirmed by the current body of evidence. Regarding the *P. betulinus* objects, it is much more difficult to find an adequate answer without leaving a firm scientific footing. As outlined in detail by Peintner and Pöder (2000), a merely alimentary use or use as some kind of commodity can be excluded; also a pure ornamentative or decorative function without any spiritual background seems very unlikely. Consequently, we have to admit that we simply do not know the Ice Man's intentions concerning these mushrooms.

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Mycoremediation: Current State and Perspectives

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Mycoremediation (also called *fungal treatment* or *fungal-based technology*) is the application of fungi in remediation of polluted soils and aqueous effluents. The fungi mostly used are wood-rot Basidiomycetes capable of degrading lignin (ligninolytic fungi). Most of these fungi cause white rot of wood, and so they are often called white-rot fungi (WRF). The ability of WRF to degrade lignin is due to a complex of extracellular enzymes—namely, lignin peroxidase, manganese dependent peroxidase, hydrogen peroxide generating oxidases, and phenol oxidases such as laccase. The lignin peroxidases were first discovered in the basidiomycete *Phanerochaete chrysosporium* Burds., and in the 1980s this fungus was the main experimental model in lignin degradation research. Due to the nonspecific character of radical-mediated reactions of ligninolytic enzymes, the degradation of a wide variety of xenobiotic compounds, having an aromatic structure like lignin, has become a subject of extensive research.

BIODEGRADING CAPABILITY OF WRF

In 1985 it was demonstrated that *Ph. chrysosporium* was able to degrade, besides lignin macromolecules, many types of organopollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls and dioxins, chlorophenols and chlorolignins, nitroaromatics, synthetic dyes, and different pesticides. Logically, the degrading ability of other species of WRF was tested as well very soon after. Several powerful degraders—e.g., *Phanerochaete sordida* (P.Karst.)J.Erikss., *Pleurotus ostreatus* (Jacq.:Fr.)P.Kumm., *Trametes versicolor* (L.:Fr.) Lloyd, *Nematoloma frowardii* (Speg.)E.Horak, and *Irpex lacteus* (Fr.)Fr.—were selected; however, the screening was mostly performed using liquid culture media.

BIODEGRADATION IN SOIL

In soil conditions the fungal degrading potential of WRE is only one prerequisite. Other factors, such as the ability of fungal mycelium to colonize soil matrix, its resistance to autochthonous soil microflora and to the toxic compounds present in polluted soils, as well as the effect of physicochemical parameters of the respective soil, will be discussed in the lecture. Step-by-step selection of a fungal degrader will be demonstrated using the model fungus *Irpex lacteus.* Provided that the respective fungal strain significantly decreased the content of the respective pollutant in the soil under laboratory conditions and, simultaneously, that applied ecotoxicological tests also documented the decrease in soil toxicity, the process can be scaled up.

SCALE-UP AND FIELD APPLICATIONS

Large-scale production of fungal inoculum does not represent a special problem; the inoculum (i.e., lignocellulosic material such as straw, sawdust, and wood chips colonized with fungal mycelium) can be

produced using facilities of a local oyster mushroom (*Pleurotus* spp.) or shiitake (*Lentinus edodes*) farm. In case of a long distance between a mushroom farm and the decontamination site, a solid-state fermentor can be used; for a very long distances the inoculum in the form of alginate-gel pellets can be prepared. Landfarming, biopiling, or treatment in containers are frequently used techniques in soil remediation. All the approaches are based on the same principle—mixg the contaminated soil with lignocellulosic substrate colonized with fungal mycelium and allowing it to work for several weeks or months under proper environmental conditions. Experience in large-scale soil mycoremediation gained in the Czech Republic, the USA, and Germany will be evaluated in the lecture.

PROBLEMS, ADVANTAGES, AND PERSPECTIVES

Mycoremediation, similar to most of other bioremediation approaches, has some drawbacks: the process is usually slow, and the removal of contaminants is rarely close to 100%. The soil matrix and bioavailability of the respective pollutant influence the final result of the treatment. However, compared to physical or chemical treatments, bioremediation is environment friendly and is a cost-effective approach. On one hand, specific features of mycoremediation are that no special equipment is needed for inoculum production (sometimes even a spent oyster mushroom substrate can be used as inoculum), and for the soil treatment, standard agricultural machinery can be used. The other advantage of mycoremediation is that the soil treated with this procedure is biologically sound and active; fungal mycelium and its career (straw, wood chips) is converted to humus, and after the decomposition of the substrate, the introduced ligninolytic fungus is not able to compete with the newly revived soil microflora and dies off naturally. On the other hand, in several cases, the degradation can be unsuccessful, and thus the process has not yet become a reliable environmental biotechnology.

In conclusion, the future success of mycoremediation depends on more intensive research into bioremediation generally and more extensive research into the biodegrading potential, physiological properties, and ecology of a large number of white rot and litter-decomposing fungi.

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Medicinal Polypores of the Forests of North America: Screening for Novel Antiviral Activity

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Polypore mushrooms have been used medicinally for thousands of years. The Greek physician Dioscorides first described the use of a wood conk, Agarikon, now known as *Fomitopsis officinalis* (Vill.: Fr.) Bond. et Singer (= *Laricifomes officinalis*), as a treatment against consumption in 65 AD. Other wood conks, such as Ling Chi or Reishi, have had a similarly long history of use in Asia. In the past 20 years, wood conks have been carefully explored for their immunomodulating and anticancer properties. More recently, mushrooms, including polypores, have and are being explored for their antimicrobial properties.

Upon submitting more than a hundred *in vitro* cultures of mushrooms to the US Defense Department's Bioshield BioDefense program, several tests show that some of these polypore mushrooms have strong antiviral activity. Within these verdant natural landscapes, trees hundreds of years old host ancestral strains of these elusive polypores. Species that are now rare, or in some cases thought to be extinct, still reside in the pristine old growth forests of the Pacific Northwest of North America. When clones from these mushrooms were grown *in vitro* and submitted for antiviral screening, several mycelial cultures produced antibiotics effective against Pox and other viruses. Notably, strains vary in their antiviral properties. Our natural genomes hold within them great potentials for staving off disease and have not yet been fully explored. The fungal diversity within these genomes may prove critical for isolating the most active strains, similar to the lessons learned from the isolation of *Penicillium chrysogenum* strains that lead to the commercialization of penicillin and saved millions of lives.

With deforestation, pollution, and industrialization, societies should reevaluate the importance of their natural forests in the context that they hold within them novel medicines of enormous socioeconomic importance. The old paradigm of viewing the forest as valuable only in terms of timber seems overly simplistic given this new knowledge.

The Importance of Culinary–Medicinal Mushrooms from Ancient Times to the Present

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Higher Basidiomycetes mushrooms have been used in folk medicine throughout the world since ancient times. For millennia, mushrooms have been valued by humankind as edible and medicinal resources. Traditional use of mushrooms as medicines has been long established among different ethnic groups. In the Far East countries, especially in China, Japan, and Korea, mushrooms have long been revered for their curative attributes.

Paramount among these is *Ganoderma lucidum* (W. Curt.: Fr.) Lloyd (Ling zhi in Chinese; Reishi, Mannentake, or Sachitake in Japanise; and Youngzhi in Korea). It is valued for both its medicinal and spiritual properties. For centures, this mushroom has been regarded in the Orient as an effective medicinal source. It is also considered a symbol of happy augury and good fortune, good health, longevity, and even life with the immortals. Very important Far East culinary–medicinal mushrooms are also *Lentinus edodes* (Berk.) Singer (Shiitake mushroom) and *Trametes versicolor* (L.: Fr.) Pilát (Turkey Tail).

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

The number of mushrooms on Earth is estimated at 140,000, yet perhaps only 10% (approximately 14,000 named species) are known. Edible higher Basidiomycetes are being evaluated for their nutritional value and acceptability, as well as 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, they present an unlimited source of polysaccharides with anticancer and immunostimulating properties. Many, if not all, Basidiomycetes mushrooms contain biologically active polysaccharides in their fruit bodies, cultured mycelia, and culture broth.

The data about mushroom polysaccharides are summarized for 651 species and seven intraspecific taxa from 182 genera of higher Hetero- and Homobasidiomycetes. These polysaccharides are of different chemical composition; the main ones comprise the group of β-glucans. β-(1→3) linkages in the main chain of the glucan and further β-(1→6) branch points are needed for their antitumor action. Numerous bioactive polysaccharides or polysaccharide–protein complexes from medicinal mushrooms are described that appear to enhance innate and cell-mediated immune responses and exhibit antitumor activities in animals and humans.

Stimulation of host immune defense systems by bioactive polymers from medicinal mush-

rooms has significant effects on the maturation, differentiation, and proliferation of many kinds of immune cells in the host. Many of these mushroom polymers were reported previously to have immunotherapeutic properties by facilitating growth inhibition and destruction of tumor cells. While the mechanism of their antitumor actions is still not completely understood, stimulation and modulation of key host immune responses by these mushroom polymers appears central. Recent evidence suggests that mushroom polymers (β-glucans) may trigger the stimulation of many kinds of immune cells in animals and humans. Several of the mushroom polysaccharide compounds (lentinan, krestin (PSK), PSP, schizophyllan, befungin) have proceeded through Phases I, II, and III clinical trials and are used extensively and successfully to treat various cancers and other diseases in Asia, but not in many Western countries, because in many cases the standards of these trials may not meet current Western regulatory requirements. The polysaccharides of some other promising medicinal mushrooms species—*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.—also show good results.

In the second half of the 20th century, mushroom-producing technologies grew enormously. Mushrooms represent a valuable source of bioactive agents with potent and unique medicinal properties. Some of recently isolated and identified substances of Higher Basidiomycetes mushroom origin express promising antitumor, immune modulating, antioxidant, cardiovascular, antihypercholesterolemia, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic effects.

Many of them are not strictly pharmaceutical products (real medicines) but represent a novel class of dietary supplements (DSs) or "mushroom nutriceuticals." The agents derived from medicinal mushroom fruit bodies, cultured mycelium, and/or culture filtrates exert a wide range of beneficial biological effects when tested *in vitro* or using animal models. Recent years have seen a surge of commercial interest in medicinal mushroom DSs, the common market value of which is approximately $13 billion US dollars.

The safety advantages of using mushroom-based DSs, as opposed to herbal preparations are the following. (1) The overwhelming majority of mushrooms used for production of DSs are cultivated commercially (and not gathered in the wild); this provides a very good chance of proper identification, and 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 our opinion, is that many mushrooms are capable of growing in the form of mycelia biomass in submerged cultures.

Merulius incarnatus Schwein., a Rare Mushroom with Highly Selective Antimicrobial Activity

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BACKGROUND

In the course of our search for new drug candidates from polypores, we studied a rare North American mushroom, *Merulius incarnatus* (Corticiaceae). The polypores and corticioid fungi are members of Aphyllophorales, a group of morphologically complex, terrestrial Basidiomycetes. Many of these fungi are saprobic wood decayers and most often are found on logs, stumps, or other dead wood. Polypores are considered by many as a major source of pharmacologically active natural products. Their secondary metabolites exhibit a wide range of biological activities such as antimicrobial, antiviral, antifungal, anticancer, cardiovascular, anti-inflammatory, antioxidant, immunostimulating, nematocidal, and other activities (Zjawiony, 2004).

Merulius incarnatus Schwein. (1822), also known under the later name *Phlebia incarnata* Nakesone et Bursdall (1994), can be found on dead logs and stumps of hardwoods, particularly those of white oak, beech, maple, and birch in the southeastern United States. It grows in overlapping clusters, usually with *Stereum ostrea* (Blume et T.Nees) Fr., mostly in the fall months (September–November). *M. incarnatus* is a rather small mushroom (2–5 cm in diameter), with a very characteristic bright coral pink color of the upper cap—hence, the common name Coral Woodcrust. The fruiting body is irregularly shaped, elliptical or semicircular, slightly convexed without stem. The pore surface is whitish and veined. The spores are white, elliptical, and 2–4 µm in diameter. The mushroom gives the yellow to orange test reaction with KOH.

MATERIALS AND METHODS

We collected *M. incarnatus* in the fall of 2001, 2002, and 2003 at Duke Forest in Durham, North Carolina. Fruiting bodies were extracted fresh with 95% ethanol and subjected to chromatographic separation, using standard and argented column chromatography followed by HPLC on Waters Delta Prep 4000 system using reverse phase Symmetry-C_8, 5 µm column, eluting with isocratic solution of MeOH-H_2O (85/15 v/v). Antimicrobial assays were conducted at the microbiological laboratories of the National Center for Natural Products Research against following pathogens: *Candida albicans, C. glabrata, C. krusei, Cryptococcus neoformans, Aspergillus fumigatus, Mycobacterium intracellulare, Staphylococcus aureus,* and methacilin resistant *S. aureus* (MRSA).

RESULTS

A crude extract of *Merulius incarnatus* exhibited significant activity against several microorganisms, particularly *Staphylococcus aureus* and methacillin resistant *S. aureus* (MRSA). The most active fraction showed IC_{50} = 3.5 µg /mL against MRSA. These results prompted us to do further studies on isolation and

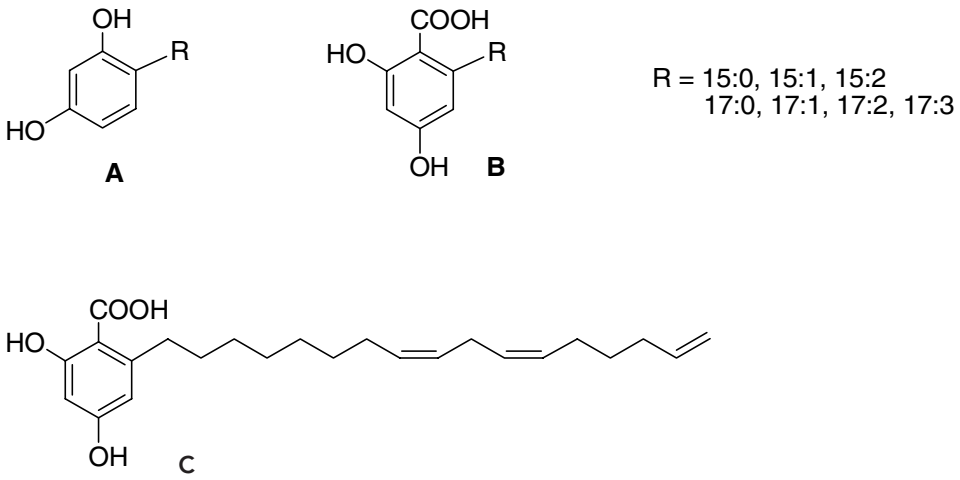

FIGURE 21. (A) Compounds with unsaturated aliphatic chains have a very specific pattern of the double bonds characteristic for resorcinolic lipids isolated from other plants, fungi, and bacteria (Kozubek and Tyman, 1999). They have either one or two double bonds in a *cis* configuration, and in some cases, also the terminal double bond. **(B)** The compounds with *cis-trans* conjugated system in place of isolated *cis-cis* were also isolated **(C)**.

structure elucidation of active secondary metabolites from this mushroom. The series of resorcinol (A) and resorcinolic acid (B) derivatives substituted with a long (C15 and C17) saturated and unsaturated aliphatic chain were obtained. For each series of derivatives (A and B) we were able to isolate seven compounds with 0, 1, 2, or 3 double bonds in the side chain.

DISCUSSION

Isolation of series of resorcinol and resorcinolic acid derivatives substituted with a long saturated and unsaturated aliphatic chain makes it the first and yet not published example of work on identification of secondary metabolites from *Merulius incarnatus*. Five compounds isolated are new and have never been found in any species. Other compounds isolated such as merulinic acid A, D, and E have been found in other mushroom species such as *Merulius tremellosus* Schrad. and *Hapalopilus mutans* (Peck) Gilb. et Ryverden (Giannetti et al., 1978; Sontag et al., 1999). Some resorcinol derivatives isolated from *M. incarnatus* are also known as the components of cereal grains (Kozubek and Tyman, 1999). All resorcinolic acid derivatives exhibited selective antimicrobial activity against methacillin resistant *Staphylococcus aureus*.

The structural similarity of all isolated compounds within a particular series made the separation and isolation process extremely challenging. It required the use of vacuum liquid chromatography (VLC), argented column chromatography (ACC), and reverse phase high performance liquid chromatography (HPLC), and in the cases of resorcinolic acid also a chemical modifications. The fact of the isolation of compounds with *cis-trans* conjugated system is unprecedented. We believe that these compounds are formed by enzymatic or photochemical isomerization *in vivo*. Further studies to understand the mechanism of formation and biological role of these compounds are needed.

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