SYMPOSIA LECTURES AND POSTER PRESENTATIONS

Mycoremediation of Engine-Oil–Polluted Soil by *Lentinus squarrosulus* Mont., an Indigenous Nigerian White-Rot Fungus

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There has been an increasing interest in the use of mushrooms in bioremediation of polluted habitats because of their ability to change the nutrient conditions in the soil and to accumulate metal ions, including heavy metals. Despite the upsurge in the research activities elsewhere, these studies are still in their infancy in Nigeria, which is one of the world's leading oil producers. *Lentinus squarrosulus* Mont., a white-rot fungus, was tested for its ability to bioremediate a soil contaminated with 0.5–40% concentrations of engine oil over a period of 3 months. Results obtained revealed that in soils contaminated with engine oil and inoculated by *L. squarrosulus,* the amount of organic matter, carbon, nitrogen, and phosphorus increased, whereas the available potassium was reduced. A relatively high percentage degradation of total petroleum hydrocarbon (TPH) was observed in low concentrations of engine oil and a considerably lower percentage for the higher concentrations of engine oil. The metal concentration of Fe, Cu, Zn, and Ni increased with the increase of engine oil concentration up to 20%, followed by a decrease showing bio-accumulation by the white-rot fungus. The improvement of nutrient content values as well as the bioaccumulation of heavy metals at 20% engine oil concentration by *L. squarrosulus* is of importance for the mycoremediation of an engine-oil polluted soil.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Submerged Culture Conditions for the Production of Exo- and Endo-Polysaccharides by *Ganoderma applanatum* (Pers.) Pat.

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Ganoderma applanatum is a perennial mushroom (Basidiomycetes) which causes white rot in broad-leaved trees. The fruit body has been used as a traditional anticancer medicine in China and is reported to have various beneficial physiological activities such as antitumor, antivirus, and immunostimulating activities. These biological activities are known to be associated with polysaccharides, and protein-bound polysaccharides were isolated from the fruiting bodies and mycelium.

Submerged cultures of edible fungi obviously have the potential for higher mycelial production or for inducing mass production of useful substances in compact space in a short time period with easy environmental control. The aim of this investigation was to determine the optimal polysaccharide production conditions from *G. applanatum* in submerged culture. In order to produce the immunostimulating water-soluble polysaccharides from the mycelium, the effects of several cultivating factors on polysaccharide production were studied. We were especially interested in the factors affecting the relationship between exo- and endo-polysaccharide production.

Carbon sources, carbon concentrations, culture temperature, and culture period influenced mostly the production of polysaccharides. The effective carbon sources for polysaccharide production were maltose, lactose, and glucose. High yield of the exo-polysaccharide required higher temperatures (25 °C), higher carbon concentrations (60 g/L), and shorter culture periods (8–12 days). In contrast, endo-polysaccharide production required lower culture temperatures (10–15 °C), lower levels of carbon (20 g/L), and a minimum of a 10-day culture period. *G. applanatum* accumulated endo-polysaccharides in their bodies at lower temperature conditions. Meanwhile, more exo-polysaccharides were produced at moderate temperatures and in the stationary growth phases. Under optimal culture conditions, the yield of water-soluble endo-polysaccharides and exo-polysaccharides reached 4 g/L and 2 g/L, respectively. The sugar compositions and chemical characteristics of the polysaccharides were also studied.

Prospective of the Cultivation of New Culinary–Medicinal Mushrooms in Israel on Agro-Industrial Waste

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Starting about 50 years ago, modern agricultural technology made the cultivation of mushrooms in Israel a year-round possibility, and since then mushrooms have earned an honored place at the table in Israeli homes.

The first mushrooms to be successfully cultivated in Israel were button mushrooms (*Agaricus bisporus* (J.Lge) Imbach). Until about a decade ago, these were the only cultivated mushrooms to be found in local markets. Edible mushroom cultivation in Israel has been highly developed in recent years because it does not require much agricultural land area or water. In the years 2001–2004 the production of mushrooms in Israel doubled. In 2001, 5000 tons were produced, of which 3500 were cultivated in northern Galilee, near the Lebanon border, and the rest in other parts of Israel. The average annual production growth in northern Galilee only is 15% and rising. The increase in production capacity has evolved by an increase in the size of the production plants and their numbers. Newer and sophisticated environmental and climate control systems have been employed.

The second cultivated species was *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm., the Oyster mushroom. Both species are cultivated in agro-industrial fashion using automated and controlled lighting, temperature, carbon dioxide, and humidity. The cost efficiency of these systems is low. The cultivators are seeking new mushroom species in order to receive higher revenues. They also wish to use the same industrial plants for the new mushrooms, to maximize their returns. The market for mushrooms is also increasing and new mushrooms are accepted. Of the 40–50 species of mushrooms cultivated in other countries, only two species are cultivated in Israel.

The growing demand in the market for fresh mushrooms is accompanied by an interest in new exotic mushrooms. Studies in research institutions in Israel mainly include the general species of genera *Pleurotus, Lentinus, Morchella,* and *Lepista nuda* (Bull.:Fr.) Cooke to produce new mushrooms in cultivation beds of the button mushrooms. Among these, the cultivation of *Pleurotus ostreatus* was developed on an industrial scale. It is based on the "satellite" concept for mushroom production. This concept includes a center for substrate preparation that serves several mushroom growers and provides technical assistance to the growers. The import of mushrooms helps to meet the increased demands for mushrooms. The market of frozen and canned mushrooms in Israel is estimated at US $20–25 million per year (or several hundred tons) and rising.

Mushroom growing is a significant tool for the restoration, replenishment, and remediation of the Earth's overburdened ecosphere. Mycelium produces extracellular enzymes and acids that break down large molecules, such as lignin and cellulose, which are the two primary components of woody plants. Many kinds of waste material that contain ligneous material are available in Israel. Commercially avail-

able agro-industrial waste and feed meal products include cotton waste, chicken manure, corncob and corn straw, banana leaves and banana pseudostems, paper and cardboard, wheat straw, peanut shells, almond shells, sunflower shells, grape residues and pomace, alfalfa, and olive oil waste.

Research has started at Haifa University to develop commercially viable cultivation methods for highly praised culinary–medicinal mushrooms *Hypsizygus marmoreus* (Bunashimeji) and *Grifola frondosa* (Dicks.:Fr.) S.F. Gray (Maitake) for export to foreign markets and local consumption using agro-industrial waste. The local market for these mushrooms is currently recognized as small due to nonexistent and/or inefficient production systems. The main goal of the research is to achieve the highest mushroom fruit body yield, grown on agro-waste material, in the shortest time and at the lowest cost. The research methodology includes:

- A survey on local need for exotic mushrooms.
- Collecting and testing of different fungal strains known for speed of growth and fruiting (from the culture collection of culinary–medicinal mushrooms of the Institute of Evolution, University of Haifa (HAI), Israel).
- Identification of suitable *Grifola fondosa* and *Hypsizygus marmoreus* strains for commercialization, based on overall quality and ease of cultivation.
- The collection and testing of different agro-industrial waste from local materials, for spawn run and fruit body production.
- Determining spawn run requirements and length.
- Development of liquid inoculation techniques for spawn production.
- Optimization of spawn storage.
- Establishing an effective and practical method of cultivation container and the type of heat treatment.
- Optimization of substrate composition and growth conditions including temperature, humidity, light, aeration, casing layer composition, and type of container for substrate.
- Studying the effect of certain additives on colonization and yield.
- Development of technological regulation for fruitbody production.
- Studying post-harvest handling.

Upon completion of the research, successful substrates and strains will be patented.

In conclusion, more research and development of mushroom cultivation biotechnology is required to satisfy the local demand for culinary–medicinal mushrooms.

Ethnomycology and Indigenous Uses of Mushrooms Among the Bini-Speaking People of Nigeria: A Case Study of Aihuobabekun Community Near Benin City, Nigeria

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In order to document the indigenous uses of mushrooms among the Bini-speaking people residing in southern Nigeria, a rural community was used as a case study. Out of the 105 households in the community, background information as well as indigenous uses of mushrooms from 74 households (or 70% of the total) was documented. The study revealed that over ninety percent (90%) of the respondents consume mushrooms as food, while 21.6% use some mushrooms for medicinal purposes.

On the average, each respondent consumes 5.8 ± 0.41 species of mushrooms. Among those that consume mushrooms, 93% do so because of taste, 90.5% use mushrooms as substitute for meat, 79% use them as soup thickener, 21.6% use mushrooms for medicinal purposes, while 20% do so because of their nutritional qualities. Two mushrooms are used in ethnomedicine in the study area: *Daldinia concentrica* (Bolton) Ces et De Not is used for curing stomach upset, skin diseases, stomach ulcer, whooping cough, and prevention of excessive growth of the foetus for easy delivery. *Calvatia* sp. is used for curing barrenness in women. It is also used for stopping chronic hiccups. Also, the sclerotium of *Pleurotus tuberregium* (Rumph.:Fr.) Singer is one of the major ingredients used for making gunpowder, apart from being used as food and medicine.

Out of the nine mushrooms mentioned by the respondents, three were neither seen nor collected. According to the respondents, they are feared to have gone into extinction due to environmental degradation. The other mushrooms mentioned are *Schizophyllum commune* Fr.:Fr., *Volvariella volvacea* (Bull.) Singer, *Auricularia auricula-judae* (Fr.)Quél., and *Lentinus squarrosulus* Mont.

Although not all the respondents were Bini-speaking, as they constituted 78.38% of the total number of households visited, Bini-speaking respondents were, however, mainly responsible for supplying all the information on the ethnomedicinal uses of mushrooms. While 85.14% of the respondents were interested in learning how to cultivate mushrooms, only 12.16% of them had knowledge of the possibility of cultivating mushrooms prior to our interaction with them. All the age categories interviewed indicated interest in learning how to cultivate mushrooms. In each age group, the number of interested persons was consistently higher than the number of uninterested respondents. The highest number of interested persons was in the age category of ≥71 years. Respondents in the age bracket of 36–40 years consume the highest number of mushroom species, with a mean value of 7.01 ± 2.5. However, there was no significant ($p = 0.05$) difference in the mean number of mushrooms consumed among the different age groups in the study area.

This study emphasizes the need to quickly document indigenous knowledge about medicinal mushrooms. This is because it was found that respondents below the age of 46 years had no knowledge of the ethnomedicinal uses of mushrooms. On the other hand, some respondents above 71 years had lost full ethnomedicinal information on the uses of mushrooms for curing some ailments. However, the latter group had the highest number of respondents that gave full information on the ethnomedicinal uses of some mushrooms. Three respondents totally refused to supply information on the medicinal uses of *Daldinia concentrica,* in particular, even though they claimed to use it in curing strokes and coughs.

No other form of mushroom preservation is practiced in the study area apart from the sun-drying of edible and medicinal mushrooms such as *Schizophyllum commune, Lentinus squarrosulus, Calvatia* sp., *Daldinia concentrica*, and the sclerotium of *Pleurotus tuberregium*. Mushroom hunting and sales appear to be gender and age related in the study area. This is because, despite the respondents' interest (85.14%) in learning mushroom production technology, 90.5% of them regard it as a job for women and children.

The results of this study underscore the need to conduct a nationwide survey on the indigenous uses of mushrooms because it can serve as a basis for intensive studies into the therapeutic effects of many mushrooms of ethnomedicinal importance. Upon further studies and trials, products from such medicinal mushrooms can then be incorporated into orthodox health management programs. Also, it is the opinion of the authors that this kind of study should, of necessity, be a prelude to the use of mushroom production technology as a tool for rural community development projects.

Nutritional and Medicinal Potential of Twenty-Three Wild Mushrooms from Northeast Thailand

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Twenty-three species of wild mushrooms (mostly polypores from genera *Trametes, Ganoderma, Schizophyllum, Inonotus, Phellinus,* and *Lentinus*) from Sakon Nakhon, northeast Thailand, were collected, and their pure cultures were kept at the Department of Plant Pathology, Khon Kaen University, for further studies.

In this paper, the nutritional value, potential of exo-polysaccharides production, and ability to produce lectins from all species were investigated. In order to produce mycelial biomass, exo-polysaccharides, and lectins culture was grown in a 250 mL Erlenmeyer flask containing 100 mL of rice bran dextrose malt peptone

(RbDMP) medium at room temperature on a rotary shaker at 125 rpm for 10–15 days. For nutritional value, the mycelial biomass was analyzed for ash, proteins, and amino acids contents. The content of ash determined by incineration of mycelia at 500 ± 10°C for 8 hours was very low (approximately 4.73 ± 0.36% of dry matter) in all species. The protein content of all species analyzed through the macro-Kjeldahl method was varied among species, and the average of crude protein was 22.2 ± 5.5% (range 13.3–26.5) of mycelium dry weight. Essential amino acids were determined by using modified techniques (Wiedmeier, Porterfield, and Hendrich, 1982) after hydrolysis of freeze-dried samples with 6 N HCl for 24 hours at 110 °C and analyzed through Shimadzu High Performance Liquid Chromatography. The amino acid patterns were shown clearly, and all amino acids contained in all species were calculated. The amount of the essential amino acids accounted for at least 30.77%, by average, of total amino acids content. Tryptophan could not be detected by the method mentioned above. Essential branch chain amino acids such as isoleucine, leucine, and valine were high, which were useful in treatments involving muscle, mental, and emotional upsets as well as for insommia and nervousness. The amount of exo-polysaccharides was obtained by mixed culture filtrate with 4 vol. of 100% ethanol, stirred vigorously, and kept overnight at 4 °C, and the precipitated exo-polysaccharides were air dried and weighted.

Exopolysaccharide production varied considerably among species ranging from 0.33 to 6.59 mg/L with an average of 2.91 ± 0.19 mg/L. Lectin extraction was performed in crude extract by using 5 times (w/v), 10 mM phosphate buffer saline (PBS contained 0.02 M sodium bisulphite, pH 7.2), stirred for 2 hours at 4 °C, filtrated and centrifuged for 15 minutes at 12,000 rpm. A hemagglutination test of a serial two-fold dilution of the crude extract and cultured broth was investigated by using Microtiter U-plates with 2% suspension of red blood cells of animals (six kinds) in PBS pH 7.2. Hemagglutination titers of lectins were positively detected at 1:1 (majority) up to 1:16 (1 species) in mycelium, cultured broth, or both in most of the species investigated. All measurements clearly indicated the potential of those wild polypore mushrooms from northeast Thailand as good sources of alternative nutritional food and potential uses as alternative medicine to promote good health and to enhance the body's adaptive capabilities.

The Clinical Use of Mushrooms from a Traditional Chinese Medical Perspective

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Mushrooms have been used in traditional Chinese medicine (TCM) for many centuries over the past two millennia. They have been used in both nutritional and herbal medicine applications. In daily life, the use of mushrooms may be thought of in terms of "folk medicine," and the Chinese have included many mushroom species in their daily cooking and also for healing purposes. For

example, Chinese people have long believed that mushrooms not only taste good but also have a detoxification effect (except for the toxic species), cleaning and cleansing the body as well as healing wounds. From the nutritional point of view, the Chinese believe that mushrooms are essential in cleansing the liver and kidneys and are often included in the main daily meal. Mushrooms have long been regarded as a great delicacy, on the dinner table of both the Chinese aristocracy and the common people.

The Chinese recognize six basic types of medicinal mushrooms, according to color—namely, white, red, purple, black, blue, and green. These colors fit into the TCM system of natural correspondences, which resonate with various diseases. For example, red is for the heart, black is for the kidneys, and green is for the liver and gallbladder.

Medicinally, the Chinese have been using mushrooms perennially for the purposes of well-being and longevity. One of the most famous examples is *Ganoderma lucidum* (W.Curt.:Fr.)Lloyd (Ling Zhi or Reishi), a reddish tree fungus, which is reputed to be an elixir. This fungus has been shown through intensive scientific research to contain many polysaccharides and secondary metabolites, which appear to have an anticancer and immuno-enhancing effect, and also have a positive effect with respect to treating autoimmunity, anti-inflammation, and various neurohumoral disorders.

Another mushroom *Trametes versicolor* (L.: Fr.)Pilát (=*Coriolus versicolor*), known as Yun Zhi, a greyish tree fungus (which has a distinctive cloud-like shape), has been used effectively over the centuries for anticancer purposes as well as for lowering cholesterol and enhancing longevity. *Cordyceps* spp., a yellowish green tree fungi, were proven valuable in harmonizing the immune system and the overall general physiology of the body.

In general, mushrooms are vitally important in treating various disorders encountered in daily clinical practice, such as respiratory disease, diabetes, and liver problems. In ancient times, decoctions were the usual method of administration. In modern TCM, mushrooms are offered as a medicine in capsules, powders, and teas and in various nutritional items such as soups and stews. They are often combined with other herbal medicines to enhance the general curative effect. Like most Chinese nutritive and herbal remedies, they work relatively slowly from the inside out, from the deep to the superficial Qi (vital energy) levels—from the internal organ systems to the meridians. The secret of the use of mushrooms in TCM is to potentiate, to complement, and to activate the effects of other TCM therapies in integration with biomedicine.

In my own medical clinic, I have found one of the most useful TCM prescriptions to be Six Treasures of Ling Zhi Mushrooms with bee pollen together with various pearls and herbs. It is in the form of a capsule, enhancing the energy/immunity of all patients as well as beneficial for cancer patients.

The Clinical Use of Mushrooms in Oncology

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Oncology, from a traditional Chinese medicine (TCM) perspective, is known as Ai Zhi, which signifies physiological accumulation of the "mass," which is pushing out and spreading to various cells and organs in the body. It is also associated with the accumulation of phlegm, which may be generally defined as a pathogenic, chronic accumulation of mucus in various organ systems, especially the stomach and the bladder organ/meridian systems, which causes severe blockage of Qi (vital energy), subsequently causing blood stagnation and mass formation. This may lead to cancer. Another major cause of cancer, according to TCM theory, is blood stasis. In TCM oncology, the major emphasis is to eliminate phlegm and blood stagnation/stasis. Various methods are used for these purposes, and nutritional/herbal medicine plays a central role. Mushrooms were proven clinically useful in fighting cancer, especially the *Ganoderma lucidum* (W.Curt.:Fr.)Lloyd (Ling Zhi or Reishi), *Lentinus edodes* (Berk.)Singer (Shiitake) species.

Mushrooms are good not only for immunoenhancement, but also to complement Western chemotherapy and radiation therapy. Mushrooms contain a number of polysaccharides and secondary metabolites, which work by countering the side-effects of cancer such as nausea, bone marrow suppression, anemia, and lowered resistance.

It is also vitally important to consider the aspects of internal body harmony in conjunction with the mental and spiritual aspects, involving the suffering patients, their families and friends, and the various physicians and other caregivers involved. In medical acupuncture clinics, certain medicinal mushroom products approved by the Canadian government and other governments are useful adjuncts in treating invasive tumors and the pain of cancer as well as the side-effects of chemotherapy, radiation, and surgery, such as the following:

- Comely: this capsule, made in Hong Kong, contains wild Ling Zhi (with several of its polysaccharides and secondary metabolites) as well as various pollens and also saponins of *Gynostemma pentaphylla* (a species of Ginseng).
- Golden Lu Bao Ling Zhi: this capsule, made in Hong Kong, is an extract of Ling Zhi, containing six different wild Ling Zhi species gathered from the mountains of the Taishan region of China.
- Ginseng, Reishi Pearl, and Pollen Tablet: made in Guangzhou, China, the main ingredients of this tablet are Ginseng (30%), wild Ling Zhi (25%), and honey enriched pollen (25%).
- Yun Zhi: a capsule made in Guiyang, China, it is 100% *Trametes versicolor* (L.:Fr.)Pilát (=*Coriolus versicolor*) (Lu Bao Yun Zhi mushroom).

The above products containing various mushroom ingredients are all sterile, purified, carefully packaged, quality-controlled products approved by the Canadian government for appropriate medical distribution. In my own medical acupuncture clinic, they are prescribed as adjunctive methods.

In the traditional Chinese medical system, acupuncture is thought to have a relatively fast effect on pain control and vital energy (Qi) balancing, whereas nutritional and herbal therapeutic modalities have a slower but deeper effect in the major internal organs. From an integrative medical perspective, cancer and other patients are required to maintain close and direct contact with their own personal family physicians and specialists, such as oncologists, for optimum health for all concerned.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Medicinal Properties of Coprinoid Mushrooms (Basidiomycetes, Agaricales)

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Myco-pharmacological investigations of bioactive metabolites and medicinal properties of mushrooms play an important role in the development of new biotech products and biopharmaceuticals. Concerning physiological activities and medicinal properties, Coprinoid mushrooms are poorly studied and not at all exploited. The majority of these organisms are fimicolous. However, many of them have also been found on trees and decaying wood (Navarro-González et al., in preparation). Based on molecular data, the traditional genus *Coprinus* Pers. has now been divided into four clades: *Coprinus*, *Coprinopsis*, *Coprinellus,* and *Parasola* (Redhead et al., 2001). For simplicity, we use the traditional nomenclature.

Important in view of biological safety, Coprini are generally nonpathogenic to humans or animals. Less than 10 cases of opportunistic mycoses by Coprini have been recorded worldwide. About 30 years ago, mycelia were isolated from two hearts of immunosuppressed patients who had undergone heart surgery and, in more recent times, from the lungs of three patients suffering from leukemia, known as non-Hodgkin's lymphoma, who received intensive cytotoxic treatment. Recently, a strain has also been isolated from human skin lesions (Kües et al., 2003). *Coprinus comatus* (O.F.Müll.) S.F.Gray may aggravate eczematous skin lesions and induce delayed-type allergic reactions by spores in individuals with atopic dermatitis. Intense efforts have been invested to identify clinically relevant allergens. *C. comatus* rCop c1, a protein of no known function, was able to induce strong specific skin reactions in sensitized individuals (Helbling et al., 2002).

Mushrooms are very good dietary sources of food (referred to as neutraceuticals) contributing to the general well-being. They are rich in proteins, carbohydrates, fibers, unsaturated fatty acids, vitamins, and minerals, but low in calories (Kües et al., 2003). While most *Coprinus* species are inherently not eaten for practical reasons (fast autodigestion of the fruiting bodies), a few species are regularly consumed by humans, and a toxic-lethal species does not appear to exist. However, among the chosen edible species is *C. atramentarius* (Bull.) Fr., users of which, in combination with alcohol consumption, describe hot flushes of the face and neck, a metallic taste, tingling sensation in the limbs, nausea, and vomiting. Occasionally, these symptoms occur also upon ingesting *C. africanus* Pegler (in Nigeria called "Ajeimutin" for *aje* = eat + *imu* = without drinking + *otin* = alcohol), *C. insignis* Peck, *C. quadrifidus* Peck, and *C. variegatus* Peck together with alcohol. The unpleasant effects are mediated by the rather unique toxin Coprine, an atypical amino acid that inhibits acetaldehyde dehydrogenase and thus blocks the metabolism of ethanol. Remission takes place within a few hours. This *Coprinus* syndrome is analogous to the non-lethal symptoms of Antabuse (disulfiram) that is applied in the treatment of alcoholism without resulting in the negative mutagenic and gonadotoxic side effects of Coprine.

Many Coprini appear to be useful natural sources

of antibacterial, antifungal, and antiviral antibiotics. For example, Coprinol, a new antibacterial cupranane (sesquiterpenoid), was isolated from fermentations of an unnamed species and was shown to be active against multidrug-resistant Gram-positive bacteria *in vitro.* Antimicrobial activity against *Bacillus cereus* and *B. subtilis* has been detected in *Coprinus plicatilis* (W.Curt.) Fr. A wide spectrum of strain-specific antimicrobial activity against Gram-positive and Gram-negative bacteria (*B. subtilis, B. mycoides, B. pumilis, Leuconostoc mesenteroides, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Comamonas terrigena,* and *Pseudomonas aeruginosa*) and microfungi (*Saccharomyces cerevisiae, Candida albicans,* and *Aspergillus niger*) was revealed in mycelia of *Coprinus micaceus* (Bull.)Fr., *C. delicatulus* Apinis, *C. comatus, C. congregatus* (Bull.)Fr., *C. radiatus* (Bolton) S.F.Gray, *C. digitalis* (Batsch)Fr., and other unidentified strains. Aqueous extracts from *C. comatus* and *C. atramentarius* inhibited vegetative growth and sporulation of *Penicillium expansum* and phytopathogens, too. A strong antiviral activity was described in *C. micaceus.*

Antibacterial and antifungal activity in mutants of *C. cinereus* (Schaeff.) S.F.Gray was due to sesquiterpenoid quinones (lagopodins, hydroxylagopodins) found in mycelial extracts and supernatants of fungal cultures. Illudins are the best studied sesquiterpenes having antibacterial and, most importantly, also cytotoxic effects against tumor cells. *C. atramentarius* was shown to produce illudin C derivates, C-2 and C-3, and *C. episcopalis* P.D. Orton to produce the three novel illudins I, I-2, and J2. *C. gonophylus* Quél. produced only illudinic acid. Antitumor activity was detected in *C. atramentarius* and *C. comatus.* Polysaccharides isolated from *C. comatus* inhibited tumor growth. 1-aminocyclopropane-1-carboxylic acid is a component of the species affecting reproductivity of male mice of the SHN line (characterized by a high risk of spontaneous mammary tumorigenesis). Furthermore, culture broth extracts of *C. disseminatus* (Pers.) S.F.Gray inhibited proliferation and induced apoptosis in certain human cervical carcinoma cell lines. An antibiotic from a culture broth of *C. radiatus* showed *in vivo* an anti-leukemia effect.

Many Coprini are characterized by strong proteolytic activity (fibrinolytic, thrombolytic, caseinolytic) that can be of medicinal interest. Galectins, a specific class of lectins detected in fruiting bodies of *C. cinereus* (Schaeff.) S.F.Gray, might be tested for therapies of muscular dystrophy or T-cell-mediated liver disorders where positive affects by mammalian galectin-1 were reported. A mild hypoglycemic effect (reducing plasma glucose concentration) by *C. comatus* described in mice might be exploited through a dietary adjunct (nutriceutical) in treatment of diabetes. Finally, hydrophobins, small nontoxic hydrophobic surface-active fungal proteins found also in *C. cinereus,* are most interesting candidates for various medical and pharmacological applications such as increasing biocompatibility of medical implants, immobilization of antibodies in a biosensor, stabilizing oil vesicles for drug delivery, and patterning molecules at the cell surface. In conclusion, Coprini are producers of various types of biologically active molecules and proteins whose further investigation will assist in obtaining new preventive and curative mushroom-based biopharmaceuticals and food additives. A more detailed review with appropriate references will be given elsewhere.

In our own screening program of Coprini, antifungal activity of *C. cinereus, C. comatus,* and *C. atramentarius* was observed in *Aspergillus niger, A. versicolor, A. flavus, Penicillium simplicissimum, P. jensenii, P. chrysogenum, Fusarium sambucinum, Cladosporium atroseptum, Stemphylium botryosum,* and *Hormiscium* sp. *C. comatus* had the highest activity, followed by *C. cinereus* and then *C. atramentarius,* while from the tested fungi, *Aspergillus* species acted in the most antagonistic way against mushroom cultures. *C. disseminatus* (Pers.) S.F.Gray and *C. micaceus* prevented or markedly suppressed the growth of numerous fungi potentially pathogenic for humans and animals (*Aspergillus candidus, A. flavus, A. wentii, Fusarium tricinctum, Stachybotris chartarum, Acremonium alternatum, Chrysosporium keratinophillum, Penicillium aurantiogriseum, P. griseofulvum, Alternaria alternata, Cladosporium cladosporoides,* and *Verticillium lecanii*). Only *A. flavus* exhibited a relatively high resistance against *C. micaceus*. Strong antiprotozoal activity against *Paramecium caudatum* was present in culture liquids of *C. comatus,* whereas different concentrations of mycelial extracts showed up to a 2.8-fold mitogenic stimulation towards the

protozoa. Culture medium of *C. disseminatus, C. micaceus, C. radiatus, C. domesticus, C. cinereus, C. comatus, C. curtus* Kalchbr., *C. strossmayeri* Schulzer, *C. xanthothrix* Romagn., and non-identified species cultures had caseinolytic (proteolytic) activity with a strong dose/effect correlation. The highest activity was observed after four weeks of cultivation, particularly in cultures of *C. xanthothrix, C. strossmayeri,* and *C. radiatus*. Cultural liquids and mycelial extracts of *C. comatus, C. disseminatus, C. domesticus* (Bolton) S.F.Gray, and *C. radiatus,* but those of *C. domesticus, C. lagopus,* and *C. micaceus* did not have a significant antioxidant activity.

ACKNOWLEDGMENTS

This work is financially supported by the grants of the Ministry of Science and Education of Armenia (# 0104), NATO (# FEL.RIG. 980764), DAAD (Pr. No 548.104401.174), and ANSEF (# 04-NS-biotech-814-73). SMB also thanks DAAD for a visiting scientist grant to work in the Göttingen University (Germany) lab financially supported by the Deutsche Bundesstiftung Umwelt (DBU). MNG holds a PhD studentship from CONACYT (Mexico).

Mycelium Macromorphology and Growth Characteristics of Culinary–Medicinal Mushroom *Flammulina velutipes* (W.Curt.: Fr.) Singer

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Recent advances in biotechnology increased the role of different groups of organisms for obtaining biologically active compounds (BAC) in the development of health-enhancing biopreparations and dietary supplements. Medicinal Basidiomycetes mushrooms are promising in the production of new biopharmaceuticals and as food additives. However, the screening of macro- and micromorphological characteristics and optimal growth conditions of mycelium of these species, selection of fast growing strains/active producers of BAC are preliminary steps in their biotechnological cultivation protocol.

The object of our study is a well known wood-inhabiting, culinary–medicinal mushroom known as *Flammulina velutipes.* Based on bibliographic data and our own observations, this species contains different groups of active compounds (polysaccharides, protein/glucan complexes, sterols, lectins, phenolic compounds, etc.) and substrate-specific enzymes. The large spectrum of pharmacological activity (immune-modulating, antitumor, antifungal, antiviral, antibacterial, fibrinolytic, thrombolytic, hypolipidic, antioxidant, spasmolytic, etc.) of *F. velutipes*' metabolites was reported.

The 21 strains of *F. velutipes,* separated from different geographical regions (Armenia: 17 strains, France: 3, and Russia: 1) and from different wood substrates (poplar: 1 strain, oak: 8 strains, grapes rod: 1 strain, ash-tree: 1 strain, pin-tree: 2 strains,

and leaves-tree stumps: 8 strains) were involved in the screening of growth and macro-morphological characteristics. Two formulas were used to calculate the colony growth rate (GR) and growth coefficient (GC): $GR=\Delta d/\Delta t$ and $GC=dgh/t$, where d = a diameter of colony growth, t = time, g and h represent the density and height of colony, respectively. The mycelial growth characteristic and morphology were analyzed after incubation of cultures on 2% malt-extract agar medium (MEA, pH = 5.5) at 25 °C. The measurements were taken for 11 days. The observations continued 2 months until primordia or fruiting body formation.

Based on GR indicators, all screened *F. velutipes* strains were divided into three main groups: I: GR <12 mm/day (9.5%), II: 12–14.5 mm/day (47.6%), and III: GR >14.5 mm/day (42.9%). The first group involved one Russian (R-9) and one Armenian (II-4) strains. The French strains possessed average GR and belonged to the second group.

Two morphological types of colony A and B within *F. velutipes* strains were described. Type A colony is farinaceous and white and later turns yellowish. The agar has yellow-brown pigmentation. Fruiting body formation was mentioned for all strains during 1–1.5 months of observation. Type A is typical for *F. velutipes* and includes average- (10 strains) and fast-growing (4 strains) separated from fruiting bodies from Armenia and France. They formed two colony "subtypes" with dense (A1) and sparse (A2) mycelium, respectively. Type B is a slowly growing morphotype of the *F. velutipes* colony with wadded and dense mycelium. The pigmentation of agar is nearly absent. The fruiting of this mycelial type was not mentioned during our observation. Type B was observed in one Russian (R-9, ash-tree) strain. However, a mixed A-B type of colony was also observed in three French and three Armenian (II-2, II-3, II-4) strains, separated from fruiting bodies collected on ash-tree and oak, respectively.

All screened strains on the 6th day of mycelial growth were divided into three groups based on GC indicators: I: GC <30 (28.5%), II: GC = 30–60 (38.1%), and III: GC >60 (23.8%). The first group involved Armenian strains with type A2, whereas group II was composed of the strains with A1 morphotype. French, Armenian (II-4), and Russian (R-9) strains were involved in group III.

We concluded that two A and B species-specific morphological types or ecotypes of colonies described in our collection of *F. velutipes* correlated with their substrate nature and geographical origination.

AKNOWLEDGMENTS

This work is financially supported by the grants of the Ministry of Science and Education of Armenia (# 0104), NATO (# FEL.RIG. 980764), DAAD (Pr. No 548.104401.174), and ANSEF (# 04-NS-biotech-814-73).

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Study of Antiprotozoal Activity and Mitogenic Effects Within Some Medicinal Mushrooms

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Many mushrooms possess both excellent nutritional values and pharmaceutical properties. Since ancient times, medicinal mushrooms have been used in traditional medicine of Asian countries. Isolated and identified substances, particularly from *Lentinus edodes* (Berk.) Singer, *Coprinus comatus* (O.F.Műll.) S.F.Gray, and *Flammulina velutipes* (W.Curt.:Fr.) Singer are used in the treatment of immune system disorders, cancer, bacterial, viral, and fungal infections, etc. They are able to decrease high glucose and lipid levels in the blood and are recommended as neuro- and vasotonics, hepatoprotective and thrombolytic agents. However, the antiprotozoal and mitogenic activities of mushrooms have not been sufficiently investigated yet.

Presented results, as a part of our Medicinal Mushrooms Myco-Pharmacological Screening Program (MMMPSP), concern the study of the antiprotozoal activity (APA) and mitogenic effects (MGE) of mycelium and fruiting bodies (FB) samples of *Lentinus edodes, Coprinus comatus,* and *Flammulina velutipes.*

Mycelia of tested species were cultivated in stationary conditions on 2% malt-extract liquid medium during 28 days. The cultural filtrate (CF) and mycelial extract (ME) samples, obtained on the 21st, 25th, and 28th days of growth, as well as FB extract of *F. velutipes* and separated polysaccharide-protein (PSP) fraction, were tested against unicellular protozoa *Paramecium caudatum* cultivated in microtiter plates using previously diluted (in water in a 1:20 ratio) Lozinskii solution (NaCl-1 g, KCl-0.1 g, $CaCl_2$-0.1 g, $MgSO_4$-0.1 g, $NaHCO_3$-0.2 g in 0.5 L distillate water, pH 7.2) at 18–20 °C. During the experiment, *P. caudatum* were fed with yeast extract.

Three different amounts (0.03, 0.06, and 0.09 mL) of five CF dilutions (not diluted and diluted in ratios 3:1, 2:1, 1:1, and 1:2) and four ME concentrations (0.01, 0.03, 0.05, and 0.1%) were tested on their APA and MGE. The APA was determined by the breakdown period of *P. caudatum* (in minutes or hours) and calculated in percents. The MGE was calculated in comparison with the mitotic division rate in control wells.

The CF and ME samples of investigated species revealed strong APA and significant MGE, respectively. A 0.09 mL CF (not diluted) of *Lentinus edodes* showed the highest APA (5 minutes), whereas previously diluted CF had relatively weaker activity (21–22 hours) (Table 1). The CF of *L. edodes* expressed no MGE. The ME (0.1 and 0.5%) possessed weaker APA compared to CF samples (48 hours). Lowering of the extract concentration brought the APA level decrease. No activity was found at 0.01% of ME. The low concentrations of ME (0.03, 0.01%, and 0.03 mL of 0.05%) possessed a 1.9–2.3-fold increase MGE.

All tested CF amounts of *Coprinus comatus* possessed strong APA. *Paramecium caudatum* were destroyed during 20 minutes. The level of activity was directly proportional to its tested amounts. No MGE

TABLE 1. Antiprotozoal Activity (APA) and Mitogenic Effect (MGE) of Medicinal Mushrooms *Lentinus edodes*, *Coprinus comatus*, and *Flammulina velutipes*

Species	Tested mushroom samples and their activities							
	CF		ME		FB extract		PSP fraction	
	APA	MGE	APA	MGE	APA	MGE	APA	MGE
C. comatus	20 min	0	0	×1.1–2.8	NT	NT	NT	NT
F. velutipes	48 hrs	×1.1–1.3	0	×1.2–2.2	0	×1.2–1.8	0	×1.1–1.4
L. edodes	22 hrs	0	48 hrs	×1.9–2.3	NT	NT	NT	NT

Notes: CF: cultural filtrate, ME: mycelial extract; FB: fruiting body, PSP: polysaccharide-protein; NT: not tested.

was observed within *C. comatus* CF. After 2 days of experiment, up to a 2.8 times increased MGE was detected within 0.1% ME samples of *C. comatus*, particularly, in the amount of 0.09 mL. Meanwhile, mitosis-stimulating activity was observed starting from 21 hours. A strong dose/effect correlation was revealed. At the same time, the tested ME concentrations of *C. comatus* did not possess any APA.

The CF samples of *Flammulina velutipes* showed weaker APA (48 hours) than tested CF samples of *Coprinus comatus* and *Lentinus edodes.* At the same time, a weak MGE was revealed when 0.03 mL of undiluted (×1.2), and diluted in ratio 2:1 (×1.3) and 1:2 (×1.1) CF of *F. velutipes* were used. All tested amounts of ME, particularly 0.06 and 0.09 mL of 0.05% and 0.1% during the 48 hours of observation, possessed up to a 2.2 times increase in MGE. It was almost absent at 0.01% of ME. No APA was observed within ME samples, FB extract, and PSP fraction separated from the extract of *F. velutipes.* However, they were able to stimulate mitosis of *Paramecium caudatum* on the 4th day of the experiment up to 2.2, 1.8, and 1.4 times, respectively.

No significant differences were found between APA and MGE levels of all tested CF and ME samples of three species obtained on the 21st, 25th, and 28th days of mycelial cultivation.

Thus, the presence of APA and MGE within Lentinus edodes, Coprinus comatus, and *Flammulina velutipes* completes the list of medicinal properties of these mushrooms and makes them suitable for further development of new mushroom-based nutritional supplements. They could be used in prevention and treatment of many intestinal protozoal infections and wound-healing processes.

ACKNOWLEDGMENTS

This work is financially supported by the grants of the Ministry of Science and Education of Armenia (# 0104), NATO (# FEL.RIG. 980764), DAAD (Pr. No 548.104401.174), and ANSEF (# 04-NS-biotech-814-73).

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Pleurotus tuberregium (Rumph.:Fr.) Singer and the Wuli People of Northwest Cameroon: Indigenous Knowledge and Ethnomyolcogy

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The Wuli constitute the population of Lus, one of the 13 villages of the administrative, cultural, and linguistic entity called Mfumte in northwest Cameroon. They have a population of about 3000 and live on either side of the wooded banks of the Mamfe River, a tributary of the Donga River, which is the border with Nigeria. The Wuli people believe and use sclerotia of *Pleurotus tuberregium*, which they call *nè*, as an important magical medicine for fighting witches that bring diseases to their people. They have no use for the sporophores regardless of their edibility. The biological characteristics, especially the white color of the inner parts of the sclerotium, and its ability to swell on absorption of water make it amenable to the Wuli's uses.

Members of a powerful society, called *wangkya*, keep the sclerotia in their sanctuary, together with sculpted objects whose aim is to fight against the witches introducing disease and death among its people. They consider that the sclerotia, when peeled, are the symbolic substitutes for the white excreta of two supernatural siamese twins. A myth holds that this strange couple, who founded the "wangkya" association, excreted strong medicine against witchcraft. Wuli people believe the magical excreta that can fight witches bringing diseases to the people are sclerotia of *P. tuberregium*. When they think that a witch has introduced a disease into the village, the initiates of "wangkya" grind the sclerotium into powder and mix it into water, warm up the mixture, and pour this mixture into a ritual pot. The pot is brought to the entrances of the village, and an initiate forces the pot to "excrete" its contents across the path or road to the village. The Wuli believe that if the evil witch passes across this barrier, her or his belly will "inflate with water," just as the *nè* did in the pot due to swelling after absorbing water. People with such swellings are believed to be witches attacked by the ritual involving sclerotia mixture, but they actually have a disease known in modern medicine as ascites. The Wuli make a metaphorical link between the biological characteristic of the sclerotium of *P. tuberregium* (ability to swell) and the intended effect of this magical practice (ascites).

The Wuli believe that this sclerotium is only growing on decayed logs of two species of trees, called *lè* (technical name yet to be determined) and *hwi*, which could be Cesalpinaceae, *Brachystegia* (cf. *nigerica*) or *Berlinia* sp. (cf. *grandiflora*), which give a female *nè* and a male *nè,* respectively. This assertion does not fit with the ecological reality and what modern studies on this fungus have shown. However, their belief is based on their ways of classifying plants and other living things in the wild—a taxonomic system which takes gender into account by "marrying" a species with another species.

This paper examines the taxonomic and religious/spiritual belief held by the Wuli people on *P. tuber-*

regium and compares them with scientific knowledge on the biology of this fungus. We attempt to understand how *P. tuberregium*'s peculiar characteristics have been integrated into the local taxonomy and how they combine with the nosological and religious system of the Wuli. A comparison of the traditional uses of this fungus for food and medicine by other tribes in Africa is also presented.

International Journal of Medicinal Mushrooms: Celebrating Seven Years

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Since ancient times mushrooms have been used thorought the world in folk medicine. Mushrooms are being evaluated for their nutritional value and acceptability, as well as their pharmacological properties. They are a nutritionally functional food and a source of physiologically beneficial and noninvasive medicine. Some of the most recently isolated and identified substances originating in mushrooms have been shown to be promising immunomodulators and have demonstrated significant antitumor, cardiovascular, antiviral, antibacterial, antiparasitic, hepatoprotective, and antidiabetic activities. For almost 40 years, medicinal mushrooms have been intensively investigated for medicinal effects in *in vitro* and *in vivo* models, and many new antitumor and immunomodulating polysaccharides have been identified and put into practical use. The information published in recent years is helpful in exploring and understanding the rich traditions of medicinal mushrooms in Eastern and Western cultures and medicine. Cultivation and development of edible and medicinal mushrooms can positively generate equitable economic growth and have already had an impact at national and regional levels.

Modern science's study of medicinal mushrooms has quickly developed during the last two decades. Publications devoted specifically to the study of medicinal mushrooms have increased geometrically. Articles appear in hundreds of biological, mycological, botanical, microbiological, forest, agricultural, food, biochemical, chemical, medical, pharmacological, and ethnobiological journals, among others.

In view of the great interest in medicinal mushrooms and the absence of a specialized journal in the field, a special journal dedicated to medicinal mushrooms, known as the *International Journal of Medicinal Mushrooms* (IJMM), was established in 1999 by Begell House, Inc. (USA) (www.begellhouse.com).

The IJMM was specifically created to help scientists all over the world share the research, information, and new techniques that might promote experimental progress in the field of mushrooms. It is a unique journal and provides a forum for healthcare professionals and research scientists to use as an international tool for education. The IJMM publishes original research articles and critical reviews on the following aspects of medicinal mushrooms: systematics, taxonomy, tissue cultures, culture collection, cultivation, biotechnology, me-

dicinal macromycetes engineering, biochemistry, chemistry, physiology, secondary metabolism, genetics, pharmacology, medicinal value, therapeutic effects, currently recognized biomedical components (and their functions), general characteristics of medicinal uses of medicinal mushrooms, and commercially useful medicinal mushrooms and their products.

The Editorial Board includes 28 famous scientists from all over the world representing 16 countries in Europe, Asia, and North and Central America. The reviewing process takes approximately six to nine months until publication. Upon receiving an article from a researcher, the Editorial Board sends this article to three reviewers. The author receives comments and suggestions and then makes the necessary adjustments for publication.

The IJMM is published quarterly and highlights new perspectives in the field of mycology and medicine. Further identification of the components responsible for health-benefiting activity has not been completely identified yet. The information presented in this journal is helpful in exploring and understanding the rich traditions of medicinal mushrooms in Eastern and Western cultures and helps to expand up-to-date knowledge of an ever increasing, rapidly developing field.

During the past seven years the IJMM has published 540 articles, including approximately 270 abstracts of the First (Kiev, Ukraine, 2001) and Third (Port Townsend, Washington, 2005) International Medicinal Mushrooms Conferences. The IJMM published articles from authors from 47 countries: North America (Canada, USA, and Mexico); South America (Venezuela, Brazil, Argentina, Colombia); Asia (R.P. China, Japan, Israel, South Korea, Singapore, Hong Kong, India, Malaysia, Taiwan); Africa (Nigeria, Namibia, South Africa); New Zealand and Australia; and 24 European countries.

The IJMM is indexed in the BIOSIS Database, International Pharmaceutical Abstracts, and Elsevier's Bibliographic Databases.

Medically Significant White-Rot Basidiomycetes: Metabolic and Biochemical Characteristics

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Well known edible and medicinal Basidiomycetes such as *Lentinus edodes* (Berk.)Singer, *Flammulina velutipes* (W.Curt.:Fr.) Singer, *Ganoderma lucidum* (W.Curt.:Fr.)Lloyd, *Pleurotus* spp., *Trametes* spp., and some others belong to white-rot xylotrophic species. These Basidiomycetes are active destructors of the woody plants due to their trophy specialization for lignin in substrates.

Cultural investigations of white-rot fungi began in the middle of the 20th century due to identification of their capabilities to grow on different nutrient substrates and to exist for long periods in the mycelium stage. This peculiarity of white-rot fungi makes possible their conservation *ex situ* in culture collections. The ability of basidiome formation during cultivation permits use of some edible

white-rot Basidiomycetes for fruit body production, identification, and verification of strains during experimental conditions. The high enzymatic potential of white-rot fungi and their ability to grow *in* and *on* substrates permits their use in various types of wastes.

Physiological investigations of white-rot fungi established different peculiarities of the second metabolism of this fungi group. It was shown that many low-molecular weight compounds isolated from white-rot Basidiomycetes determine their flavor, pigmentation, and biological activities. The properties, structures, and characteristics of activities of many terpenoid, alkaloid, phenylquinoid, and anthraquinoid metabolites were established. At present some mushrooms produce substances used in aromatherapy and in the preparation of sanative beverages (i.e., *Marasmius* sp., *Gloeophyllum odoratum* (Wulfen) Imazeki, *Lentinus edodes*). Others are used in the preparation of medicinal drugs containing mucidin, inotodiol, and ganodermanes (i.e., *Oudemansiella mucida* (Schrad.)Höhn., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Ganoderma lucidum*). High-molecular weight substances such as polysaccharides are also the products of the second metabolism of white-rot basidiomycetes. Today, polysaccharides are the main constituents of some drugs and dietary supplements well known in countries of the southeast regions on Earth. These medicinal products are obtained by fermentation—white-rot Basidiomycetes such as *Lentinus edodes*, *Trametes* sp., *Schizophyllum commune* Fr.:Fr., and some others.

Investigations of the hydrolytic enzymes of white-rot fungi allow indicating the presence of different types of proteases and obtaining the secreted fibrinolytic proteases from *Flammulina* and *Coprinus* species and milk clothing enzymes from *Mycena pura* (Pers.:Fr.) P.Kumm., *Irpex lacteus* (Fr.) Fr., and *Sparassis crispa* (Wulfen) Fr. In the last three decades, the enzymatic activity of white-rot Basidiomycetes has been causing great interest, resulting in active investigations. It was shown that among Gem-, Flavin-, and Cu-containing oxidases produced by these fungi, the main role in degrading lignin belonged to lignin peroxidases, manganese peroxidases, and laccases. Biochemical investigations of some species from families Polyporaceae, Ganodermataceae, and Strophariaceae have shown that these white-rot Basidiomycetes produce different oxidases, among which laccases were the main enzymes. Some characteristics of isolated laccases were investigated in cooperation with colleagues from the Bach Biochemistry Institute RAS. Great interest in the practical use of oxidative enzymes of Basidiomycetes is explained in their chemical and catalytic features. The possibility of oxidation of different aromatic, principally phenolic substances to quinones is now very well known in different areas of industry and medicine.

ACKNOWLEDGMENTS

The research on conservation of Basidiomycetes *ex situ* is supported by RFFR (Russian Fund of Fundamental Researchers) grant 03-04-49604; biochemical investigations of the lignin-destroying Basidiomycetes and their oxidases are supported by INTAS grant 03-51-5889.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Antioxidant and Gene Protective Effects of Medicinal Mushrooms *Inonotus obliquus* (Pers.:Fr.) Pilát and *Phellinus robustus* (P. Karst.) Bourd. et Galz.

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Fungal melanin pigments were shown to display high antioxidant activity. This work was designed to isolate melanin pigments from *Inonotus obliquus* and *Phellinus robustus* to study their antioxidant and gene protective properties during metabolic activation of aromatic amines by the peroxidase pathway of oxidation.

Investigation of the element composition of exo- and endomelanins of *Inonotus obliquus* and *Phellinus robustus* showed that these species distinguish one from another insignificantly. However, it has been demonstrated that the content of carboxyl groups in endomelanins of both investigated species was two times higher in comparison with exomelanins. IR- spectra of exo- and endomelanins of *I. obliquus* and *Ph. robustus* were also similar. The molecular weight of exo- and endomelanines from *I. obliquus* was 35–60 kDa, and from *Ph. robustus* 40–60 kDa.

The obtained data indicate that the alkaline fraction of exo- and endomelanins of *I. obliquus* had a molecular mass of 55–60 kDa, *Ph. robustus* 65–70 kDa, the alcohol fraction of *I. obliquus* 25–30 kDa, and *Ph. robustus* 40–45 kDa. The melanins of both species also contained a small quantity of substances with the molecular mass of 100–120 kDa.

Melanins isolated from both mushrooms and applied at a concentration above 20 μg/mL inhibited the reaction of peroxidase-mediated oxidation of *o*-dianisidine (DA). It was demonstrated that the ability of melanin isolated from *Ph. robustus* to inhibit peroxidase-mediated free radical oxidation of DA was considerably lower than that of the pigment from *I. obliquus*. The highest degrees of inhibition of peroxidase-mediated DA oxidation with melanins isolated from *Ph. robustus* (75 μg/mL) and *I. obliquus* (75 μg/mL) mushrooms were 75% and 80%, respectively. Our data showed that melanin isolated from *I. obliquus* inhibited peroxidase-mediated oxidation of DA more efficiently than it prevented damage to DNA induced by oxidation products. In contrast, melanin from *Ph. robustus* applied at a lower concentration inhibited the formation of DNA/DNA crosslinks more readily than it prevented DA oxidation. Such properties of melanins can be used in developing new anticarcinoganic drugs.

Microstructures in Medicinal Mushroom Cultures

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The correct identification of the taxonomic position of mushroom cultures due to their biotechnological application is a task of paramount importance. A dolipore septae and clamp connections are basic characteristics of Basidiomycetes cultures. A detailed study of clamp connections, anamorphs, and vegetative mycelium structures allows for more accurate morphological characterization of taxa introduced in pure culture.

Vegetative mycelium microstructures of more than 150 macromycetes species (Basidiomycota and Ascomycota) were studied using scanning electron microscopy (SEM). New data were obtained on the fine microstructures in cultures of medicinal mushroom species of the genera *Pleurotus, Ganoderma, Trametes, Agaricus, Auricularia, Oudemansiella, Coprinus, Marasmius, Morchella,* and species *Lentinus edodes* (Berk.) Singer, *Hericium erinaceus* (Bull.) Pers., *Grifola frondosa* (Dicks.:Fr.) S.F.Gray, *Hypsizygus marmoreus* (Peck) H.E. Bigelow, *Schizophyllum commune* Fr.:Fr., *Piptoporus betulinus* (Bull.: Fr.) P.Karst., *Omphalotus olearius* (DC.:Fr.) Singer, *Laetiporus sulphureus* (Bull.:Fr.) Murrill, *Polyporus squamosus* (Huds.) Fr., etc.

The presence and dislocation of clamp connections on hyphae are essential taxonomic characteristics for some species. Some species have clamps of an original form, namely *Oudemansiella mucidum* (Schrad.) Höhn., *Auricularia auricula-judae* (Fr.) Quél., and *Lentinus tigrinus* (Bull.:Fr.) Fr.; whereas *Piptoporus betulinus* (Bull.:Fr.) P. Karst., *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm., and *Lyophyllum decastes* (Fr.) Singer are characterized with clamp connections of various forms and sizes. Single clamps, whorls of clamps, coupled clamps, and sprouted clamps were observed in cultures of these mushrooms. Clamps are constant in cultures belonging to species of *Pleurotus, Coprinus, Oudemansiella, Panus, Lentinus,* and *Pholiota*, but very rarely occurred in vegetative mycelium of *Agaricus* spp. (*A. subperonatus* (J.E. Lange) Singer, *A. arvensis* Schaeff., *A. bernardii* Quél., *A. comtulus* Berk. et Broome, *A. campestris* L.:Fr., *A. maskae* Pilát, *A. bernardiiformis* Bohus, *A. comtulus,* and *A. brasiliensis* S. Wasser et al.).

Different structures of asexual reproduction (anamorphs) may serve as taxonomic criterion at the species level. Arthroconidia have been found in the mycelial cultures of *Oudemansiella* spp. *Lepista nuda* (Bull.:Fr.) Cooke, *Omphalotus olearius, Agaricus abruptibulbus* Peck, *A. bernardiiformis, A. fissuratus* (F.H. Møller) F.H. Møller, *A. macrocarpus* (F.H. Møller) F.H. Møller, *A. maskae, A. squamuliferus* (F.H. Møller) Pilát, *A. cupreobrunneus* (Jul. Schäff. et Steer) Pilát, *A. silvaticus* Schaeff., *A. arvensis, Hypsizygus marmoreus, Lyophyllum ulmarium* (Bull.:Fr.) Kühner, *Paxillus acheruntius* (Humb.) J. Schröt., *Polyporus squamosus* (Huds.: Fr.) Fr., etc. Formation of coremia is known only for *Pleurotus abalonus* Y.H. Han, K.M. Chen et S. Cheng and *P. cystidiosus* O.K. Mill. Single globose conidia, or excretory cells, on simple conidiophores, resembling a sterigmata of the basidium, are formed

on hyphae in cultures of *Pleurotus* spp. and *Schizophyllum commune*. Chlamydospores in dicaryotic *Hericium erinaceus* cultures, dichohyphidia, and intercalar chlamydospores of *Grifola frondosa* are of taxonomic significance. An anamorphic state of oidium and *Costantinella* type is characteristic for Morchellaceae. Chlamydospores were discovered in cultures of *Agaricus bisporus* (J.E. Lange) Imbach, *A. arvensis*, *Leucocoprinus birnbaumii* (Corda) Singer, *Macrolepiota subsquarrosa* (Locq.) Bon, *Handkea excipuliformis* (Scop.) Kreisel, *Langermmania gigantea* (Batsch.) Rotsk., *Boletus edulis* Bull.:Fr., *B. quletii* Schulzer, *Suillus bovinus* (Pers.) Kuntze, *Marasmius androsaceus* (L.:Fr.) Fr., *Hypsizygus marmoreus*, *Coriolus zonatus* (Nees) Quél., *Auricularia auricula-judae*, *A. polytricha* (Mont.) Sacc., etc.

For *Coprinus cinereus* (Schaeff.) S.F.Gray, *Crinipellis shevczenkovi* Buchalo, *Agaricus gennadii* (Chatin et Boud.) P.D.Orton, and *Leucocoprinus bresadolae* (Schulzer) S.Wasser cultures sclerotia are known. Strand-like mycelial cords were found in cultures of *Agaricus arvensis*, *A. bisporus* (J.E. Lange) Imbach, *A. bitorquis* (Quél.) Sacc., *A. campestris*, *A. subfloccosus* (J.E. Lange) Pilát, *A. vaporarius* Krombh., *A. brasiliensis*, *Macrolepiota procera* (Scop.) Singer, *M. excoriata* (Schaeff.) M.M. Moser, *M. mastoidea* (Fr.) Singer, *Omphalotus olearius*, *Russula ionochlora* Romagn., *Phallus impudicus* L., *Lycoperdon pyriforme* Schaeff., *Scleroderma citrinum* Pers., and *Tulostoma berteroanum* Speg.

Hyphae ornamentation observed in *Lyophyllum* spp. cultures may serve as taxonomic characters. Warty ornamentations were detected in *Oudemansiella brunneomarginata* Lj.N. Vassiljeva and *O. mucida* on the loop forming hyphae. Lacunose structured hyphae are described in some species of Morchellaceae. Formation of calcium oxalate crystals was observed in all investigated species of *Agaricus*. Polygonal, hair-like crystals and crystals of other shapes were observed in *Hypsizygus marmoreus*, *Hericium erinaceus*, *Lentinus edodes*, *Armillariella mellea* (Vahl) P. Karst., *Pholiota jahnii* Tjall.-Beuk. et Bas, *Clitocybe odora* (Bull.:Fr.) P. Kumm., *Kuehneromyces mutabilis* (Schaeff.) Singer et A.H. Sm., *Peniophora gigantea* (Fr.) Massee, *Omphalotus olearius*, *Coprinus comatus* (O.F. Müll.) S.F.Gray, *Agaricus fissuratus*, *A. subfloccosus*, and *Montagnea arenaria* (DC.) Zeller.

Can Bioactive Polysaccharides and Proteo-Polysaccharides from Medicinal Mushrooms Benefit Women with Malignant Breast Tumors?

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In view of the staggering statistics of women, especially menopausal women, who are afflicted with malignant breast tumors, there is an urgent need to seek effective measures to combat such sweeping health problems. The World Health Organization (WHO) predicts that more than 1.2 million women worldwide may develop breast cancer this year. In the United States of America alone, 1,370,910 new cases of breast cancer may be documented in 2005, estimated by the American Cancer Society. One in eight women may experience breast cancer in her lifetime.

Prominent species of medicinal mushrooms have a long-standing history of being used for health benefits, including antitumor effects, particularly in China, Japan, and Russia. We are examining bioactive polysaccharides and proteo-polysaccharides from selected species of medicinal mushrooms, in adjunct use with conventional cancer treatment. Our interest is in center therapy with immunoceuticals, imunomodulating components from medicinal mushrooms, which are effective by oral intake. Bioactive polysaccharides and proteo-polysaccharides in the category of imunoceuticals from *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd, *Grifola frondosa* (Dicks.: Fr.) S.F.Gray, and *Trametes versicolor* (L.:Fr.)Pilát will be examined for their potential to alleviate the devastating side effects of conventional cancer treatments, such as presurgery hormonal therapy or chemotherapy to shrink the size of tumor, surgery, as well as postsurgery radiation therapy, chemotherapy, or hormonal therapy. Exciting initial results of a case study of a stage II breast cancer patient who uses bioactive polysaccharides from medicinal mushrooms during chemotherapy will be reported.

The project is designed as a forum to raise questions, evoke discussions, and seek evidence-based information. The intended audience is laymen at large and medical professionals, who are currently in practice. Sample questions in brainstorming include cell-receptor for glucans, autoimmune diseases, antioxidants, use of vitamin C, potential cross-drug interaction, toxicity, safety in use, contraindications, who should avoid using such components, and how can breast cancer patients benefit.

To provide bioactive mushroom polysaccharide and proteo-polysaccharide in adjunct use to conventional cancer treatment is a vitally important area, to which medicinal mushroom science and industry can contribute. More quality large-scale longitudinal clinical studies as well as case studies with specific information are encouraged. Attention is called to mushroom growers to produce high-standard, organically grown mushrooms as a source of effective polysaccharides and proteo-polysaccharides with efficacy for cancer and other uses.

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Comparative Analysis of Cordycepin, Minerals, and Vitamins on Cultivation Media of *Cordyceps militaris* (L.:Fr.) Link

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Cordyceps militaris, belonging to Clavicipitaceae, Ascomycotina, has long been considered to have natural medicinal properties, compounds that are homeostatic, mycolytic, and antiasthmatic, and it is used as an expectorant. It has also been found as an insect parasite under humid ground in the mountains of Korea, China, and Japan.

Cordycepin, a nucleoside analogue 3′-deoxy-adenosine with a broad spectrum of biological activity, was first extracted from *C. militaris*. The quantitative analysis of cordycepin is important in studies of their functions in biological systems.

The purpose of the present study was to develop a simple, fast, and sensitive LC/MS method for simultaneous separation and determination of an active component in the oriental medicinal mushroom mentioned above. Based on this work, the contents of cordycepin in *C. militaris* fruiting, cultivated on various media, were determined and compared. And also, the nutritional components such as minerals and vitamins were determined in order to provide useful information to the consumers. The optimum separation for cordycepin was achieved using a solvent gradient consisting of a mixture of 0.1% formic acid in methanol (solvent B) in a background of 0.1% formic acid in water (solvent A) as a mobile phase and a 3.0 × 150 mm Waters Xtera column. Selective ion monitoring (SIR) mode ([M+H]+ at m/z 252) was used for quantitative analysis of cordycepin. The cultivated *C. militaris* on various media contained 1~14 mg/g of cordycepin, 0.65~1.08 mg% of thiamine, 0.86~7.17 mg% of riboflavin, and 3.01~5.26 mg% of niacin. The content of mineral components varied on categories, especially on those containing 500~3500 mg% of potassium as a major mineral. Cordycepin, niacin, and potassium were much higher in the fruiting cultivated with soy powder media (gold 10) than other media.

Enzymes of *Phanerochaete chrysosporium* Burds. and *Irpex lacteus* (Fr.)Fr.: Decolorization of Dyes and Effluents

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White-rot Basidiomycetes (WRB) play a central role in global carbon cycles as a result of their innate ability to mineralize the lignin, which has a complex polymeric structure. Shallow stationary cultures of *Phanerochaete chrysosporium,* grown on low-nitrogen mineral medium, produced manganese peroxidase (MnP) and lignin peroxidase (LP). Sulphonphthalein (SP) dyes were decolorized by MnP activity. Decolorization of SP dyes occurred optimally at pH 4.0. An increase in the halogenation (bromine group) of the SP dyes decreases its substrate specificity for MnP. The presence of additional auxochromes (position, type, and number) on the SP dye chromophore influence the suitability of SP dyes as an MnP substrate.

The methyl group in the *ortho* position (in the case of *o*-cresol red) is favored over that in *meta* position (in the case of *m*-cresol purple). Oxygen scavenger (sodium metabisulfite) and hydroxyl radical scavengers (thiourea and mannitol) did not have any influence on MnP decolorization activity. EDTA, a metal chelator, inhibited MnP-catalyzed decolorization reaction. The results highlight the SP dyes as substrates of MnP and provide another class of chromogen for the detection and estimation of ligninolytic peroxidases. Also, the results emphasize as a model for the bioremediation program of the structurally similar xenobionts and recalcitrant compounds stressing over enzyme(s) of white rot basidiomycete *P. chrysosporium* as a potent biochemical tool.

In another study, white-rot basidiomycete *Irpex lacteus* decolorized the textile dyes on solid medium. Decolorization on solid medium was of older mycelium. Extracellular enzyme extract prepared from *I. lacteus*-infested wheat straw possessed ligninolytic enzymes manganese peroxidase (MnP), manganese-independent peroxidase (MIP), and lignin peroxidase (LP). Decolorization of triphenyl methane dyes is attributed to MnP activities.

The same color dyes (red, orange, and blue) were decolorized variably on the solid medium as well as by the ligninolytic enzymes of *I. lacteus*. The presence of a halogenated compound, cyanuric chloride, in the structure of reactive orange 13 makes it a suitable substrate for MIP, whereas MnP decolorizes reactive orange 16 that has a naphthalene ring with an hydroxyl group, presenting the dye as a simpler phenolic moiety. Decolorization of triphenyl methane dyes methyl blue (MB) and fast green (FG) was attributed to MnP activities. Brilliant green (BG) resisted decolorization by ligninolytic activities. The presence of two 3-[2-(ethyl amino) ethyl] benzene sulfonate groups in FG and three 4-amino benzene sulfonate groups in MB favor the decolorization, whereas the presence of two *N,N*-diethyl amine groups in BG resist the decolorization by ligninolytic activities. Enzymatic decolorization of effluents led to the complete loss of the peak in the UV-region. Ligninolytic activities associated to decolorization of effluents showed no influence of pH and decolorized the effluent over the pH range of 3–5. The results highlight the efficacies of the ligninolytic system in designing a modest bioremediation program and also provide the means to distinguish among the peroxidases generated as a part of the ligninolytic system.

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Hypocholesterolemic Effect of the Oyster Mushroom, *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. and Its Isolated Polysaccharides

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Unfavorable developments in the incidence of hypercholesterolemia (high cholesterol) and clinical complications related to atherosclerosis throughout the industrialized world makes the search for natural substances with hypocholesterolemic effects highly important. The role of dietary fiber in lipid metabolism and athergensis has been extensively studied; however, the focus has been on the fibers of vegetable, fruit, and cereal origin. Mushrooms contain high fiber content, sterols, proteins, and microelements. These, along with their low caloric content, makes mushrooms ideal for a nutrition program aimed at the prevention of cardiovascular disease.

This work has been carried out to investigate the hypocholesterolemic effect of oyster mushroom *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. and its ethanolic extract on hamsters fed a hyperlipidemic diet. The chemical analysis of the oyster mushroom indicates that the fruit bodies contain 54% carbohydrate, 16% protein, and 6% lipid along with lower concentrations of many other chemical substances. The physicochemical properties of the mushrooms and their extracts were extensively investigated. The molecular weight of the active fraction of the mushroom ethanolic extract was 304 kilodaltons. The NMR spectra indicated that the extract is glucan in nature. In order to investigate the hypocholesterolemic effect of the mushrooms, it was decided to evaluate the effect of this fungus on several biochemical criteria associated with atherosclerosis and coronary heart disease. The hamster model was chosen because its cholesterol metabolism has been found to be clearly similar to that of humans. The present work revealed that the addition of mushrooms to the diet did not affect the intake of other foods or the final body weight of the hamsters. Results showed a significant decrease in the tested serum and liver lipid parameters in hyperlipidemic hamsters given mushroom fruit bodies and their extract. The effect of mushroom ethanolic extract on acyl-CoA—cholesterol actyltransferase (ACAT) activity, apolipoprotein content, and phospholipid profile—were studied. Results indicated that mushroom ethanolic extract reduced cholesterol absorption.

Our data confirm the importance of ACAT for the absorption of dietary cholesterol in hamsters. The lethal dose value of ethanolic mushroom extract administered in hamsters was 660 mg/kg body weight. Estimation of enzymes representing liver function and glucose concentration was done in this study to illustrate if there were any side effects due to administration of mushroom extract to these hyperlipidemic hamsters. The hypocholesterolemic effect of mushroom ethanolic extract was less pronounced than the effect of a diet containing whole oyster mushroom. Similarly, the favorable redistribution of cholesterol in lipoproteins was less evident in the group given mushroom ethanolic extract compared to a diet containing whole mushroom.

This indicates that the ethanol does not extract all hypocholesterolemic substances present in oyster mushrooms. Histological studies on arteries from different groups showed much thickened artery walls in hamsters fed a hyperlipidemic diet. The thickening of the wall was partially prevented by using mushroom extract.

In conclusion, the favorable hypolipidemic activities of oyster mushrooms, as evidenced by the decrease in lipid parameters and the ACAT inhibition in animal models, show that further investigation is warranted. This is an area under investigation by a number of laboratories. There is an implicit role suggested for this drug class as therapy for coronary artery and other atherosclerotic diseases in humans.

Proteolytic Enzymes of Basidiomycetes (Taxonomical and Ecological Aspects)

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The proteolytic activity (PA) of cultures and fruit bodies of Basidiomycetes belonging to various taxonomical and ecological groups was studied. The study involved a total of 700 strains and the fruit bodies of 400 species.

Fermentative activities of the mycelium and fruit bodies were revealed and estimated by means of its influence on a certain substrata, as in fibrin films (fibrinolytic activity, FA), human blood clots (thrombolytic activity, TA), milk (milk-clotting activity, MCA), and casein (caseinase activity, CA). A milk-clotting activity fairly often accompanies TA and FA. A caseinase activity should be considered as a nonspecific indicator of PA. Our main attention was paid to the study of ferments, which can execute the lysis of fibrin films and thrombi (FA and TA, respectively).

The current results allow the estimation of a certain level and character of proteolytic process for Basidiomycetes of various taxonomical groups as well as specific connections of certain types of enzymatic activity with a taxonomical position and trophic preference of macromycetes under investigation. Besides, it becomes possible to determine some functions of fibrinolytic and thrombolytic proteinases in mushroom vitality and to estimate an opportunity of practical use of these ferments in certain branches of medicine.

Generally, it is possible to ascertain that the dominant type of activity within saprotrophs (together with xylothrophs) is CA, since it was observed among half of the studied species. The fibrinolytic activity was demonstrated only by representatives of 20–25% of total species. Within a symbiotrophic species the caseinase activity (as a nonspecific parameter of PA) was registered only at a quarter of investigated species, whereas FA is mentioned only at 12% of total species number.

It was established, within the aphyllophoroid fungi, the most expressed proteinase biosynthesis is characteristic to the families Polyporaceae and Schizophyllaceae, whereas within the agaricoid Ba-

sidiomycetes the families Pleurotaceae, Tricholomataceae, and Coprinaceae demonstrated the greatest activity. The active species of aphyllophoroid fungi, in most cases, belong to the xylotrophs, which produce a white rot, no less than active agaricoid ones, which are the lignotrophs, decomposing the lignocellulose complexes of wood debris, forest litter, various composts, etc. The symbiotrophic species (families Amanitaceae, Cortinariaceae, Russulaceae, etc.) were characterized by a complete absence of fibrinolytic and thrombolytic activity (a few exceptions would include the genus *Tricholoma* sect. *Tricholoma* and some representatives of the order Boletales).

It seems the revealed pattern of distribution of proteolytic ferments of different substrate specificity (FA, TA, MCA, CA) in various taxonomical and ecological groups of Basidiomycetes certainly allows the prediction of the challenge of species with the mentioned biochemical activities and, besides, in some cases the ability to be useful taxonomically.

During the studies of fibrinolytic and thrombolytic activity of macromycetes cultures, the ability of Basidiomycetes to synthesize the proteinases of four types—namely, the aspartyl, the serine, the thiol, as well as the metalloproteinases—were confirmed. From the cultures of some representatives of the genus *Coprinus*, characterized by a high level of FA and TA, the proteinases of a serine type were allocated and described, and from the cultures of *Cerrena* and *Coprinus* species the metalloproteinases were received for the first time. The comparison of the biochemical and physicochemical characteristics of the proteinases received from the investigated basidiomycetes to those mentioned in the literature as bacterial, micromycetous, and vegetable proteinases allows us to conclude that there exists a principal similarity of these proteolytic enzymes.

It was also shown that Basidiomycetes of various trophic preferences synthesize the proteolytic fermentative complexes combining the proteinases of different classes. The metalloproteinases bring the basic contribution in the fibrinolytic activity among all the trophic groups. For example, the fibrinolytic activity of soil saprotroph *Coprinus domesticus* (Bolton) S.F.Gray is determined by the metalloproteinases on 47%, the serine proteinases on 30%, the thiol proteinases on 17%, and aspartyl only on 6%. The fibrinolytic activity of another soil saprotroph, *C. cinereus* (Schaeff.) S.F.Gray, is determined by these types of proteinases on 53, 21, 13, and 13%, respectively. Such type of activity for litter saprotroph *Clitocybe josserandii* (Singer) Singer is caused by these types of proteinases in the ratio 40, 23, 28, and 9%. In the xylotrophic species, *Flammulina velutipes* (W.Curt.:Fr.) Singer and *Cerrena unicolor* (Bull.) Murrill, the contribution of metalloproteinases in FA is 70–90%, whereas in symbiotrophic *Tricholoma portentosum* (Fr.) Quél. the fibrinolytic activity is caused by metalloproteinases completely (100%).

During the complex analysis of fibrinolytic and trombolytic activity of this large group of fungi, the impression is created that there is a real dependence between the type of synthesized proteinases and the trophic preference of the fungi in question. High-level FA and TA in trophically specialized groups of saprotrophs and the occurrence of similar ferments in some symbiotrophic species can testify to an opportunity of transition of such taxa from obligate symbiotrophy to a facultative one. Such dependence of the type of synthesized proteinases and the trophic features of the fungi in question could be considered an additional criterion for estimation of the trophic amplitude of macromycetes in nature.

Protoplast Fusion between *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. and *P. citrinopileatus* Singer

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Protoplast fusion has been used as a method to create mushroom hybrids, especially when using conventional methods cannot achieve this result. *Pleurotus ostreatus* and *P. citrinopileatus,* along with many other species of the genus *Pleurotus,* are widely industrially cultivated in many parts of the world, including Thailand.

Protoplasts fusion between *P. ostreatus* and *P. citrinopileatus* in this experiment was carried out by growing 4-day-old monokaryotic mycelia derived from a single spore isolate of each species on malt extract broth. The mycelia were agitated at 100 rpm for 2 hours with 9 mg membrane-filtration sterilized Lysing Enzyme (Sigma L-1412) in 1 mL osmotic stabilizer (0.6 M $MgSO_4(7H_2O)$ in 0.05 M sodium maleate buffer, pH 5). The freshly prepared protoplasts were then mixed and incubated in 40% PEG (polyethylene glycol 6,000)/0.05 M $CaCl_2(2H_2O)$ for 20 minutes at room temperature. All protoplasts were regenerated on regeneration medium containing 30 g agar, 20 g malt extract, 20 g glucose, 1 g peptone, and 1000 mL 0.6 M sucrose for 7–12 days. There were 487 regenerated colonies detected, but only three of them were selected as fusants by posessing clamp connections on their mycelia. The fusants (Fu1, Fu2, and Fu3) were proved to be "hybrids" of *P. ostreatus* and *P. citrinopileatus* as the experiment progressed.

The mycelia of the fusants were significantly faster in growth and larger in size than the parental strains, which were relevant to the theories that fusants, which are dikaryotic ($n + n$), grow faster (Toyomatsu and Mori, 1987) and have larger hyphae (Abe et al., 1982) than their monokaryotic (n) parental strains. The experiments were performed by subculturing the mycelia of each fusant and of the two parents on MEA plates and incubating for 9 days at room temperature followed by determining the diameters of each colony for at least 10 replications as the mycelial growth and measuring the diameters of the mycelia microscopically using a calibrated eyepiece micrometer for 100 replications as the hyphal size.

The fusants showed esterase and malate dehydrogenase isozyme bands common to those of their parents when esterase, malate dehydrogenase, and alcohol dehydrogenase were studied as the method modified from that of Pasteur et al. (1988).

The fruiting bodies of Fu1, which was the only fusant successful in fruiting on sawdust plastic bags, showed recombined characteristics of the parents. Its fruiting bodies were yellow in color, which was similar to *P. citrinopileatus,* but its spore print was creamish color, which was similar to *P. ostreatus. P. ostreatus* has a creamish color both in its fruiting bodies and spore prints, while *P. citrinopileatus* has golden yellowish color in its fruiting bodies but is pinkish grey in spore sprints.

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Screening Antitumor Activity of Low-Molecular-Weight Compounds Obtained from the Fruit Bodies of Family Agaricaceae Chevall. (Higher Basidiomycetes)

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Numerous higher Basidiomycetes are an important source of medicinal substances, applied in the treatment of a wide range of ailments. The anticancer activity of mushroom-derived components has long been known and is well documented. The majority of active substances are high-molecular-weight (HMW) (200–400,000 Da) polysaccharides and polysaccharide complexes, active hexose correlated compounds (AHCC), polysaccharide-peptides, nucleosides, triterpenoids, complex starches, and other metabolites. Of the known 918 Agaricaceae species (Kirk et al., 2001), only 37 species of eight genera were screened on HMW antitumor active compounds (Ohta et al., 1999), and three species (*Agaricus langei* (F.H. Møller et Jul. Schäff.) Maire, *Leucoagaricus carneifolius* (Gillet) S. Wasser, *L. leucothites* (Vittad.) M.M. Moser ex Bon), were screened for low-molecular-weight (LMW) antitumor-active substances (Yassin et al., 2003). While activity of HMW substances is attributed to the immune-modulating function, LMW substances might show a direct antitumor effect against cancer cells.

The current study examined the anticancer activity of extracts, obtained using different organic solvents (dichloromethane: DCHM, ethyl acetate: EtAc, and methanol: Meth). Overall, 13 species (eight *Agaricus*, three *Macrolepiota*, one *Leucoagaricus*, and one *Lepiota*) were screened. Six lines of three types of cancer were studied: prostate cancer cell lines LNCaP (androgen-dependent prostate cancer; the cell line carries mutation T877A in the androgen receptor); DU145; PC3 (both lines androgen-independent); breast cancer cell line MDA-kb2 (MDA-MB-453); patient-derived chronic myelogeneous leukemia cell (CML) lines (K562); and laboratory model of CML, Baf3/p185 Bcr-Abl. All cell lines were subjected to 36 extracts, each of which was tested in two concentrations. When setting up the experiment, attention was focused on extracts able to inhibit nonselectively all cell lines used, as well as extracts that would inhibit growth of a specific type of cancer selectively.

Extracts showing significant growth inhibition (50% or more) against all cell lines used were considered to be nonselective. Only *Macrolepiota excoriata* (Schaeff.) M.M. Moser extract inhibited growth of all cancer cell lines by more than 50%.

Extracts that inhibited tumor growth of each cancer cell line by 50% or more and showed minimal activity (30% or less) against other cell lines used were considered as selective mushroom extracts. A total of 30 extracts were shown to be selective for the Baf3/p185 Bcr-Abl CML line. Out of these, 22 extracts were active at a concentration of 0.25 mg/mL and thus are considered most promising for further research, especially with *Agaricus xanthoderma* Genev.,

A. fissuratus (F.H. Møller) F.H. Møller, *A. arvensis* Schaeff., *A.* cf. *subrufescens*, and *Lepiota americana* (Peck) Sacc. extracts. Extracts from two latter species demonstrated the highest inhibition rate of the Baf3/p185 Bcr-Abl cell line (Table 1).

All of them are known medicinal species of the family Agaricaceae (Didukh et al., 2003, 2004). *Agaricus xanthoderma* is a poisonous species of group I (causing gastrointestinal disorders) and is known to contain psalloitin, an antimicrobial substance of unknown nature, active against gram-positive bacteria *Salmonella* sp. (Shivrina, 1965). DCHM extracts

TABLE 1. Extracts of Agaricaceae Species Fruit Bodies Selectively Inhibiting Growth of Baf3/p185 Bcr-Abl Leukemia Cell Line

Species	EC, mg/mL	Solvent	Growth inhibition, %	
			Baf3/p185 Bcr-Abl	K562
Agaricus arvensis	1	DCHM	70.37	27.48
	0.25	DCHM	56.04	–3.54
	0.25	Etac	57.81	21.21
Agaricus campestris	1	Meth	74.95	23.22
	0.25	DCHM	60.73	20.14
Agaricus fissuratus	0.25	Etac	54.46	13.04
	1	Meth	70.86	16.11
Agaricus pequinii	0.25	DCHM	63.44	7.47
		Etac	59.89	–0.75
Agaricus cf. *subrufescens*	0.25	Etac	55.71	–5.00
	1	Meth	62.98	19.62
Agaricus vaporarius	0.25	DCHM	65.98	10.251
	0.25	Etac	62.66	2.20
	1	Meth	57.51	-6.57
Agaricus xanthoderma	0.25	DCHM	66.46	22.82
	0.25	Etac	75.93	15.23
	1	Meth	66.68	9.99
	0.25	Meth	62.08	–5.62
Macrolepiota procera	0.25	Etac	50.29	24.01
	0.25	Meth	54.66	26.15
Macrolepiota rachodes	0.25	Etac	63.18	8.30
	1	Meth	69.10	8.87
	0.25	Meth	62.02	-6.52
	0.25	Meth	65.79	29.45
Leucoagaricus leucothites	0.25	Etac	58.64	25.79
	0.25	DCHM	54.01	18.10
	1	Meth	63.15	0.253
	0.25	Meth	52.37	-6.40
Lepiota americana	0.25	Meth	53.43	21.08
	0.25	Etac	71.01	29.19

Note: EC: extract concentrations; SD: standard deviation; DCHM: dichloromethane; EtAc: ethyl acetate; Meth: methanol

are able to inhibit growth of *Escherichia coli*, *Biomphalaria glabrata*, and *Aedes aegypti*. Of the three *A. xanthoderma* extracts used in this study, EtAc extract was most active against Baf3/p185 Bcr-Abl.

Agaricus arvensis is known to help in the case of hypertension, in inhibiting cells of Sarcoma 180 and Erlich's carcinoma, and in inhibiting the growth of bacterial cells (Ying et al., 1989). *A. fissuratus* is very close to *A. arvensis*. Some authors (Nauta, 2001) consider these two species as conspecific. Despite their similarity, the species differed in extracts selective to Baf3/p185 Bcr-Abl: DCHM extract for *A. arvensis* and EtAc extract for *A. fissuratus*.

Agaricus cf. *subrufescens* extract 7a, extracted with DCHM, inhibited growth of cells by 89.76%. *A.* cf. *subrufescens* is closely related to the important culinary–medicinal species *A. brasiliensis* S.Wasser et al. The latter species is known for its anticancer, antiviral, and antimutagenic effects, as well as other activities.

Lepiota americana contains the aromatase-inhibiting fraction (Kim et al., 2000). However, active compounds of the fraction have not been elucidated. Aromatase-inhibiting function plays an important role in breast cancer cell proliferation.

None of the extracts were selective for other cell lines tested. Thus, the major part of active extracts revealed in the study was neither strictly selective nor strictly nonselective.

Based on the concentration at which an extract is active, the percent of growth inhibition, and standard deviation values, *Agaricus xanthoderma* and *Lepiota americana* extracts appear to be most promising in the study of the Baf3/p185 Bcr-Abl cell line.

Application of extracts against other cell lines seems to be less promising as growth of the prostate cancer cell lines (DU145, LNCaP, PC58) and breast cancer (MDA-kb2) were inhibited by extracts in high concentrations. PC58 line turned out to be most resistant to all kinds of extracts used.

Thus, the screened extracts turned out to be most active against chronic myelogeneous leukemia cell lines. Most promising for further research are *Lepiota americana* and *Agaricus xanthoderma* extracts, selective to Baf3/p185 Bcr-Abl. Dichloromethane turned out to be the most efficient solvent for extraction of low-molecular-weight biologically active substances from fruit bodies.

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Nontimber Forest Products: Their Role and Potential in the Ecology and Economy of the Inland Northwest US

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Currently several hundred species of plants, fungi, invertebrates, and mammals are harvested or hunted annually from America's public forests for either commercial, traditional, or personal use. Regulation is irregular, and many nontimber forest products (NTFPs) change hands as a cash commodity, on a per-pound or per-unit basis.

Valued on a per-acre basis, NTFP production equals or frequently far exceeds the value of the harvested timber, when calculated on a 100-year cutting cycle. Longer timber harvest cycles favor increased NTFP production in many cases, as do land treatments such as selection logging or strip logging over clear-cut logging.

From a socioeconomic perspective, the trade in wild mushrooms is a multimillion-dollar industry that provides a large number of jobs per pound of commodity. These seasonal jobs are created in poor, rural, and remote communities in which economic development projects typically flounder. The perishable nature of the fungi makes it a cash commodity, and this in turn attracts mushroom pickers who are often young, mobile, and economically disadvantaged.

NTFP development has been stymied for years by Forest Service accounting procedures which return all income from permit fees, etc., to the timber fund, which then allocates (or not) money for administering a commercial morel harvest or issuing salal or beargrass harvesting permits. This procedure has effectively stunted any meaningful management of these resources.

A realistic use of our forest resources requires first that these resources be identified. One of the challenges to using NTFRs is identifying the resource, whether it is (or is not) a foodstuff or medicine, or if it can be employed for some commercial purpose or for environmental remediation. This presentation will examine several species of fungi found in the intermountain west and the Pacific Northwest and address their current use and potential for economic or ecological development.

The following species and their potential or current use will be addressed: (1) Commercial food species: *Morchella* sp., *Tricholoma magnivelare* (Peck) Redhead, *Cantharellus* sp., *Boletus edulis* Bull.:Fr. group, *Pleurotus* sp.; (2) species with potential as commercial foodstuffs: *Russula xerampelina* (Schaeff.:Secr.)Fr. group, *Rozites caperata* (Pers.: Fr.) P.Karst., *Naematoloma* (*Hypholoma*) *capnoides* (Fr.:Fr.) P.Kumm., *Lyophyllum decastes* (Fr.) Singer group, *Coprinus comatus* (Mull.:Fr.) S.F.Gray, *Calvatia* sp.; (3) species with potential as food/commercial products: temper using *Coprinus*, *Lyophyllum decastes*, etc.; biopulp—delignifying wood using fungi instead of chemicals: specie of genera *Cerisporiopsis, Phlebia, and Hypodontia*, brown cuboidal rot—water storage/retrieval; (4) restoration and remediation using species: *Pleurotus* sp. in riparian areas, *Lyophyllum decastes* against knapweed infestations.

All the above species will be pictured and aspects of habitat and conditions discussed.

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Medicinal Uses of Fungi by New Zealand Maori People

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Māori are the indigenous people of New Zealand, arriving about a thousand years ago from Polynesia and developing a tribal society based on natural resources of the forest, the sea, and fresh water. In the absence of written language prior to European colonization, transfer of knowledge by the Māori was oral. Early European explorers and ethnographers documented many significant features of Māori traditional knowledge. Much detail of their knowledge relating to fungi, however, appears to have been lost through disruption of oral transmission from elders to the young. This is indicated, for example, by the lack of known correlation between many of the 183 Māori names for different species of fungi and Latin binomials and lack of knowledge of the use of most species.

Fungi were not recognized as a natural grouping by Māori, and only recently has the word for *Armillaria* spp. ("harore") been selected as the most appropriate generic term in Māori for all fungi. Consumption of mushrooms was the main use of fungi by Māori although mushrooms were not considered a prize food. Other uses included tinder and fire carrying (*Laetiporus portentosus*), a source of charcoal as pigment for "ta moko"—tatoo (*Cordyceps robertsii*), and as an environmental indicator (*Armillaria* spp. and mushrooms in general).

A recent attempt to collate Māori knowledge about fungi included a survey of published reports as well as interviews with Māori elders and younger Māori. Documented medicinal uses appear to be limited to six fungal species, and only one of these was known to interviewees. Detailed information is often lacking about preparation and application of the mushrooms and the definitive symptom(s) that were treated:

- *Agrocybe parasitica* (tawaka) for treatment of fever, and given to expectant mothers (purpose unclear) and for those poisoned by toxic plants (*Corynocarpus laevigatus,* karaka, and *Coriaria arborea*, tutu).
- *Auricularia cornea* (hakeke) for those poisoned by toxic plants (karaka and tutu).
- *Calvatia* spp. (pukurau) as an anaesthetic, to staunch bleeding, and as pain relief following scalding.
- *Cordyceps robertsii* (awheto) for asthma.
- *Laetiporus portentosus* (putawa) as a wound protector, to soften and ease a difficult labor.
- *Usnea* sp. (lichen) skin treatment.

There is an urgent need to encourage documentation of indigenous knowledge of fungi from other nations to avoid potential loss of this knowledge. From a New Zealand perspective, further understanding of uses of fungi by other South Pacific peoples could indicate additional species that might have been used for medicinal purposes by Māori.

Medicinal Mushrooms of Southwest Siberia

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As of today, there are more than 1500 known species of macromycetes that are grown in various plant cooperatives in southwest Siberia. Approximately 250 species of macromycetes are edible. The list of poisonous mushrooms contains approximately 50 species. Many edible and poisonous mushrooms have become the subject of research for the purpose of investigating their bioactive substances. According to corresponding literature, in the southern part of West Siberia there are more than 150 species of Basidiomycetes with medicinal properties. Of these, the most well known in Siberian folk medicine are *Amanita muscaria* (L.:Fr.) Hook., *Boletus edulis* Bull., *Inonotus obliquus* (Pers.:Fr.) Pilát, and *Fomes officinalis* (Vill.:Fr.) Bond. et Singer.

Medicinal and stimulating effects of *Inonotus obliquus* (Chaga) have been known from time immemorial. It is scientifically proven that the chaga mushroom increases the immune system of ill people and activates the circulation of brain tissue elements, thus increasing bioelectric activity of the brain's cortex. The Siberian population actively uses Chaga's infusion. In practical medicine, Befungin was the first manufactured Chaga product, which has been confirmed by Russian scientists. The chemical composition and biosynthetic activity of this mushroom were researched by the many scientists. There is confirmed information of the use of Befungin to cure psoriasis. Pharmacies in the city of Novosibirsk offer a broad selection of manufactured products that contain Chaga: dried chaga, chaga infusions, chaga syrup, tea, and lotions-balsams to cure arterial and joint diseases as well as in the healing of wounds.

The Center of Fungi-therapy, which was created in St. Petersburg's suburbs, actively uses chaga, along with shiitake mushroom (*Lentinus edodes* (Berk.) Singer), in cancer therapy. Lately, there has been a lot of information about medicinal products made from *Cordyceps militaris* (L.) Link. In natural environments of southwest Siberia, there have been only isolated discoveries of this particular type in the mountainous Altai and Kemerovskaya province.

To the class of rare medicinal mushrooms in Siberia, the following species should be added: *Sarcosoma globosum* (Schmidel:Fr.) Rehm, *Sparassis crispa* (Wulf.:Fr.) Fr., *Ganoderma lucidum* (W.Curt.: Fr.) P. Karst., *Dictyophora duplicata* (Bosc) E. Fisch., *Phallus impudicus* L., *Grifola frondosa* (Dicks.:Fr.) S.F. Gray, *Hericium coralloides* (Scop.:Fr.) Pers., *Pleurotus calyptratus* (Lindbl.) Sacc., *Polyporus umbellatus* (Pers.:Fr.) Fr., *Lyophyllum decastes* (Fr.:Fr.) Singer, *Oudemansiella mucida* (Schrad.:Fr.) Höhn., and *Suillus bovinus* (Pers.:Fr.) Kuntze. In this region, it is very popular to use as food products such edible types as *Kuehneromyces mutabilis* (Schaeff.:Fr.) Singer et A.H. Sm., *Armillaria mellea* (Vahl:Fr.) P. Kumm., *Flammulina velutipes* (W. Curt.:Fr.) Singer, *Leccinum aurantiacum* (Bull.) S.F. Gray, *L. scabrum* (Bull.) S.F. Gray, *L. versipelle* (Fr. et Hök.) Snell, *Suillus granulatus* (L.:Fr.) Snell, *S. grevillei* (Klotzsch:Fr.) Singer, *S. luteus* (L.:Fr.) S.F. Gray, *Cantharellus cibarius* Fr.:

Fr., *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm., *P. pulmonarius* (Fr.:Fr) Quél., *Lactarius deliciosus* (L.:Fr.) S.F. Gray, *L. torminosus* (Schaeff.:Fr.) S.F. Gray, and representatives of genus *Russula*, the majority of which produce a good harvest in favorable (salubrious) weather conditions in birch-pine, deciduous, and taiga woods in valleys and mountainous areas. Unfortunately, people still use *Paxillus involutus* (Batsch:Fr.) Fr. for food, even though it has been acknowledged as a poisonous type.

Among the common forest's species there are also *Laccaria laccata* (Scop.:Fr.) Fr., *Lepista nebularis* (Fr.) Harmaja, *L. nuda* (Bull.:Fr.) Cooke, *Lycoperdon perlatum* Pers.:Pers, *L. pyriforme* Schaeff.:Pers., *Fomes fomentarius* (L.:Fr.) J.J. Kickx, *Fomitopsis pinicola* (Sw.:Fr.) P. Karst., *Piptoporus betulinus* (Bull.:Fr.) P. Karst., *Ganoderma applanatum* (Pers.) Pat., *Laetiporus sulphureus* (Bull.:Fr.) Murrill, *Schizophyllum commune* Fr.:Fr., and several others. In the valleys and meadows of Novosibirskaya province, the Altai Region, and Mountainous Altai, *Agaricus arvensis* Schaeff.:Fr., *Calvatia lilacina* (Berk. et Mont.) Lloyd, *Handkea excipuliformis* (Scop.) Kreizel, *H. utriformis* (Bull.:Pers.) Pers., as well as the less common *Langermannia gigantea* (Batsch.:Pers.) Rostk. and *Pleurotus eryngii* (DC.:Fr.) Quél. are commonly found. In various habitats, it is possible to find *Coprinus atramentarius* (Bull.:Fr.) Fr. and *C. comatus* (O.F. Müll.:Fr.) S.F. Gray, but their harvest is rarely abundant.

Radioactive Contamination of Ukrainian Mushrooms

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Even 18 years after the Chernobyl catastrophe, radioactive contamination of wild growing mushrooms, in some cases reaching very high levels, created human health problems resulting from their nutritional and medicinal use. According to radioecological evaluations, the internal dose of irradiation in this case may reach 40–60%. A combination of macro- and micromycetes mycelial biomass and its capacity to uptake and retain radionuclides play very important roles in their migration and distribution. Actually, knowledge about the mechanisms involved in the accumulation and retention is incomplete. There is a positive correlation among the levels of soil contamination, degree of humidity, pH, rainfall, and radionuclide accumulation in mushrooms, although the species and ecological specificities of uptake are also observed. To measure species specificity of uptake, species-hyperaccumulators—for instance, radiocaesium—were used as bioindicators of contaminated soils. Among them, the widely spread and common species, *Lactarius rufus* (Scop.:Fr.) Fr. and *Paxillus involutus* (Batsch:Fr.) Fr., not consumed by the population because of their inedibility and toxicity, are now the most convenient test objects for long-term monitoring.

During the last few years in about 80% of mushroom samples traditionally included in the Ukrainian diet—species of Boletaceae (*Xerocomus* spp., *Suillus* spp., *Leccinum scabrum* (Bull.:Fr.)

S.F.Gray, *Boletus edulis* Bull.:Fr.) and *Cantharellus cibarius* (Fr.:Fr.) Fr. taken from areas with an average soil contamination of 0.1–5 Ci/km^2 (presently, the collecting and consuming of wild growing mushrooms are practically not controlled)—the ^{137}Cs levels exceeded permissible limits adopted in the Ukraine (2500 Bq/kg d.w.). Mushrooms growing in the Exclusion Zone, in the vicinity of the Chernobyl Power Plant (with ^{137}Cs contamination density of 837 Ci/km^2) accumulated up to millions of Bq/kg d.w. In 2004 ^{137}Cs activity in samples from the location of Novo-Shepelychi was within (in Bk/kg d.w.) 340,000 (*Armillariella mellea* (Vahl.:Fr.) P.Kumm.) to 20,000,000 (*Xerocomus subtomentosus* (L.:Fr.) Quél.), and ^{90}Sr within 2000 (*X. subtomentosus*) to 13,000 (*Stropharia aeruginosa* (W.Curt.:Fr.) Quèl.). Transfer factors for ^{137}Cs were in *X. subtomentosus* 645, *P. involutus* 548, *Lactarius turpis* (Weinm.)Fr. 280, *L. deliciosus* (L.:Fr.) S.F.Gray 222; for ^{90}Sr Tf were in *S. aeruginosa* 6.0, *L. turpis* 5.1, *A. mellea* 3.1. The ratio of ^{137}Cs/^{90}Sr was within the range of 7.5–10,000.

Mushrooms growing on the highly contaminated territories are permanently subjected to chronic irradiation. Taking into account polymorphism peculiar to mushrooms, the direct finding of morphose for them is difficult. Microstructural morphology of three bioindicative species using light and electron scanning microscopy was studied. It was statistically established that the spore length in *Xerocomus badius*(Fr.:Fr.) Kühn. ex Gilb., *Paxillus involutus,* and *Lactarius turpis* samples collected in the Exclusion Zone was bigger than in the controls. In some cases, in the hymenophore of studied samples, parts with abnormally large as well as with very small spores were found. The observed variability indicated that high dosages resulting from radionuclides incorporated by mycelium and fruiting bodies from contaminated soils affect the hereditary structures of mushrooms.

In nature mushrooms accumulate less ^{90}Sr than ^{137}Cs. In cultivation conditions, increasing levels of radiostrontium accumulation for *Lentinus edodes* (Berk.) Singer and *Pleurotus ostreatus* (Jacq.:Fr.)P. Kumm. (Grodzinskaya and Kuchma, 2004) were observed. This phenomenon can be connected to the higher biological availability of ^{90}Sr from mixed and watered substrates. An increase of ^{90}Sr activity in wood (observed in the last few years in contaminated territories) inevitably will cause an increase in its content in lignotroph species. Therefore, the selective control of ^{90}Sr accumulation in cultivated lignotrophs, even in territories with low surface soil contamination, is strongly recommended.

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International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Immunostimulating and Antitumor Effects by *Inonotus obliquus* (Ach.:Pers.) Pilát

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Although it has been reported that Chaga *Inonotus obliquus* (Hymenochaetaceae) shows immuno-enhancement activity and antioxidant activity, protective effects against radiation have not been investigated. In addition, although a few studies on antitumor effects *in vitro* have been reported, it is unknown whether these effects also occur *in vivo.* Many studies on the protective effects of Propolis against radiation and its antitumor activity have been undertaken using water and ethanol extracts, but studies on the whole lysate, in which the original Propolis is completely dissolved, have not been done. It is unknown what mechanisms are involved in the protective effect against radiation and the antitumor activity in these substances.

Therefore, in this study, we investigated the protective effects against radiation and *in vivo* antitumor effects of *Inonotus obliquus*. In addition, we investigated the quite novel protective effect against radiation and antitumor activity of the whole Propolis lysate. Blood cells are one of the indices for evaluating protective effects against radiation. Hemopoietic tissues and peripheral lymphocytes are highly sensitive to radiation, and a decrease of immunity caused by a decrease in white blood cells and myelocytes is remarkable after exposure to radiation. Therefore, in this study, we focused on the antioxidant activity and immuno-enhancement activity of *Inonotus obliquus* and Propolis and examined the *in vivo* effect of radiation on the number of peripheral blood cells. Furthermore, measurement of T lymphocyte subsets, SOD-like activity, antioxidant activity, radical scavenging activity based on chemiluminescent methods, and absolute amounts of free radicals based on ESR were carried out, and radical scavenging activity, which is a mechanism of protection from radiation, was examined. In addition, we examined antioxidant activity and immuno-enhancement activity, both of which are closely related to tumor suppression, and further investigated effects on suppressive effects on tumor growth and antitumor effects of tumor necrosis factor (TNF).

The current major therapies for tumors are surgery, radiation, and chemotherapy. Immunotherapy is hoped to become a fourth therapeutic modality in the future. All of the three main therapies impose a burden on the body and weaken immunofunction. However, we hope that a combination of immunotherapy, using natural materials such as *Inonotus obliquus* and Propolis with immuno-enhancement action, may increase the percentage of patients who recover.

Radioprotection and Antitumor Effect by *Lyophyllum decastes* Singer and Propolis in Mice

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In this study, we examined the radioprotective effects of treatment with *Lyophyllum decastes* (Tricholomataceae, higher Basidiomycetes) and Propolis. It would obviously be counterproductive if the antiradiation effects of these substances prevented the antitumor effects of the radiation. Therefore, we examined the effect of radioprotective agents on the growth of tumors in combination with radiation therapy. Thus, the aim of this study is to take the first step to apply *L. decastes* and Propolis as new radioprotective agents and to obtain data for the development of these agents in the future.

As a possible mechanism, a crude polysaccharide fraction (ATF) was prepared promoting the invasion of NK cells into tumor tissues and inhibiting the appearance of the S phase in mitotic cycles of tumor cells. Many chemotherapeutic drugs cause adverse reactions. Not only tumor cells, but also other proliferating normal cells are damaged, and the function of the intestine, kidney, spinal cord, and immunity are affected because these drugs act by inhibiting DNA synthesis. Therefore, in general, antitumor drugs with fewer adverse reactions are desired. If antitumor drugs with fewer adverse reactions exist, the point of action would not be mediated by effects on DNA and RNA, which are common to both tumor cell and normal cells and may be associated with some specific tumor cell function involved in the relationship between the host and tumor cells. For example, antitumor effects can be caused by activation of the immune system and actions on vascularization caused by tumor tissues. In this study, *L.decastes* alone did not show a marked antitumor effect. One possible reason is that *L. decastes* does not show direct antitumor effects like those of artepillin C, which is present in Propolis, but acts secondarily from an immunological aspect.

After a topical 6 Gy irradiation (therapy), the *L. decastes* group showed a tendency to suppress the tumor growth, as compared with the group that was only irradiated. It is thought that *L. decastes* does not show a direct antitumor effect. Therefore, the radioprotective effect reduces the decrease in immune cells, and a secondary antitumor effect generates a slight difference, as compared with the group that was only irradiated.

Thus, in radiation therapy, *L. decastes* shows an antitumor effect caused by a radioprotective effect on the immune cells, in addition to the antitumor effect caused by irradiation.

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Antitumor Immunity in *Agaricus* sp., *Paffia paniculata,* and Propolis

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We investigated the antioxidant activity, lymphocyte versus polymorphonuclear leukocytes enhancement activity (L/P activity), and antitumor activity of three simple substances (*Agaricus* sp., *Paffia paniculata,* and Propolis) as well as a mixture of these three kinds of substances (ABP).

A powdered mixture (ABP) of these three substances were prepared using an agate mortar. For the extraction, 2000 mL of water was added to 300 g of finely powdered *Agaricus* sp. and then stirred for 2 hours in a water bath of 40 °C. After centrifugation for 10 minutes at 5000 rpm, the supernatant was filtered using folded filter paper. Distilled water (2000 mL) was added to the precipitates, and extraction was repeated in the same way. Dried *Agaricus* sp. was obtained by combining the supernatant from the first extraction with the supernatant from the second extraction (yield: 48.0 g, recovery: 16%). Propolis (100 g) was powdered, and 300 mL of 70% ethanol was added. After drying at room temperature, filtration was carried out using folded filter paper. The filtrates were dried using an evaporator. Dried Propolis was obtained by freezing and thawing (yield: 53.5 g, recovery: 53.5%). Distilled water (2000 mL) was added to 200 g of finely powdered *Paffia paniculata*. Dried *P. paniculata* was obtained by the same technique (yield: 83.6 g, recovery: 41%).

With respect to radical scavenging activity, water and *P. paniculata* had no radical scavenging activity and showed no marked difference, whereas *Agaricus* sp. showed slight radical scavenging activity. Both ABP and Propolis showed greater radical scavenging activity than 0.2 mM Trolox, which was used as a positive standard.

Ten neonatal Swiss-Webster mice were divided into two groups. Saline was injected intraperitoneally into one group, and *Agaricus* sp. was injected intraperitoneally into the other group at a dose of 200 μg/mouse. L/P ratios after the administration of ABP (200 μg/mouse) were 0.91 ± 0.07 (day 6), 3.23 ± 0.39 (day 10), and 4.82 ± 0.46 (day 14), whereas L/P ratios in the control group were 0.43 ± 0.04 (day 6), 0.96 ± 0.08 (day 10), and 1.43 ± 0.39 (day 14). The L/P ratio after the administration of Propolis was significantly elevated ($p < 0.01$), as compared to the control group.

When the ABP mixture was administered at a dose of 400 μg/kg for 34 consecutive days, remarkably high antitumor activity (suppressive ratio: 85.1%, $p < 0.01$) was shown.

In Sarcoma 100 solid carcinoma, when *Agaricus* sp. (200 mg/kg B.W./day), *Paffia paniculata* (60 mg/kg B.W./day), and Propolis (80 mg/kg B.W./day) were orally administered for 34 consecutive days, suppressive ratios were 60.3% ($p < 0.05$), 54.8% ($p < 0.05$), and 62.6% ($p < 0.05$), respectively. When the ABP mixture was administered at a dose of 400 mg/kg B.W./day for 34 consecutive days, remarkably high antitumor activity (suppressive ratio: 83.5%, $p < 0.01$) was shown.

A Newly Isolated Anamorph of the Medicinal Fungus *Cordyceps sinensis* (Berk.) Sacc. (Ascomycetes): A Review of Its Identification, Cultivation Parameters, Chemical Composition, and Antioxidant and Antitumor Activities

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Cordyceps sinensis (Cs) (DongChongXiaCao in Chinese), is a rare and precious Chinese herb facing a stringent shortage of natural supply (i.e., overharvesting and continued destruction of habitat) due to increasing world demand. This increasing shortage in supply has driven the price of wild collected Cordyceps up to previously unimaginable levels, making wild collected *C. sinensis* the most costly medicinal raw material available today. The cultivated mycelium of the *C. sinensis* fungi has been shown to significantly lower the cost and has proven to be a feasible alternative to the natural species for herbal medicine, pharmaceutical, and functional food use. Since the mid-1980s there have been a number of different anamorphs of *C. sinensis* isolated and brought into cultivation. While these modern biotechnologically cultivated anamorphs appear to hold great potential for relieving the worldwide shortage of this valuable medicine, the sheer number of different anamorphs and their widely differing chemical and cultivation parameters pose many questions about the effectiveness and equivalence to the wild collected *C. sinensis.*

In this work, we investigated and elucidated a fungus species, which was isolated from the wild Cs herb, which we have identified as a new anamorph of Cs. Its UV, IR, and HPLC spectra all exhibit high similarity in chemical composition to those of natural Cs herbs. As in the case of the wild fungus, this fungus (extracted by water or ethanol) exhibits strong antioxidant properties, scavenging free radicals (as shown by DPPH assay), and inhibits lipid peroxidation (shown by TBA assay). It also shows strong antitumor activity in a number of different tumor cell lines, both *in vitro* and *in vivo*, and shows potent inhibition of tumor growth in the Murine model.

These results suggest that this newly isolated Cs anamorph more closely mirrors the chemical composition and pharmacological activity of the natural Cs than has any previously identified anamorph, indicating the potential for an improvement upon the existing fungal lines in Cordyceps cultivation. The morphological characteristics, genetic identification, biotechnological potential, and detailed growth parameters in both liquid cultivation and solid state fermentation for this fungal mycelium are fully evaluated and indicate this new anamorph as having great potential as an effective and low-cost substitute for the natural Cs herb.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

The Known Hallucinogenic Species of Psilocybe (Agaricomycetideae) in the World: Traditional Uses and Distribution

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A discussion of the known species of *Psilocybe* is presented with special attention to those possessing hallucinogenic effects. Guzmán in 2005 considered 227 species, 144 of them belonging to hallucinogenic mushrooms. It is interesting to observe that 718 names have been proposed as *Psilocybe* in the literature and that Guzmán, in 1983, accepted only 144 species in the genus, of which 91 were considered hallucinogenic. Another important observation is that more than 80 species of *Psilocybe* have been described by Guzmán from almost all continents since 1968. This latter observation shows the lack of studies in the genus in contrast with the oldest original studies by Fries in 1821 and 1836, and by Ricken in 1915.

Genera considered in *Psilocybe*, now independent are *Hypholoma, Galerina, Melanotus, Naucoria, Panaeolus, Pholiota, Psathyrella,* and *Stropharia,* among others. Modern studies in *Psilocybe* began with Singer in 1949; however, it was with the discovery of hallucinogenic mushrooms in Mexico in 1956 that the interest in the genus began. Modern studies in *Psilocybe* were also contributed by Bon and Roux in 2003, Noordeloos in 1999 and 2001, and Pegler in 1977, 1983, and 1986. Noordeloos added the genera *Hypholoma, Melanotus,* and *Stropharia* in *Psilocybe,* which a long time ago were considered independent from *Psilocybe*. The hallucinogenic species in *Psilocybe* were considered by Singer and Smith in 1958 in the section *Caerulescentes,* which was distinguished by the bluing feature in the basidioma.

However, Guzmán, in 1983, divided this section in 10 of all of the 18 considered in the genus, including some modifications, as well as a new section discussed by him, both in 1995 and 2004. Guzmán's classification is based on the form and structure of spores and the presence or absence of cystidia, including the bluing feature of the basidioma, and its farinaceous taste and flavor, although in some species—e.g., *P. mexicana* R. Heim and *P. semilanceata* (Fr.) P.Kumm. It is sometimes difficult to see the bluing reaction because it depends on the development of the basidioma. Moncalvo et al. (2002), in a molecular study of several Agaricales, divided *Psilocybe* into two genera: *Psilocybe* s.s. and *Psychedelia* Moncalvo et al., the latter genera including all hallucinogenic species that have psilocybin and psilocin. This means that there is, at the present, a discussion about what makes up the taxonomic point of view of the hallucinogenic species of *Psilocybe*. Certainly the fact that all of them are bluing weigh heavily in the discussion, but this feature is also present in other mushrooms, as is found in *Copelandia* and some Boletaceous fungi. In addition, psilocybin and psilocin are present in other genera such as *Copelandia, Gymnopilus,* and *Pluteus.*

Related to traditional uses of hallucinogenic species, in Mexico several groupts of Indians still use these mushrooms in religious ceremonies. There are probably some tribes in Papua New Guinea that use *Psilocybe kumaenorum* R.Heim for religious purposes, as reported by Heim in 1967. Some primitive tribes

in North Africa probably used some unknown *Psilocybe* in traditional ceremonies, as shown by Samorini in 2001 in primitive paintings found in a cave. Unfortunately, after the discovery of hallucinogenic mushrooms in Mexico, young people started to use this drug for recreational purposes. This changed the traditional uses of hallucinogens in Mexico, and an economic purpose was found in the ceremonial uses. Also, the easy culturing of these fungi made an illegal trade of the hallucinogens possible in the US, Europe, and Japan, in spite of the fact that the hallucinogenic mushrooms were forbidden by several governments.

Species of *Psilocybe* are distributed in all the continents. However, Mexico is the country with the highest number of species, 53, while Europe has only 16 and the US and Canada, 22. In Latin America, excluding Mexico, there are around 60 species. Africa has only four species. In Asia (Japan, Nepal, Vietnam, Thailand, and Java) there are 15 species (besides five that Horak and Desjardin have under study). In Australia, New Zealand, Papua New Ginea, and New Caledonia there are 19 species. It is possible to see that the tropics and subtropics in the world have the majority of the species, with more than 100, in comparison with temperate regions, which have around 40 species.

Furthermore, it is supposed that the origin of the hallucinogenic species of *Psilocybe* started in the Austral hemisphere in the old Gondwanian Continent. Another important observation in the distribution of the species is that the majority was found in hygrophytic or mesophytic forests, known also as subtropical cloud forests, and grew in humid mountains at 900–1400 m altitude close to the sea. In Mexico it was observed that more than 90% of the known species are in this kind of vegetation. *Psilocybe antioquesis* described from this kind of vegetation in Colombia recently was found in Mexico and in Cambodia in the same vegetation. An example of an abnormal distribution of a temperate species is *Psilocybe semilanceata,* very common in Europe, common only in the northwest of the US, unknown in Mexico, and reported one time from Chile and New Zealand.

Considering all of the above observations and more, the author is in the process of preparing the second edition of his monograph of 1983 on the genus *Psilocybe.*

p-Terphenyl Compounds Possessing Antioxidative Activity from Japanese Inedible Mushrooms

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We are continuing to study biologically active substances from inedible mushrooms. In this paper, we report 36 new *p*-terphenyl compounds possessing antioxidative activity from fruit bodies of four Japanese inedible fungi named *Thelephora aurantiotincta* Corner, *T. terrestris* Ehrn., and *Hydnellum caeruleum* (Hornem.)P.Karst., belonging to Thelephoraceae; and *Paxillus curtisii* Berk., belonging to Coniophoraceae. Eight new *p*-terphenyl compounds, thelephantins A–H (1–8) (Quang et al., 2003a,b) and five known compounds—atromentin, 2-*O*-methylatromentin, gabajunins C and E, and thelephorin A—were isolated from *T. aurantiotincta*. Eight new *p*-terphenyl compounds—thelephantins I-P (9–16) (Quang et al., 2004) together with two known compounds, 2-*O*-methylatromentin and dihydroaurantiacin—were isolated from *H. caeruleum*, seven new *p*-terphenyl derivatives—terrestrins A–G (17–23) and two new pregnane-type steroids, terrestrones A and B (24, 25) from *T. terrestris*. In addition, 13 new *p*-terphenyl derivatives named curtisians E–Q (26–38) (Quang et al., 2003c–e) along with two known compounds—curtisians C and D–were also obtained from *P. curtisii*. These absolute structures were determined by 2D NMR, MS, IR, and UV spectra, X-ray crystallographic analysis, and chemical reactions.

Antioxidative activities of these new *p*-terphenyl compounds were evaluated by diphenyl-*p*-picryhydrazyl (DPPH) radical scavenging effects. Some new *p*-terphenyl compounds showed equivalent or strong activities compared with vitamin C, α-tocopherol, and BHA (tert-butylhydroxyanisole).

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Effect of Substrate Composition and Heat Pretreatment on Vegetative Growth of *Agaricus brasiliensis* S. Wasser et al. and *A. subrufescens* Peck

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Approximately 670 million broiler chickens and 43 million turkeys are produced in North Carolina annually, generating wastes that are causing environmental problems and concerns among residents. These waste products can be used as substrate for growing specialty mushrooms. Mushroom farming can enable farmers to obtain additional crops (mushrooms), while bioconverting the agricultural wastes to more environment friendly products such as organic fertilizers. *Agaricus brasiliensis* and *A. subrufescens* are culinary–medicinal mushrooms currently produced in the US. These mushrooms can be alternative crops generating high income in the place of tobacco for the North Carolina farmers.

Various combinations of sawdust, poultry litter, and cotton waste were made and loaded in 3 × 12 cm glass test tubes. The tubes containing substrate were sterilized at 121 °C for 1, 2, or 3 hours. A set of 8 tubes per substrate combination was inoculated with *A. brasiliensis* or *A. subrufescens*. Similar substrate combinations were pasteurized at 90 °C for 48 hours and then inoculated.

Growth rates of each substrate combination/heat treatment were determined, based on the time for achieving total substrate colonization in each tube. To determine the effect of nutrient supplementation on mycelia growth and development, the substrate combination/heat treatment that performed best in the above treatments was supplemented with wheat bran or millet or combinations of both at 10, 15, and 20% proportions. Non-supplemented substrate served as a control.

Substrate combinations containing cotton waste did not show significant growth and development in either *A. brasiliensis* or *A. subrufescens*. Sterilization was ineffective at suppressing contaminants in any substrate combination that had poultry liter. Pasteurization of poultry litter containing substrates at 90 °C for 48 hours was sufficient to suppress contaminant. Poultry litter and sawdust in a 1:1 ratio proved to be the best substrate for supporting mycelia growth, where total colonization took an average of 15 days, reaching primordial formation in 60 days. Both fungi responded well to nutrient supplementation, showing an average of 81.5% increase in mycelia growth over the control. In both fungi tested, there was consistent increase in colonization rate as supplementation increased; however, incidence of contaminants increased with the increase of supplementation.

Further work on optimization of substrate pretreatment and environmental conditions that best support the development of primordial through mature fruit body formation in both fungi are currently being researched.

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Spore Germination and Breeding Pattern in *Grifola frondosa* (Dicks.:Fr.) S.F. Gray

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Grifola frondosa (Meripiliaceae, Polyporales), commonly called Maitake, grows on hardwood trees in the northern temperate forests of the eastern United States, Canada, Europe, and Asia. It produces sporophores that are edible with high organoleptic properties. In addition to Maitake's use as food, it is known to exhibit medicinal properties and is thus a fungus of high economic importance today.

The attempt to develop commercial strains from isolates of *G. frondosa* distributed in the US revealed the difficulties encountered at spore germination. Therefore, it became necessary to investigate various physicochemical factors that can affect spore germination of *G. frondosa*. Plant hormones, gibberellic acid (GA) and indole-3-acetic acid (IAA), temperature, pH, sonication, mushroom extract, and soil extract were tested in laboratory experiments. Temperature and pH showed significantly higher spore germination values compared to the control (2.8 spores per plate). In 30 °C temperature pretreatment, an average of 28.6 spores germinated at pH 7 resulted in an average germination of 32.2 spores per plate. Some of the concentrations of plant hormones showed germination values that were significantly higher than the control. The concentrations of plant hormones that were not significantly higher than the control were 1.0 mg/L GA, 0.01 mg/L IAA, and 0.05 mg/L IAA. The effect of sonication on spore germination was also significantly higher than the control in all the time periods tested. Soil extract treatment had a negative effect on spore germination as the values were significantly lower than the control. It was therefore concluded that pH and heat pretreatments were the most appropriate factors to stimulate spore germination in *G. frondosa.*

A preliminary study to determine the breeding pattern in *G. frondosa* was also conducted. Single spore isolates (ssi) were selected and examined for clamp connections, and 10 clamp free ssi were selected for use in the mating experiment. Cross mating was conducted by co-cultivating pairs of single spore isolates on 2% PDA plates and incubating for 14 days. All ssi were crossed in all possible combinations. Examination to determine formation or absence of clamp connection under ×100 and ×400 magnification conducted on all crosses was conducted, and the results analyzed for the breeding pattern. The results indicated that *G. frondosa* exhibits a tetrapolar breeding pattern. Among the 10 isolates used, the distribution of mating alles were 2 ssi A1B1, 2 ssi A2B2, 3 ssi A1B2, and 3 ssi A2B1. It was observed that clamp connection formation in *G. frondosa* is more than 70% and 50% less than what we normally observe in dikaryotic cultures of commercially cultivated species of *Pleurotus* and *Lentinus*.

Biology, Food, Medicinal, and Biotechnological Applications of the Tropical Mushroom *Pleurotus tuberregium* (Rumph.:Fr.) Singer

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Pleurotus tuberregium (Syn. *Lentinus tuberregium*), a tropical Basidiomycete, is the only species of *Pleurotus* known to produce fruit bodies from a true sclerotium. *P. tuberregium,* often collected in the field growing on decaying logs, can also be cultivated on many different lignocellulosic substrates. Fruit bodies can be found growing out of the sclerotia in the wild and can be induced to do the same if subjected to soaking followed by exposure to warm temperature and humid conditions. This fungus is reported to be distributed in most of equatorial Africa, Madagascar, India, Sri Lanka, South East Asia, Papua New Guinea, New Caledonia, and Northern Australia.

Until recent times, the taxonomic position of *P. tuberregium* has been problematic. The leathery fruit bodies and dimitic hyphal system with intercalary skeletal hyphae possessed by this fungus led authorities such as E. J. H. Corner and D. N. Pegler to place *P. tuberregium* in the genus *Panus* and *Lentinus,* respectively. However, more recent studies have shown *P. tuberregium* to have nematotoxic abilities, which supports the classification of this fungus in the genus *Pleurotus* by R. Singer in 1986. Molecular systematics using ribosomal DNA sequences have shown *P. tuberregium* to be nested with the genus *Pleurotus*.

Phylogeny studies of *P. tuberregium* based on ITS sequences have shown that the African isolates studied showed very little variation, indicating a homogenous gene pool of *P. tuberregium* in Western Africa. In contrast, there was more gene flow in the Australasia–Pacific region, which could suggest that the origin of diversification for *P. tuberregium* is the Australasia–Pacific region.

In nature, during the early months of the rainy season, sporophores germinating from fruit bodies discharge spores. These spores, having adequate humidity and warmth in the environment, germinate and seek compatible mates to combine with and begin the dominant dikaryotic mycelia stage in their life cycle. The life cycle of *P. tuberregium* is different from most other Basidiomycetes in having the sclerotial stage between the mycelia stage and fruit body formation. However, we have also observed in nature and in the laboratory that the genetic background and substrate conditions can cause some isolates of *P. tuberregium* to go from the mycelia stage directly to sporophore formation. Field observations and laboratory studies have revealed that *P. tuberregium* is a white rot fungus that secretes laccase- and manganese-dependent peroxidase as its main enzymes used in lignin degradation. Cultivation studies have also shown that *P. tuberregium* can grow on virtually any lignocellulosic substrate to produce sclerotia and sporophores.

The advantage derived from this quality is the possibility of mass producing the highly-sought-after

FIGURE 1. Sclerotium of *Pleurotus tuberregium* (Rumph.: Fr.) Singer.

sclerotia in West Africa, where until recent times, collection from the wild was the only source.

Also, the understanding of the breeding pattern, which is tetrapolar, as well as breeding and selection studies have led to the development of improved strains for use in commercial cultivation of this fungus. Similarly, its high temperature requirements in addition to its ability to degrade many lignocellulosic substrates has made it a potential candidate for biotechnological approaches to waste recycling and bioremediation of recalcitrant pollutants.

There are many reports that document traditional uses of *P. tuberregium* for food and medicine in Africa. Nutritional analysis has shown both sclerotium and sporophores of *P. tuberregium* to be an excellent source of protein, carbohydrates, and essential minerals. Scientific studies into the medicinal properties of *P. tuberregium* are active areas of research today. Extracts from sclerotia of *P. tuberregium* have been shown to have antibacterial and antitumor activities. Protein-bound polysaccharides isolated from *P. tuberregium* have been shown to have high antitumor activities against Sarcoma 180. Liquid cultivation of mycelia of *P. tuberregium* has been reported. Mass production of mycelia in bioreactors in developed countries and sclerotia in developing countries, where adequate environmental conditions prevail, will be the source of materials from *P. tuberregium* needed in medicinal products preparation and biotechnological applications.

Toxicological Evaluation of *Lentinus squarrosulus* Mont. (Polyporales), an Indigenous Nigerian Mushroom

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Lentinus squarrosulus Mont. (Syn. *Lentinus subnudus* Berk.) is a highly prized Nigerian mushroom, which is appreciated for its meaty taste and texture. The mushroom is of immense value in traditional medicine, and it features considerably into Nigerian folklore and mythology (Oso, 1977). Fasidi and Kadiri (1990) showed that mature *L. squarrosulus* fruit bodies are rich in ascorbic acid and amino acids, and protein is their most abundant nutrient. Fasidi and Kadiri (1993), Kadiri (2002), and Kadiri and Arzai (2004) were able to cultivate fruit bodies of *L. squarrosulus* successfully on uncomposted and composted agricultural wastes and woodlogs of tropical hardwood plants.

Defatted powdered samples of mushroom fruit bodies, dissolved in methanol and centrifuged, were found to show an absence of amatoxins and phallotoxins, following the method of Wieland and Faulstich (1978) and using α-amanitin and phalloidin as standards. In the oral toxicity test of mushroom water

extracts, three different dosages were used—namely, 60 g/kg, 90 g/kg, and 120 g/kg per rat body weight. The dosages were concentrated, diluted to 30 mL each, and given to 6-week-old rats as drinking fluid in six-rat replicates per dosage. The control rats were given 30 mL of distilled water each as drinking fluid. The treated and control rats were provided with 12 g of rabbit pellets per animal per day. Throughout the 35-day study period, the treated and control rats did not show clinical symptoms or death.

In the intraperitoneal toxicity test, which was carried out according to the method of Block et al. (1955 a,b) and Kadiri et al. (1996), Wister rats, aged 6 weeks and weighing 70–75 g, were observed for 1 week prior to the toxicity test. The water extracts of 60 g/kg, 90 g/kg, and 120 g/kg mentioned above were concentrated by evaporation to 1.5 mL. One rat was used for the application of each dosage level, and the treatment was repeated five times using five rats. The 1.5 mL dosages of the different water extracts of 60 g/kg, 90 g/kg, and 120 g/kg were administered into the rats by injection intraperitoneally. The control animals consisted of three groups. The first group was injected intraperitoneally with 0.5 mL of 1 mg/mL α-amanitin solution, the second group with 0.5 mL of 4 mg/mL phalloidin solution, and the third group with 0.5 mL of sterile distilled water. Phalloidin and α-amanitin are lethal mushroom toxins.

The treated and control rats were given 100 mL distilled water and 12 g of rabbit pellets per animal per day. During the 35 days of experimental study, the treated and control rats were observed for clinical symptoms or death, the weights of rats that died during the study were recorded, and the surviving rats were weighed at the end of the study period.

Rats injected with phalloidin and α-amanitin died on the first and second day, respectively, whereas rats injected with distilled water and mushroom water extracts did not die during the 35-day study period. Phalloidin and α-amanitin injected rats showed loss in body weight, while rats injected with extract of *L. squarrosulus* showed a gain in body weight.

These results of increases in fresh weights of rats given oral and intraperitoneal toxicity tests, and the death of rats injected with α-amanitin and phalloidin indicate that the constituents of *L. squarrosulus* are nontoxic. In conclusion, *L. squarrosulus*, which historically has been known to be edible as food and ethnomedicine, and in the present study is confirmed to be nontoxic, is, therefore, recommended as an edible mushroom.

AKNOWLEDGMENT

I thank the International Foundation for Science, Sweden for awarding the research grant, E/2396-3.

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International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Differential Chromatographic Behavior of Some Lignolytic Enzymes from White-Rot Basidiomycetes on Immobilized Metal Chelates

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The lignocellulosic materials are the most abundant on earth, which has attracted great attention for production of useful substrates from this waste (Volc and Kubatova, 1994; Takakura and Kuwata, 2003). The white-rot fungi (Basidiomycetes) have the ability to degrade lignocellulosic substrates by synthesizing several lignolytic enzymes of industrial and medical interest such as xylanases (EC 3.2.1.8), celulases (EC 3.2.1.4), glucose 2-oxidase (EC 1.1.3.10), peroxidase (EC 1.11.1.7), pyranose 2-dehydrogenase, superoxide dismutase (EC 1.15.1.1), and laccase (EC 1.10.3.2). White rot fungi are believed to be the most effective lignin-degrading microorganisms in nature.

The overproduction of lignolytic enzymes (pyranose 2-dehydrogenase, glucose 2-oxidase, laccase, xylanases, and superoxide dismutase) from several fungal strains—(*Agaricus bisporus* (J.Lge) Imbach, *Trametes versicolor* (L.:Fr.)Pilát, *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd, *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm., and *Fusarium* sp.)—was carried out by optimizing the composition of the culture media. As far as the composition of culture media is concerned, several agricultural wastes were used, such as rice husks, corn cobs, and rice bran. The effect of specific inducers for overproduction of enzymes was also investigated using these fungal strains.

In order to devise a simple and rapid one-step purification procedure for lignolytic enzymes, the chromatographic behavior of these enzymes (laccases, glucose 2-oxidase, and superoxide dismutase) on immobilized metal chelates was investigated as a function of pH, nature of metal ion, length of spacer arm, ligand concentration, and nature of matrix. The adsorption of enzymes was investigated by using Metal (II)–iminodiacetic acid metal chelates containing Cu (II), Ni (II), Zn (II), Co (II), and Ca (II) as a function of pH. The adsorption to immobilized metal chelates was pH dependent, as evidenced by the fact that high adsorption was observed at pH 8.0. The adsorption of enzymes on metal (II)-IDA chelates was due to the available histidine residues on enzyme molecules, shown by the fact that the addition of imidazole in the buffer system abolished the binding of enzymes to these columns.

Once the experimental conditions of immobilized metal affinity chromatography (IMAC) for enzyme purification were optimized, they were purified in one step by IMAC on Cu(II)-IDA agarose column at pH 6.0 and 8.0 with a high recovery of enzyme activity as well as a high degree of purity. Purified preparations of enzymes were apparently homogeneous on native PAGE and SDS-PAGE. The differential chromatographic behavior of enzymes on metal(II)-IDA chelates is apparently due to the number and spatial distribution of available histidine residues on these enzyme molecules.

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Role of Women in Mushroom Cultivation: Indian Perspectives

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Women in rural and lower-middle-income societies make an economic contribution to agricultural and healthcare markets. They are the custodians of traditional knowledge that is of great significance in rural medicine. Whereas women not only retain a high and widely shared level of general knowledge about wild foods, medicinal plants, and other natural resources, they also acquire new "men's knowledge" as roles and duties change. In tribal pockets of India in general and Central India in particular, tribal women sell edible mushrooms—e. g., *Agaricus bisporus* (J. Lge) Imbach, *Termitomyces heimii* Natarajan, *Pleurotus sajorcaju* (Fr.)Singer, and *Cantharellus cibarius* Fr.: Fr. The tribal women collect these naturally growing mushrooms from the forests and sell them in local markets. This enables them to contribute to their families' income. These mushrooms are commercially important and their cultivation can be done. Some non-governmental organizations also involve tribal women in cultivation of mushrooms such as *Pleurotus sajorcaju* and *Agaricus bisporus.*

Mushroom cultivation is an income-generating activity. This, on one hand, will develop self-reliance among the rural women and save them from tiring manual labor, and on the other hand, will provide them with more opportunities for cultural, societal, and technical education in improving the quality of family and community life by income generation.

Biotechnological packages for women can be introduced in the weaker sections of the society in order to improve health avenues for livelihood and in supplementing their family income. The need-based integrated setup can be used for exploitation of resources of the region for their physicoeconomic upliftment. This would create new avenues of employment for the rural populations. Women should be involved not only in collecting edible mushrooms, but also in cultivating medicinal mushrooms.

A majority of species of edible fungi have not been successfully cultivated because it is not feasible to recreate their growing conditions in isolation from their normal environment. Advances in molecular biology help us in identifying and selecting mushroom strains and understanding their association with environmental factors and cultivation methods. Hybrid mushrooms can be generated by tinkering with their genes to produce specimens that have desirable characters in terms of nutritional value, flavor, or resistance to environmental conditions. Study of strains of certain mushroom species that have not yet been used in the neutraceutical and pharmaceutical industries and as new dietary supplements, cosmetics, and pharmaceutical products can be developed from edible mushrooms.

The main goal of the present paper is to discuss the active roles of women in cultivation of edible and medicinal mushrooms of central India as an income generation activity.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Growth Characterization and Triterpenoid Derivatives Quantification of *Ganoderma* spp. Using HPLC

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Mycelium of *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd grew on the surface of molasses to form mycelial mats after about 12 days. When the mycelial mats became older, the color changed to a darker hue and wrinkled. It took about 9 days for the mycelium of *G. tsugae* Murrill to cover the whole surface of the molasses. Mycelial mats of *G. tsugae* were soft, and the old mycelia turned to yellow in color. Harvesting three pieces of mycelial mats of *G. lucidum* was enough to get 0.96 g of dried biomass. For *G. tsugae,* the mycelial mats were lighter. On wheat grains, the mycelium of *G. lucidum* needed 20 days to permeate throughout the grains. The mycelium of *G. tsugae* needed only 15 days. Both *Ganoderma* species produced primodia for approximately 65 days, and the mature basidiocarps were formed in about 100 days. The pH of extracts from both fungi became less acidic when harvested after longer periods. All the separation spots of the samples had an Rf value below 0.92. The TLC method detected the presence of triterpenoids in the extract. The different concentrations of thymol salt were used to obtain the standard curve through HPLC method. The values of triterpenoid concentrations, which refer to the peaks as P_1, P_2, P_3, P_4, P_5, and P_6 in the samples, were calculated based on this standard curve. P_1 was obtained in every stage of incubation. P_3 and P_6 were detected only in the fruiting body, whereas P_2, P_3, P_4 were the major triterpenoids of the mycelium. The highest triterpenoid production was found after 110 days; the fruiting body stage of *G. tsugae* is P_5 with 1212.00 μg/mL. *G. lucidum* showed triterpenoid concentration and derivatives six times higher than *G. tsugae*.

Health Benefit of the *Pleurotus sajorcaju* (Fr.) Singer (Oyster Mushroom) in India

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Mushrooms have attracted the attention of man from very ancient times, and the use of mushrooms as food is as old as human civilization. The cultivation of mushrooms can be considered as the most economic method of converting lignocellulogic agricultural wastes to consumable, protein-rich biomass. Use of edible fungi forms are an important step in the process of microbial biotechnology, in which a useful edible product is obtained from agricultural or industrial waste, which may otherwise pose environmental problems. Studies were carried out on cultivation of oyster mushrooms; nutrient analysis was performed at different stages of maturity; *in vivo* digestibility studies on albino rats were conducted; and effects of cooking methods on nutrients and studies on non-insulin–dependent rats were described. The effect of fiber on diabetic patients was observed, and recipes were developed for consumer awareness.

Mushroom cultivation was carried out using paddy straw and paddy husk, and the results indicated that the yield obtained from paddy straw was high (500 g/bed) when compared with the paddy husk (400 g/bed). Paddy straw is considered the best substitute for cultivating *Pleurotus sajocaju* (Fr.) Singer in terms of yield. In rural areas a women can easily manage 4–10 beds, depending on the space available, helping them to earn Rs.180–450 per week as supplementary income.

Mushrooms provide high-quality proteins and are low in calories. The protein in *P. sajorcaju* is rich in all the essential amino acids required for an adult. Approximately 25–35% of total amino acids occur as free amino acids. They are also known to be excellent sources of riboflavin, niacin, and patothenic acid. The mineral content in fresh mushrooms is higher than is found in many fresh vegetables and fruits.

Three different stages of maturity were described: immature (2 days before harvesting), mature (at the time of harvesting), and dried (matured and sun-dried). No significant difference was observed between immature and mature stages. Dried *P. sajorcaju* contained more nutrients.

Studies on *in vivo* digestibility of *P. sajorcaju* protein carried out on young male albino rats given 10% levels of protein revealed 87% digestibility of mushroom protein, compared to 93% for edible soy bean cake, which is significantly different at 5% level.

The three common methods were used in this study—namely, boiling in water for 10 minutes, shallow frying in refined oil for 10 minutes, and deep frying in refined oil for 10 minutes—revealed that the loss of nutrients were greater in shallow frying, followed by deep frying, and minimal in boiling. Vitamin C losses varied from 50–75% based on the type of cooking. Heat treatment did not affect the crude fiber, fat, and ash contents. Inclusion of paddy straw mushroom powder in the hypercholestremic diets significantly reduced total plasma lipids, total cholesterol, and the level of glycerides, whereas free fatty acids and phospholipids levels were not much affected.

The effect of fiber (at 10% and 15%) from *P. sajotcaju*, spinach, and isalbgol on blood glucose response

in 24 non-insulin dependent diabetic patients (NIDDM) from a diabetic clinic was investigaged. Fifteen percent of fiber from mushroom was found to be effective. Mushroom powder was incorporated effectively in weaning foods, which served as a useful protein source and also contributed major nutrients such as protein and calcium.

With the idea of popularizing the mushrooms among the local communities, some recipes such as mushroom soup, mushroom curry, creamy mushrooms, pizza stuffed capsium, pan cakes, pakoda, omlet, sandwichs, ketchup, chutney, pickle, baked mushrooms, mushroom flavoured rice, mushroom garlic sauce, mushroom flakes, mushroom chips, mushroom instant soup powder, noodles, and macaroni were developed. The organoleptic analysis indicated that the overall acceptability is very good. Mushrooms can be preserved for a longer period, significantly through pickles, powder, soup powders, masala powders, and noodles, etc.

Isolation and Regeneration of Protoplasts from *Cantharellus cibarius* Fr.:Fr., an Edible Ectomycorrhizal Mushroom

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Mushroom mycelia are now considered as a potential source of protein, amino acids, vitamins, and minerals. Commercial production of mushroom mycelia could provide a promising source of proteinaceous food. *Cantharellus cibarius* is an ectomycorrhizal mushroom, which grows during the rainy season in association with wild bamboo in India in general and in Central India in particular.

The mushroom belongs to the order Cantharellales and the class Basidiomycotina. The mushroom is sold in open markets in the tribal pockets of Melghat (Maharashtra), Tamiya, and Patalkot in District Chhindwara; the Balaghat District of Madhya Pradesh; and the Bastar region of Chhatisgarh states of India. The ectomycorrhizal mushroom *C. cibarius* was collected from Melghat, Amravati. The gills of this mushroom were used for isolation of the fungus on "Modified-Fries-Medium." Protoplastation is interesting and useful in the case of ectomycorrhizas, in which most of the exchange of solutes takes place in apoplastic space in the hartig net area. These fungi have advantage over arbuscular mycorrhizal fungi because they can be grown on defined synthetic media and thus can be manipulated apart from the plant partner. The isolation of the protoplast can also be used to understand the mystery of symbiosis between the fungal partner and mycelia protoplast can be used to address questions pertaining to metabolic exchange.

Protoplasts of *C. cibarius* were isolated from mycelium grown in potato-dextrose broth. When mycelia were treated with a combination of macerozyme, chitinase, and cellulase and with 0.6 M sucrose as the osmotic stabilizer, the protoplast yield was 1.0×10^6 per gm fresh weight of mycelium within 3 hours of incubation. The protoplasts were regenerated in solid MFM agar plates. The regeneration frequency was between 20 and 25% and depended on culture conditions.

The main aim of isolation of the protoplast from *C. cibarius* was to study its regeneration potential.

The Receptor for β-Glucan of *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd in Immune Cells

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The mechanisms on how polysaccharides of medicinal mushrooms can be absorbed and used in the potentiation of immunity in human bodies has not been elucidated until recently. In 2001, dectin-1 was identified as a β-glucan receptor, which is calcium dependent and whose gene is located on mouse chromosome 6 and human chromosome 12. The dectin-1 mRNA is highly expressed in dendritic cells. Dendritic cells and macrophages have pattern recognition molecules for binding β-glucan. Dectin-1 was also found in murine organs, spleen, thymus, lung, and intestines. Treatment of macrophages with β-glucan of *Ganoderma lucidum* (GLG) resulted in an increase of IL-6 and TNF-α in the presence of LPS. But GLG alone did not increase IL-6 or TNF-α. The results suggest that dectin-1 may cooperate with CD-14 to activate signal transduction, which is important in immune responses.

Screening Systems for Medicinal Basidiomycetes Antitumor Extracts

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Prevention and treatment of oncological diseases is one of the most important fields of possible practical application of preparations on the basis of medicinal Basidiomycetes. The present experimental data demonstrate the ability of mushrooms to synthesize metabolites, revealing anticarcinogenic effects, possessing antitumor activities due to cytostatic or/and immunostimulating action, and inhibiting the process of metastasis. One of the most important current problems in our view is the development of screening systems for mushroom antitumor preparations, including their effectiveness and cost. The present investigation is the first step in this direction.

Strains of G*anoderma lucidum* (W.Curt.: Fr.) Lloyd, *Hericium erinaceus* (Bull.: Fr.) Pers., *Lentinus edodes* (Berk.) Singer, *Trametes versicolor* (L.: Fr.) Pilát, having taxonomic proximity and affiliation with the group of xylosaprotrophes (causing white mould) due to their ability to use lignin, was studied.

The action of extracts of submerged mycelium, fruit bodies, and basidiospores from Russian and Chinese mushroom farms was investigated.

Submerged cultivation was carried out in the Gause Institute of New Antibiotics of Russian Academy of Medical Sciences. The strategy for developing methods of submerged cultivation of the species studied, which secures maximal shortage of cultivation costs owing to possibilities of strain diversity, reduction of process length 2–4 times, and increase of biomass yield was worked out. The methods of submerged culture were developed individually for each studied object and included the strain of certain mushroom species, liquid media composition, initial pH, aeration, lighting conditions, temperature rate, and process duration.

Various methods of extraction were tested on the mycelia and fruit bodies of the studied mushroom species. For a number of cases, fractionating of extracts was done and monosaccharide composition of extracted polysaccharides was analyzed.

Obtained materials were investigated *in vivo* on hybrid mice (C57Bl/6J × DBA/2) F1 (B6D2F1) with transplantable ascites lymphoma EL_4 as a model. Extracts were inoculated orally and intraperitoneally.

Some groups were treated with cyclophosphan (CF) (50 mg/kg) with the aim of inhibiting leukocyte suppressors. General host status of mice, body weight changes, tumor engraftment and its growth, and the survival rate of mice were observed during the experiment. The cause of mice death, if it occured, was determined by analysis of observation data and dissection results.

Investigation of *Ganoderma lucidum* extract antitumor activity showed that the antitumor activity of submerged mycelium extract is much higher than the antitumor activity of Russian and Chinese basidioms and basidiospore extracts.

Ganoderma lucidum water extract (introduced intraperitoneally) inhibited tumor growth in young mice, and in 9-month-old adult mice antitumor growth was not effected by itself but authentically strengthened the CF effect. A similar effect was observed in adult (5-month-old) mice after oral introduction of *G. lucidum* mycelium combined extract. Thus, the dependence of the antitumor effect of the mushroom extract on the age of the laboratory animal age was determined.

Some additives were selected to strengthen the antitumor effect of *G. lucidum* mycelium extracts. The highest self-activity was shown by orally introduced mycelium polysaccharide fractions with various ratios of monosaccharides.

Unlike basidiom extracts, submerged mycelium extracts of *Hericium erinaceus* showed self-antitumor activity on a solid lymphoma model, resulting in inhibition of tumor growth. This effect was distinctly increased by the single prior introduction of a low dose (50 mg/kg) of CF.

A *Lentinus edodes* extract study with a solid lymphoma model did not reveal any differences between submerged mycelium and basidioms. Oral administration of these extracts substantially inhibited growth of subcutaneous lymphoma. This effect was also distinctly increased by the single prior introduction of a low dose (50 mg/kg) of CF. A new way to rapidly receive submerged mycelium extracts of *L. edodes* with marked antitumor activity was proposed.

Intraperitoneal administration of water extracts of submerged mycelium of *Trametes versicolor* results in the inhibition of ascites lymphoma growth. On day 27 the amount of mice in the experimental group was reliably (χ^2 $\rho = 0.03$) bigger than in untreated group.

Summing up the obtained results, we should underline the principle conclusions. The selection of raw material for drugs, biologically active supplements, and other functional food products should be done individually for each basidiomycetes species. The chemical purification expediency of the active substances depends on the observed effects. Thus, the necessity of initial extract fractionating for obtaining well defined antitumor effects was shown for *Ganoderma lucidum.* At the same time, the results for *Lentinus edodes* gave proof for practical uses of crude extracts. We should keep in mind the dependence of the obtained results

on the laboratory model used and the direction of clinical studies. At the present time, the question of which is the preferable choice of Basidiomycetes species for definite oncological disease treatment is discussed. The problem of specific individual medicinal Basidiomycetes species antitumor action in different patient age groups is also very important.

Mushrooms as a Source of Polyprenols

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Accumulation of long chain polyprenols in leaves of plants was first described 50 years ago. Polyprenols were found in a number of angiosperm plants and conifer trees (Chojnacki and Vogtman, 1984). These compounds have a broad spectrum of physiological activity. Dolichols are partially hydrogenated polyprenols. The physiological activity of dolichols is several times higher than polyprenol activity (Grigor'eva and Moiseenkov, 1989). Dolichols occur in mammalian tissues and yeast, and they are also found in some plants (Mankowski et al., 1976; Kukina et al., 1991). Bactoprenols were found in bacteria, but the data about "fungoprenols" are absent in scientific literature.

Polyprenols were isolated from the following mushrooms: *Armillaria mellea* (Vahl:Fr.) P. Kumm., *Fomes fomentarius* (L.:Fr.) J.J. Kickx, *Tricholoma populinum* J. Lge, *Lycoperdon perlatum* Pers.:Pers., and *Suillus luteus* (L.:Fr.) S.F. Gray. The samples of fresh mushrooms were extracted by a mixture of hexane and isopropanole in a ratio of 1:1. Adding water to the extract formed two layers of liquid. The hexane included the nonpolar substances and alcohol included a number of polar components. These fractions may be investigated separately. Samples of extracts were investigated by high-performance liquid chromatography with the ester of tocopherol as an internal standard. HPLC was performed as described previously (Mankowski et al., 1976) for plantaprenols from sea buckthorn leaves. The yields of extractive substances from these samples and content of polyprenols in extracts are presented in Table 1.

The distribution of polyprenols in free and esterified forms is summarized in Table 2. The accurate analysis of chromatographic data led to conclusions about the differences of polyprenols. *Armillaria mellea, Lycoperdon perlatum,* and *Tricholoma populinum* contain predominantly acetylated polyprenols. Polyprenols from *Suillus luteus* were investigated after saponification because the polyprenols were esterified with fatty acids. *S. luteus* probably contain small amounts of dolichols. The polyprenols in *Fomes fomentarius* and *S. luteus* are present in minute amounts.

According to this research, mushrooms are being considered as new sources of polyprenols.

TABLE 1. Polyprenols Accumulation in Different Species of Mushrooms

Species	Yield of hexane extract (% from dry weight)	Content of polyprenols in hexane extract	Yield of alcohol extract (% from dry weight)	Content of polyprenols in alcohol extract
Armillaria mellea	3.0	3.2	10.2	Traces
Fomes fomentarius	0.7	0.2	12.0	Traces
Tricholoma populinum	2.5	2.0	8.2	Traces
Lycoperdon perlatum	0.7	1.1	4.0	Traces
Suillus luteus	2.5	0.5	30.0	Traces

TABLE 2. Distribution of Polyprenols

Species	Total polyprenols (% of fresh weight)	Content of components (%)							
		14	15	16	17	18	19	20	21
Armillaria mellea	0.01								
Free	0.003	5.2	10.5	17.5	32.5	32.6	8.4		
Acetylated	0.007	6.3	17.0	35.7	27.4	9.7	3.8		
Fomes fomentarius	0.0005		+	+	+	+			
Tricholoma populinum	0.007								
Free	0.0024	9.3	15.6	26.7	26.0	22.2	Tr.		
Acetylated	0.0056	13.5	21.7	32.7	28.5	3.6			
Lycoperdon perlatum	0.007								
Acetylated	0.007					11.8	52.9	23.5	11.8
Suillus luteus	0.00007			+	+	+	+		

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Cytotoxic Activities of Ergosta-4,6,8(14), 22-tetraen-3-one from the Sclerotia of *Grifola umbellata* (Pers.) Pilát

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Grifola umbellata (also known as *Polyporus umbellatus*) is a mushroom that is used as a diuretic in Chinese medicine. Herein, we report on the isolation and identification of a cytotoxic compound from the sclerotia of *G. umbellata.*

For the isolation of the compound, a hexane soluble fraction of the sclerotia of *G. umbellata* was subjected to column chromatography on silica gel and/or Sephadex LH-20 column eluted with organic solvents. The structure of the isolated compound was elucidated using IR, MS, ^{1}H-, and ^{13}C-NMR spectra, and the structure of the compound was determined as ergosta-4, 6, 8 (14), 22-tetraen-3-one (erogone).

Cytotoxic activities of erogone compared to human cancer cell lines, HT-29 (colon cancer), HeLa 229 (cervix cancer), Hep3B (liver cancer), and AGS (stomach cancer) were compared using the XTT assay kit. Ergone inhibited all cell lines as the dose was increased. In the case of Hep3B and HT-29 cell lines, maximal cytotoxic activities of erogone were achieved at the concentrations of 10 and 15 μ/mL, respectively. However, the cytotoxic activities of erogone compared to Hela 229 and AGS were much weaker than those of Hep3B and HT-29 cell lines. Values of 50% inhibitory concentrations (IC_{50}) of erogone against Hep3B, HT-29, HeLa 229, and AGS were 5, 7.2, 26.3, and 22 μ/mL, respectively.

Cytotoxic activities of ergone against various tumor cell lines were evaluated in this study for the first time. Therefore, other mushrooms were also examined for the presence of the compound. In the present study, we measured the content of erogone in eight mushrooms—*Grifola umbellata, Lentinus edodes* (Berk.) Singer, *Ganoderma applanatum* (Pers.: Wallr.) Pat., *Tricholoma matsutake* (S.Ito et S.Imai) Singer, *Sarcodon aspratus* (Berk.) S.Ito, *Ramaria botrytis* (Pers.) Ricken, *Pleurotus eryngii* (DC.:Fr.) Quél., and *Sparassis crispa* (Wulf.)Fr.—and four mycelia—*Grifola umbellata, Lentinus edodes, Ganoderma applanatum,* and *Tricholoma matsutake*—using HPLC. The contents of erogone in mushroom and mycelium were in the range of 4.8–29.0 μ/g and 15.5–38.0 μ/g, respectively. Among mushrooms and mycelia tested, the mycelia of *Grifola umbellata* and *Tricholoma matsutake* had the highest amounts of erogone (38.0 μ/g).

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Effect of Selenium on the Nutritional Components of *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd

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Selenium (Se) is not an essential microelement because some organisms—i.e., yeasts and plants—do not need selenoproteins, but in some animals and humans Se play an essential nutritional role. Selenium is an intergral component of several enzymes and proteins, and according to their roles it can be concluded that Se is an antioxidant and an antimutagenic agent, and it prevents the malignant transformation of normal cells and activation of oncogenes.

The effect of Se on the nutritional components of Ling Zhi or Reishi mushroom *Ganoderma lucidum* (GL) such as polysaccharides, proteins, amino acids, and mineral elements was studied using Se-enriched *G. lucidum* (Se-GL) of different Se content as tested samples. The Se-GL was obtained by cultivating *G. lucidum* in substrate with different Se contents. The results showed that Se-GL with good yield and high Se content could be obtained by cultivating *G. lucidum* in a substrate with Se content of 200~250 μg/g. Low concentration of Se (<100 μg/g) in the substrate facilitated the synthesis of total protein and amino acids in *G. lucidum,* but high concentration of Se (>150 μg/g) played a reverse role. However, Se of all concentrations facilitated the synthesis of polysaccharides. Se concentration in the culture had no significant effect on the distribution of the amino acids and proteins. In addition, the effect of Se on the contents of mineral elements of Se GL was rather complicated. With the increase of Se content in Se-GL, the contents of Cu and Mo increased; however, the contents of other elements such as Fe, Ca, and Sr decreased.

Morphological, Ecological, and Genetic Characterization of the *Pleurotus eryngii* Species Complex in Israel

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The genus *Pleurotus* (Jacq.:Fr.) P.Kumm. (Pleurotaceae, higher Basidiomycetes) covers a group of ligninotrophic mushrooms that are edible and medicinal. Species of the genus *Pleurotus* are important mushrooms because of their ease of cultivation, their nutritional value, and their medicinal properties. Traditional medicine attributes medicinal properties to *Pleurotus* spp. Scientific evidence supports their importance as producers of substances with antibiotic, antitumor, anti-inflammatory, and hypo-cholesterolaemic activities. Scientists in many countries, including Israel, contribute to the research of the genus *Pleurotus.* The evolutionary connections of species in the genus *Pleurotus* are still not clear, and many taxonomic problems are still controversial. The geography plays an important role in the evolutionary studies of this genus, but the ecological/genetic perspectives of the genus have not been investigated satisfactorily.

The present study focuses on the taxonomic, ecological, distribution, and genetic characterization of this species complex in Israel. The objective of this study was to characterize the ecogeographical variation of *Pleurotus eryngii* species complex in Israeli populations. The morphology characterization of *P. eryngii* defined the exact geographical distribution of this species. The taxonomic study and the collection from the Sataf springs (Judea Mountains) described *P. eryngii* var. *tingitanus* Lewinsohn et al. (Lewinsohn et al., 2002, Mycotaxon LXXXI, pp. 51–67) as a new variety. The ecogeographical study indicates that Israeli populations are well adapted to the hot and dry climate and can tolerate extreme temperature and aridity conditions. This study also showed that heat stress can influence the growth rate of the isolates subsequently grown under optimal temperature conditions. The ecogeographical distribution and habitats of *P. eryngii* showed a high correlation between the coefficient of growth and the mean colony diameter growth rate.

Comparison with European genotypes showed that the Israeli genotypes are better adapted to hot and dry climates. Humidity and rainfall have a stronger effect on the adaptability of this complex to different environments (Lewinsohn et al., 2000, Mycological Research 104:1184–1190). The genetic study included random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), which was used to assess the genetic diversity in 12 populations (a total of 144 genotypes) of *P. eryngii* complex, sampled in Israel. Results show a higher level of diversity of RAPD polymorphism in *P. eryngii* populations especially in the drier, stressful climatic regimes. The 12 primers used in this study amplified 164 scorable RAPD loci, of which 163 (99.4%) were polymorphic and only one was monomorphic. Out of

the 164 loci, 123 (75%) varied significantly ($p < 0.05$) in allele frequencies among populations. This total proportion (75%) of significant polymorphic loci far exceeds the 5% level expected by chance (binomial test, $p < 0.000001$). The levels of polymorphism and gene diversity appeared to be significantly different between the populations. Sixty-eight percent of the RAPD diversity was within populations, and 32% was between populations. Interpopulation genetic distances showed positive association with geographic distance, which was confirmed with spatial autocorrelation analysis of RAPD frequencies.

Spearman rank correlation revealed a strong positive association between high polymorphism and the aridity index. In multiple regression, the coefficient of determination of polymorphism and gene diversity was explained by climatic variables linked to temperature and humidity ($R^2 = 53.6\%$, $p = 0.032$). These findings further demonstrate the validity of the "environmental theory of genetic diversity" hypothesis within *P. eryngii* populations in Israel. The results suggest that natural selection develops a high level of RAPD polymorphism as adaptation to stressful and temporally heterogeneous environments (Lewinsohn et al., 2001, Mycological Research 105(8):941–951).

In conclusion, these results showed great ecogeographical variation of *P. eryngii* species complex in Israeli populations, distributed along a transect of increasing aridity. These differences are reflected in morphological, genetic, and adaptive polymorphisms that can be used in the future to select varieties with commercial value for cultivation of this important fungus species.

Culinary–Medicinal Mushrooms Affecting the Development of Adipose Tissue Development in Rats and Depending on the Growth Conditions for Their β-Glucan Production

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Two different studies have been conducted, and the results are compiled herein. Fırst, genetically homogenous rats were used for investigation of the effect of the most commonly cultivated mushrooms on their plasma lipids and adipose tissue localization. Therefore, the rats were randomly divided into five groups of six individuals and subjected to commercial rodent fodder or the same fodder supplemented with 5% (DW/ DW) oat flower, shiitake (*Lentinus edodes* (Berk.) Singer), button mushroom (*Agaricus bisporus* (J.Lge) Imbach), or blue oyster mushroom (*Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm.) with free access to water and dry food for 8 weeks. The plasma LDL, HDL, and total cholesterol levels were measured with the Technicon RA-XT analyzer. Distribution of adipose tissue was determined. All non-intramuscular adipose tissue was dissected and weighted, and subcutaneal, intraperitoneal, mesentery, and interscapular adipose tissue measured. The group fed with shiitake

had the lowest plasma lipid levels. Shiitake and blue oyster mushroom had the most profound affects on the accumulation and localization of adipose tissue. Possible explanations are discussed.

In a second study, six *Lentinus edodes* and four winter mushroom (*Flammulina velutipes* (W. Curt.: Fr.) Singer) strains, varying by their optimal substrate preference, were cultivated in Petri dishes during the vegetative phase in order to investigate the effect of pH and substrate composition on their mycelia performance and β-glucan production. Substrate was either the most commonly used MYPA with malt and yeast extract, or peptone or MYPA with $CaSO_4$. The vegetative growth speed was monitored by measuring the daily radial growth. After mycelia reached the dish margin, the pH of the substrate was measured, the biomass of mycelia weighted, and β-glucan content analyzed. Marked statistical differences were discovered between strains and substrates in growth speed, biomass production capabilities, post-growth substrate pH, and β-glucan content. According to these findings, it is possible to modify the functional quality of *Lentinus edodes* and *Flammulina velutipes*.

Studies on Laccase and Biomass Production *In Vitro* and Culture by a Mexican Wild Strain of *Agaricus bisporus* (J.Lge) Imbach: a Comparison with Commercial Strains

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Until recently, many scientists believed that *Agaricus bisporus* was an extracted European mushroom. However, recent findings showed the opposite. In Mexico, several strains of *A. bisporus,* growing under *Cupressus benthami* trees, have been isolated recently. Commonly known as the white button mushroom, most of the 2.4 million tons of cultivated *A. bisporus* around the world are still genetically closely related to the hybrids cultivated commercially. The improvement of genetic diversity of *A. bisporus* is essential for scientists to enhance productivity and diversification of cultivated mushrooms to meet the world market requirements. Some studies showed that wild strains of *A. bisporus* had better yield than several commercial strains. Moreover, some wild strains seem to be more resistant to brown blotch disease, produced by *Pseudomonas tolaasii,* than some commercial strains. Therefore, improvement of yield and natural resistance through wild strains can be considered in mushroom breeding programs. The aim of this paper is to evaluate comparatively three *A. bisporus* strains (two commercial and one Mexican wild strain) on *in vitro* enzyme laccase and biomass production as well as commercial mushroom production on compost.

Four different culture liquid media were used for the laccase and biomass experiment: malt extract (ME: 10 g/L malt), yeast malt extract (YME: 20 g/L malt, 2 g/L yeast), compost malt extract (CME: 10 g malt/600 mL compost extract and 400 mL distillated water), and yeast malt extract added with water soluble lignin derivates (YME-WSLD: 20

g/L malt, 2 g/L yeast, 3.7 g/L of Indulin AT fitted to a 1 μmol concentration of phenols/L of water). Strains were grown on static culture in glass of 250 mL containers, containing 10 mL of sterilized media. Ten replicates by each medium and strain were made. The samples were incubated for 7 and 14 days in the dark at 24°C. Biomass production was determined by weight differences filtering mycelia through filter papers (90 mm medium size pore). After filtration, the liquid medium was recuperated and used to determine laccase production with ABTS [2,2′-azino-bis (3 ethylbenz-thiazoline-6-sulfonic acid)] as enzymatic substrate.

For mushroom production, spawn was prepared on wheat grains, which were cooked for 15 minutes, left in hot water for 10 minutes, and then drained. The grains were added with 0.5% (dry weight) of a 1:1 proportion of calcium carbonate and calcium sulfate. The mixture was autoclaved at 121°C for 90 minutes in polyethylene bags (250 g). Strains were cultivated following the general commercial methodology. Commercial compost was inoculated with spawn of each strain at 1%. Plastic trays (3.8 cm long, 32 cm wide, and 16 cm high) with 5 kilograms of compost were used. Ten replications were made by strain. Mushrooms were harvested while the veil was still intact and were weighed once the stalk was cut off as for selling on the commercial market. Produced mushrooms were grouped by means of cap size: G1 1–5 cm and G2 5.1–10.0 cm. Yield was evaluated as means of fresh mushroom weight.

There were significant differences in biomass and laccase production of the three strains. The wild strain produced significantly less laccase and biomass than the commercial strains. Significant differences were found also regarding the culture media. Strains cultured on ME showed a higher production of laccase and biomass, followed by the MCE. Relationships among laccase, biomass, and yield cannot be determined. On the other hand, full substrate colonization was observed between the 11th and the 19th day (wild strain growth was faster). Although casing was made at the same time for the three strains, pinning of the wild strain was observed the next day after the casing. On average, the wild strain produced 1694.3 g for a 5 Kg sample, whereas commercial strains produced 1056.2 and 760.9 g. All three strains produced more fruit bodies on G1 than on G2.

Production of Ligninolytic Enzymes by Oyster Mushroom *Pleurotus ostreatus* (Jacq.:Fr.) P. Kumm. Under Different Nutritional Conditions

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One of the effective methods for regulating and increasing the quantity of ligninolytic enzymes is the addition of the appropriate inducers to the nutrient medium. The ligninolytic enzymes of some white-rot fungi are synthesized only after the primary growth phase in response to nitrogen and carbon limitations in the medium. In the screening program, *Pleurotus ostreatus* appeared to be one of the best producers of extracellular laccase (Lac) and manganese peroxidase (MgP). Therefore, we compared the effect of different carbon and nitrogen sources, including lignocellulosic substrates, on *P. ostreatus* 98 oxidative enzyme production.

The secretion of Lac, MgP, and versatile peroxidase (VP) were significantly dependent on 11 types of carbon sources when species were grown in a medium with 2 g/L NH_4NO_3. Mandarin peels, which were used as one of the possible substrates, ensured very high yields of Lac (2375.5 U/L). Increasing its concentration in the nutrition medium from 20 g/L to 40 g/L resulted in a more than six-fold increase in Lac activity. Carboxylmethylcellulose, gluconic acid, glucose, and cellobiose (10 g/L) appeared to be poor growth substrates for Lac production. Moreover, results of this study showed that the activity of the enzyme was at its lowest level on medium containing avicel and mannitol.

Lignin degradation of *P. ostreatus* 98 on culture media with 10 g/L glucose and 11 types of nitrogen sources (0.5 g/L) was suppressed. This species showed high Lac activity in the presence of peptone, kazein acid, and corn step liquor (887.1 U/L, 802.3 U/L, and 674.3 U/L, respectively). In contrast, medium with $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ inhibited this enzyme accumulation.

Secretion of VP and MnP by the investigated strain was comparatively low. Adding different carbon sources to the medium caused variation of VP activity from 0.24 U/L (medium with xylan) to 32.4 U/L (medium with mandarine peels). The results demonstrated that MnP activity was almost two-fold lower than VP activity on all of the used sources. The content of kazein acid and corn step liquor exerted the most favorable influence on the activity of VP and MnP. On the other hand, NH_4NO_3, NH_4Cl, and $(NH_4)_2SO_4$ were rather poor nitrogen sources for the production of these peroxidases.

In conclusion, it was shown that the synthesis of Lac, MnP, and VP by *P. ostreatus* 98 differed in various nutritional conditions. Among the different carbon and nitrogen sources, mandarine peels and peptone were optimal substrates for high production of the investigated enzymes.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Inhibitory Effects of Extracts from *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae) on Immediate Type Allergy Induced Compound 48/80 in ICR Mice

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The expression of interleukin (IL)-12 (a key role in Th1 differentiation) and IL-18 (a proinflammatory cytokine in enhancing Th1 immune response) mRNA in a macrophage-like cell line, RAW264.7, stimulated with a polysaccharide purified in *Agaricus brasiliensis* S. Wasser et al. (Agaricomycetideae) (ABSW), were investigated using RT-PCR. We have reported that mRNA expression in IL-18 indicated no drastic changes during stimulation with polysaccharide purified in ABSW for 12 hours, but its level rapidly increased to 5.6-fold at 24 hours as compared with the initial level. The level of IL-12 p40 mRNA expression was different from those of IL-18. After 6 hours of stimulation, its level had not been reached at all, but started to increase at 12 hours and continued to increase by 24 hours. Moreover, polysaccharides from ABSW changed the percentage of splenic Thy 1.2- and L3T4 (CD^4)-positive cells in the T cell subsets in ABSW-treated mice. It was presumed from the results that this mushroom possessed the ability to differentiate from naïve T cells to T-helper type 1 (Th1) and showed antiallergic activity.

To test this hypothesis, in this study, extracts from ABSW (3.6 mg/mL) were dissolved in distilled water and ingested by 8-week-old male ICR mice as drinking water for 14 days. No drastic differences were observed in the body weights of the mice. The cumulative number of scratching behaviors for 30 minutes in saline-treated mice (control) was approximately 2300 after intradermal injection of compound 48/80, which is a pruritogenic agent, whereas those in ABSW decreased to approximately 960. To determine the effects of ABSW on degranulation in mast cell activation, histamine contents in blood were measured. ABSW treatment suppressed histamine release (36% compared to the control). Similarly, suppression of degranulation from must cells also was detected histochemically by toluidine blue staining.

The differentiation of naïve T cells to the Th1 and Th2 subset of effector cells was regulated by the cytokines to which T cells were exposed at the time of antigenic stimulation. Interferon (INF)-γ and IL-12 promote Th1 development, whereas IL-4 promotes differentiation to Th2. To confirm which ABSW could change the differentiation of naïve T cells to Th1 or Th2, contents of INF-γ, IL-12, and IL-4 in the spleen of mice were measured with or without Concanavalin A (Con A; T-cell mitogen). The cytokines did not show any difference without Con A. When splenocytes were incubated with Con A, contents of INF-γ and IL-4 secreted from T cells significantly increased compared with the control, but IL-12, which was produced from macrophages, did not. Moreover, ABSW induced INF-γ more potently than IL-12 in splenocytes incubated with Con A. These results indicate that ABSW possessed immunomodulating properties that might be involved in the development of Th1 cells, culminating in an inhibition of immediate type allergy caused by compound 48/80.

A Study on the Effect of *Ganoderma lucidum* (W.Curt.: Fr.) Lloyd in Indian HIV Carriers

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Over the past decade, substantial progress has been made in defining strategies for treatment of disease caused by human immunodeficiency virus (HIV), in which natural products can serve as a source of structurally novel chemicals that are worth investigating as specific inhibitors of HIV as well as its essential enzymes, protease (PR), and reverse transcriptase (RT). Therefore, it is necessary to identify and develop new anti-HIV agents without adverse side effects and viral resistance. *Ganoderma lucidum* (Reishi), belonging to the higher Basidiomycetes, appears to be very safe because oral administration of the extract did not display any toxicity. A literature survey revealed that the medicinal effect of *G.lucidum* was not investigated in India, and, hence, the present study was carried out to investigate the effect of supplementation of *G. lucidum* in Indian HIV carriers.

Ganoderma lucidum is one of the valuable crude drugs, which has been used in China and Japan as a traditional Chinese medicine or folk medicine for the treatment of various diseases. In the present study, 25 confirmed HIV-1 human subjects were selected based on the inclusion and exclusion criteria and administered with fruit body of *G. lucidum* for 90 days. Prior to and after 90 days of supplementation with *G. lucidum,* the level of HIV viral load, lymphocyte enumeration (percentage of CD_3, CD_4, CD_8, and absolute CD_4, CD_8 counts), total leukocyte count, WBC differential count, and IgA, IgG, IgM, and liver function tests were estimated.

Estimation of HIV viral load revealed that viral load was not increased in HIV carriers after supplementation of *G. lucidum,* which indicates that the replication of the human immunodeficiency virus was inhibited in HIV carriers. But there was no significant decrease of HIV viral load. It was observed that the mean percentage of CD_3 and CD_8 were not changed significantly after supplementation, but the percentage of CD_4 was significantly increased from 18.77 ± 3.25 to 23.89 ± 4.04. However, the increase was statistically significant ($p < 0.01$). Similarly, mean absolute CD_8 count was not altered, but mean absolute CD_4 count also increased from 211.60 ± 56.97 (cells/mL) to 355.60 ± 90.73 (cells/mL). This increase was statistically significant ($p < 0.01$). Total leukocyte count, WBC differential count, and IgA, IgM, and IgG levels were not altered significantly in HIV carriers. The results of liver function tests obtained also indicated that the supplementation of *G. lucidum* was harmless because they did not show any toxic effects in HIV carriers. It is concluded that the supplementation of *G. lucidum* mushroom may reduce the risk of developing AIDS in Indian HIV carriers.

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A Study of Pharmacological Components from Submerged Culture Mycelia of *Phellinus linteus* (Berk. et Curt.) Teng

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Phellinus linteus is a polypore of class Hymenomycetes and a heart-rot fungus occurring especially in groves of *Morus bombycis* Koidz. In Japan this fungus has been named Meshimakobu because it was found mainly in Meshima of the Danjo Islands in Nagasaki Prefecture. In Chinese medicine it has been called Souou and is used as a medicinal ingredient. About 30 years ago, certain pharmacological effects of this fungus were examined, and it was revealed that this fungus exhibited the highest antitumor activity among the Hymenomycetes mushrooms (Ikekawa et al., 1968). In this study, we found some biological activities of the mycelia extract and succeeded in isolation and structure-determination of the active principles in the extract (Nakamura et al., 2000, 2002). The results are as follows:

- The scavenging activity of superoxide anion radicals of the extracts was detected. As a result, caffeic acid was isolated as an antioxidant (Nakamura et al., 2002). The IC_{50} of this principle was 3.05 μg/mL (16.9 μM). This was the first report in which caffeic acid was isolated from mushroom mycelia. It was proposed that caffeic acid may partly contribute to the pharmacological activity of *Ph. linteus*.

- The anti-allergic activity of the extracts was demonstrated by *in vivo* assay using NC/Nga/mice. Oral administration of mycelial extracts of *Ph. linteus* exerted type-I allergy suppressive effects by inhibiting the production of serum IgE and inhibiting the formation of dermatitis-like skin lesions. Hot-water extracts of the mycelia was more active than the culture filtrates.

- The antitumor activity of the extracts was examined by using Sarcoma 180/mice, p.o. As a result, all the fractions of *Ph. linteus* mycelia showed antitumor activity in the solid tumors implanted in mice. The highest antitumor activity (81.2%) was observed in the protein/glucan complex obtained by the precipitation of 24% NaOH extract at pH 6.0. The protein/glucan complex consisted of 39.3% polysaccharide and 49.4% protein. Its ^{13}C and ^{1}H NMR data showed that the main glucan part of the complex was primarily α-1,3-glucan chains (Nakamura et al., 2004).

It was demonstrated that the mycelia component of *Ph. linteus* showed useful activity for the treatment of allergic disease and cancer. These two diseases are recognized as very serious illnesses in Japan. The antioxidant activity (superoxide scavenging activity) of the mycelia component may contribute to keeping people's health.

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Antimicrobial Colossolactones from a Nigerian Polypore *Ganoderma colossum* (Fr.) C.F. Baker

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A number of synthetic antimicrobial agents have been developed to kill microorganisms effectively. However, drug resistance often occurs when these agents are used long-term. As a result, there has been a tremendous interest in the antimicrobial properties of plants and fungi. *Ganoderma* species are widely used, especially in Asia, for the treatment of chronic diseases such as cancer, hepatitis, bronchitis, asthma, and haemorrhoids. Many publications have also described the antimicrobial properties of compounds isolated from various polypores, including *Ganoderma* species. Nigeria has a rich biota of polypores, but the antimicrobial activity of these fungi has not been reported previously.

The antimicrobial activity of a new colossolactone, 23-hydroxycolossolactone E, and two known colossolactones isolated from *Ganoderma colossum* (Ganodermataceae) were tested against gram-positive and gram-negative bacteria. Fruiting bodies of *G. colossum* were collected from a dead log of *Delonix regia* (Fabaceae), Yaba College of Technology, Lagos, Nigeria. The polypore was morphologically determined as *G. colossum* by the truncate, ornamental, yellow basidiospore that is 11–17 × 7–11 cm in diameter. A voucher specimen accession K (M) 120802 was deposited in the herbarium at the Royal Botanic Garden, Kew, UK. Bioassay guided fractionation of compounds from a fungal sample was carried out using various chromatographic techniques. A ground sample was extracted with 100 mL of *n*-hexane: dichloromethane. The residue was reconstituted in methanol and subjected to column chromatography. Seventeen fractions obtained were fractionated using analytical thin layer chromatography (TLC). Chromatograms were inspected under UV light at 254 and 366 nm.

The activities were evaluated by the thin layer chromatography agar overlay method. Activity against *Bacillus subtilis* and *Pseudomonas syringae* was detected at different zones on the TLC plates: R_f 0.10–0.70, 0.80, and 0.98 by fractions (4–12). The active compounds were detected by spraying with anisaldehyde/sulphuric acid. The combined fractions (4–12) were evaporated and chromatographed by preparative HPLC using 50/50% methanol in water, a linear gradient for 20 minutes, and having a flow rate of 4.5 mL/min. The compounds were eluted at 14.2 minutes (1, 5.0 mg), 19.2 minutes (2, 0.9 mg), and 15.7 minutes (3, 0.8 mg) in the preparative HPLC. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400MHz using tetramethylsilane (TMS) as an internal chemical shift reference for samples at 30 °C, with chemical shift values of 0.00 ppm in both ^{1}H and ^{13}C NMR spectra.

All NMR data were recorded using $CDCL_3$. The structures of compounds 1–3 were determined by ID ^{1}H selection NOESY and ^{1}H and ^{13}C NMR spectroscopy. The sequence of carbon and hydrogen atoms was settled by two-dimensional heteronuclear multiple bond correlation spectroscopy, and two-dimensional heteronuclear single quantum coherence spectroscopy (HSQC, HMBC). The compounds were stable. Compounds 1, 2, and 3 (spotted on a TLC plate) were detected by deep green, violet, and deep green staining, respectively, with anisaldehyde in sulphuric acid. Compound 1 was identified as 23-hydroxylcolossolactone E. Compounds 2 and 3 were identified as colossolactone B and E, respectively. Antimicrobial activity of compounds was performed with Gram-positive and Gram-negative bacteria by the thin layer chromatography agar overlay method. Aliquots (1.6–8.0 μg) of compounds were spotted on the TLC plates and eluted with chloroform:methanol (9:1). Seeded medium (50 mL) was evenly spread on the TLC plates. Chloranphenicol was used as the positive control. Plates were incubated overnight at 37 °C, after which ρ-iodonitrotetrazolium violet solution was added. The results showed that colossolactone E and 23-hydroxycolossolactone E were active against *Bacillus subtilis* and *Pseudomonas syringae*. Colossolactone B was not active against the bacteria. The activities of Colossolactone E and 23-hydroxylcolossolactone E were stronger against *B. subtilis* than *P. syringae*. The zones of inhibition of bacteria by 23-hydroxylcolossolactone E were found to be similar to those of chloramphenicol.

As yet, there is no publication on the use of isolates of *Ganoderma* from Nigeria in the treatment of infections, despite its well-documented medicinal uses in Asia. Nigeria is a country where immune-suppressive diseases, such as HIV, are endemic, and the availability of antibacterial products that could be sustainably harvested could be significant to poor rural communities.

Mycomedicine and Ethnomycology: The Nigerian Experience

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There is a dearth of information in the scientific literature on the indigenous uses of mushrooms in Nigeria. There is also a poor documentation of ethnoknowledge among our people, which is still contributing to a loss of vital information, on almost a daily basis. This paper, therefore, sheds light on the current status of mycomedicine and ethnomycololgy of mushrooms in Nigeria. It also emphasizes the need for documenting indigenous knowledge and the empirical confirmation of our local people's claims about the therapeutic effects of mushrooms in our environment. The authors stress the need for progress in mycomedicine if serious attention is given to eliciting the vital information from rural community dwellers, where reliance on natural materials for treatment of ailments is still much observed.

Pertinent questions that may reveal the relevance of ethnomycology include: How fast can we progress in mycomedicine without ethnomycology? How much has ethnomycology contributed to the present status of mycomedicine? How do we gather basic information on medicinal mushrooms? Is it not more cost-effective to seek indigenous knowledge on the uses of mushrooms, along with the legends with which they are associated (ethnomycology), than to study the medicinal relevance of all the mushrooms in the wild?

Mycological concepts and their applications are also much observed in Nigerian cultures. Many proverbs and other sayings involve mushrooms and show age-long interactions with mushrooms. Documentation of ethnomycological and ethnomycomedicinal information is being advocated to encompass all the fungi used in one way or another by a particular culture.

Another aspect of this presentation includes soil fertility information, based on the ethnoknowledge of a particular tribe in Nigeria, and how this can be used to educate the custodians of this knowledge on soil fertility involving mushrooms. Ethnoknowledge among the diverse Nigerian cultures also includes the use of fungi in food and drink fermentation. Notable among such foods are "yan olude" from maize grains, "pito" from the grains of *Sorghum bicolor*, and "shekete" from plantain (*Musa parasidiaca*). Out of the three fermented products, the one that involves direct use of culture of organisms is pito. Much effort is being made to preserve the culture at different stages of the preparation of the drink. It is also important to note that some of the ethnomycological practices cannot be explained by science, while quite a number of them can be confirmed and improved upon in the laboratory.

In addition, many precautions observed by the indigenous people can certainly be explained by science. A good reference point is the observation by the indigenous cultures in southern Nigeria that fermented drinks would "spoil" if exposed to orange fruits or even to the peels. Orange peels are usually kept at quite an appreciable distance from such fermented drinks, during and after preparation.

Furthermore, the dregs from palm wine are usually reserved for newly married young men. This is because the dregs are believed to contain "something" that will energize the drinker before and after meeting his wife. The size, behavior, and habitat of mushrooms are used by many witch doctors as tools for impoverishing or favoring their adversaries. For example, "Olu oron" (*Termitomyces microcarpus* (Berk. et Br.) R.Heim) is used as an instrument for reducing the status of their enemies because of its small size. Hence, the incantation that accompanies the concoction refers to the fact that 200 sporocarps of the mushroom are not enough for a pot of soup.

On the other hand, the sclerotium of *Pleurotus tuberregium* (Rumph.:Fr.) Singer is a major ingredient in a concoction that is used for effecting a bumper harvest. This is done by some cocoa (*Theobroma cacao*) and yam (*Dioscorea* sp.) farmers in the southern part of Nigeria. The mode of preparation and the accompanying incantations of these concoctions leave no doubt that some unseen force/powers are at work. One can't just discard these claims and practices as unscientific, because the positive and negative effects of these indigenous practices evoke regard, dislike, and fear (as the case may be) among the general populace.

Finally, the paper highlights the need to put more effort into the documentation of indigenous knowledge about edible and medicinal mushrooms.

Cultivation of *Lentinus squarrosulus* (Mont.) Singer on Sawdust of Selected Tropical Tree Species

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Lentinus squarrosulus (Mont.) Singer was cultivated on the sawdust of five economic tropical tree species. The sawdust of *Chlorophora excelsa, Celtis* sp., *Guera cedrata, Nesogordenia papaverifera,* and *Brachystegia nigerica* were collected during processing at the sawmill. The pure culture of the mushroom used in this study was that of strain LS001UBNIG, obtained from the mushroom bank of the Botany Department, University of Benin. The planting spawn of this mushroom was raised on a sorghum-based material. The different sawdust types were then separately sun-dried for several days until the weight became constant. The experiment had two controls. The first set of control comprised individual sawdust types without any supplement, while the second control was done by supplementing each sawdust type with 1% calcium carbonate ($CaCO_3$) and 1% sugar. In one treatment, each sawdust type was supplemented with 1% $CaCO_3$, 1% sugar, and 10% wheat bran. The second treatment was achieved by supplementing each sawdust type with 1% $CaCO_3$, 1% sugar, and 20% wheat bran. The moisture content of each sawdust type was adjusted to 70%. Five replicate bags, each containing 300 g oven-dried weight equivalent of the moistened sawdust were prepared for individual sawdust type. Thereafter, the substrate-filled bags were each covered with cotton wool and steamed for 4 hours on 2 consecutive days.

The pasteurized substrates were then allowed to cool down, and subsequently, were inoculated with

spawn of the mushroom at 5% level of spawning. The mushroom was able to colonize all the different substrate/supplement combinations, except the sawdust of *Celtis* sp., which totally failed to support the growth of the mushroom mycelium at the wheat bran supplementation of 20%. Time for mycelium colonization of the substrate ranged from 7.80 ± 0.49 days in *Celtis* sp., supplemented with 1% sugar, 1% $CaCO_3$, and 1% sugar; to 17.50 ± 6.50 days in the sawdust of *C. excelsa* supplemented with 1% $CaCO_3$, 1% sugar, and 10% wheat bran. The earliest time of primordial emergence was 20.60 ± 0.16 days (on sawdust of *B. nigerica* without any addition of supplements), while the longest time (42.00 ± 1.00 days) of the same parameter was observed on the sawdust of the same tree species supplemented with 20% wheat bran. The highest yield of 16.17 was found when the sawdust was supplemented with 1% $CaCO_3$, 1% sugar, and 10% wheat bran, while the lowest value for the same parameter was found in the sawdust of the same tree species when supplemented with 1% $CaCO_3$, 1% sugar, and 20% wheat bran.

The results of this study show that the mushroom *Lentinus squarrosulus* did not show any particular trend with an increase in the level of supplementation. Rather, in some cases, the yield of the mushroom was reduced following an increase in supplementation. The results, therefore, suggest the need for appropriate balancing of the carbon/nitrogen ratio in order to achieve maximum yield. Also, the study underscores the need for guided use of sawdust types along with the required appropriate supplements.

Commercial Cultivation of Edible Mushrooms in Nigeria

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Situated in the western part of Africa south of the Sahara, Nigeria has a climate that favors the natural growth of mushrooms. In the southern part of Nigeria, the rainfall is high, and this region is predominantly covered by the rainforest ecosystem, making it very favorable for mushrooms to thrive.

In many parts of Nigeria, almost every ethnic group has a traditional knowledge of mushrooms and harvesting them in the wild. However, due to past unpalatable experiences with poisonous mushrooms, people are skeptical about their safety. As a result, the consumption of wild mushrooms is now becoming unpopular except for a few species of *Pleurotus* and *Auricularia* that are so common that their identity cannot be mistaken.

Taking a census of opinions on the love/desire for mushrooms would reveal that Nigerians, indeed, yearn for the commercial production of edible mushrooms so that they can eat them without fear of being poisoned. Several researchers have been working on the growth requirements of some edible species as well as the development of new desired strains. The major hindrance in this pursuit is lack of funds. Facilities for biotechnological studies are lacking, and the government does not seem interested in the cultivation of mushrooms as a good source of nutrients and as curative /preventive agents of many diseases for the ever-increasing population.

One way out of this problem is that researchers should seek collaboration from other countries in which the technology is advanced and well funded so as to speed up research on the cultivation in Nigeria. Government support is also solicited. The nutritive and medicinal values of mushrooms far outweigh any sacrifice for bringing their commercial production to fruition.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Sociocultural and Ethnomycological Uses of Mushrooms Among the Esan People of Nigeria

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Esan is one of the major tribes in Edo State, but it is a minority among the approximately 250 tribes that exists in Nigeria. Different villages and towns of this tribe trace their histories to many other big tribes across the country. However, similar farming practices, eating habits, social norms, and cultural practices, in addition to a common language (Esan) with highly variable dialects within the tribe, are the common factors that make this tribe different from its neighbouring tribes of Ika, Owan, Etsako, and Bini. The use of mushrooms in food, traditional medicine, and other sociocultural practices is reported here.

The Esan word for mushroom is *utun*. Divination is very prevalent among the Esan people, who depend upon it for solving mysteries. People afflicted with diseases and misfortunes that are usually affiliated to punishments from the gods and ancestors go to seek divinations from priests, in order to know what sacrifices will appease the gods. What we found is that many of the diviners use mushrooms to wash and clean their faces so that they can see the future or the cause of their problems. The sclerotium *Pleurotus tuberregium* is the fungus used for this purpose.

In the cultural dance practiced by the Ogwa people, dancers are known to jump to high and dangerous positions and fall to the ground without signs of injuries. This is usually when they are in a trance. A mushroom yet to be identified is a critical ingredient in the preparation they eat before the dancing begins. It is clear now that this mushroom has hallucinogenic properties.

Termites are a major problem among the local people in the rural areas because their wooden homes are usually infested and eaten up by termites. Sclerotium of *P. tuberregium* is used in addition to other herbs to prepare a powdered substance that is spread or sprinkled around the houses during the raining season in order to ward off the termites.

Many mushrooms are used as food by the Esan people. Such mushrooms are usually collected in the wild during the early periods of the rainy season. It is a practice mainly engaged in by women and children. Such mushrooms are used as meat substitute, sold fresh in local markets, or dried for use during the dry seasons when meat becomes very scarce. The elderly women lead the younger women and children in such collections (forays), identifying the edible mushrooms from poisonous ones, from those that are suitable for fresh sales or drying for preservation purposes. Collections of such mushrooms have been made, and so far the following species followed by their local names have been identified: *Pleurotus tuberregium*, *Lentinus squarrosulus* (Asikhia), *Volvariella* sp. (Ameinmen), *Agaricus* sp. (Ojutun), *Auricularia* sp. (Ehor-ofen), *Termitomyces* sp. (Utun-okhan), *Schizophyllum commune* (Ikpekpelu), and *Coprinus* sp. (Utun-uzo). Studies to identify about 13 other mushrooms used for food are in progress.

Sclerotia of *P. tuberregium* are the most widely used for medicinal purposes. The local people usually com-

bine sclerotium with different herbs to treat whooping cough, dysentery, malaria fever, and energy-providing patties for pregnant women and babies suffering from malnutrition. An extract from the combination of sclerotia and the plant *Ocimum gratissimum* L. (a plant in the tea family locally called Alhanmhonkhor) is used to treat ear infection in children.

Ongoing studies on formulations and administration of mushrooms based on traditional medicines and comparison of usage with the practices of others tried in the West African region will be presented. Many mushrooms known today to have medicinal benefits, which arose from observations of their uses in China and Japan, are objects of scientific studies that continue to determine the active components in such mushrooms. Whereas the documentation of this knowledge is important for the understanding of the sociocultural practices of the Esan people, it is equally good for science, because detailed scientific studies of mushroom uses and practices of the Esan people can lead to the discovery of new drugs and nutraceuticals.

Novel Antibacterial Compounds Obtained from Some Edible Mushrooms

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Mushrooms are considered to be natural nutraceuticals and are cultivated for both edible and medicinal purposes. Many edible mushrooms possess enriched proteins and some medicinal properties such as antibacterial, antifungal, antiviral, and anti-AIDs.

Based on both nutritional and medicinal properties, the present study is focused on the antibacterial substances and their efficacy. Antibacterial substances were isolated from culture filtrates, fresh mycelia, and dried fruiting bodies (basidiomata) of an Indian milky mushroom, *Calocybe indica* Purkayastha et A. Chandry (Tricholomataceae), and an oyster mushroom, *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm. (Pleurotaceae). Antimicrobial compounds were extracted with different organic solvents such as acetone, chloroform, ethanol, ethyl acetate, and methanol. The antibacterial activity against some human pathogenic bacteria, such as *Bacillus* spp. *Escherichia coli, Vibrio cholerae,* and *Salmonella thiphi,* was studied using agar diffusion method.

The maximum inhibition was observed in the dried fruiting bodies of *Calocybe indica,* extracted with the solvent ethyl acetate, followed by *Pleurotus ostreatus.* In both cases, two different colored compounds of blue and green were visualized under UV and recorded Rf values of 0.86 and 0.95, respectively, when the compounds were separated by thin layer chromatography.

These compounds could be responsible for pathogen inhibition, and the significance of the principally active compounds is worth future analyses by mass spectrum and nuclear magnetic resonance (NMR).

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

The Growing Effect of Vineyard and Winery Wastes on the Production of Mycelia and Fruit Bodies of Edible and Medicinal Fungi

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It is well known that huge amounts of waste and residue are produced annually during agroindustrial activities, and their utilization could allow self-sustainable processes and products, having a beneficial effect on soil as efficient environmental protection. Many of the lignocellulosic wastes cause serious environmental pollution effects if they are allowed to accumulate in the vineyards, or much worse, to be burned on the soil. At the same time, these wastes could provide an environmentally efficient way to solve the problem by the application of micro-organisms and processes in vine waste treatment and management.

The main aim of this work was to find the best way of recycling vine wastes by using them as a growing source for edible and medicinal mushrooms, in order to extend the food chain in vineyard ecosystems. According to this purpose, two fungal species from the Basidiomycetes group—*Lentinus edodes* (Shiitake) and *Pleurotus ostreatus* (Oyster Mushroom)—have been used to determine the growing effect of lignocellulosic vineyard wastes used as culture composts as well as winery wastes on the production of mycelia and fruit bodies that could be processed and marketed as useful products, such as food and drugs.

The experiments of this research work were achieved by growing all these fungal species in special culture rooms, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies. During the experiments, the effects of culture compost composition (carbon, nitrogen, and mineral sources) as well as other physical and chemical factors (such as temperature, inoculum size, CO_2 and O_2 concentration, air humidity, watering, light intensity, incubation time) on mycelial net formation and, especially, on fruit body induction were investigated.

The registered data revealed that lignocellulosic vine wastes can be used as substrates for mushroom growing only after some mechanical pretreatments to break down the whole lignocellulose structure in order to be more susceptible to fungal enzyme action. All these pretreated lignocellulosic wastes were disinfected by steam sterilization at 120 °C for 60 minutes. The final composition of culture composts was improved by adding grain seeds (wheat, rye, rice), $CaCO_3$, and $NH_4H_2PO_4$, to each kind of culture medium composition, depending on the fungal species used to be grown.

All the culture composts for mushroom growing were inoculated using liquid inoculum, aged 5–7 days, and the volume size ranging between 5% and 7% (v/w). The optimal temperatures for incubation and mycelia growth were maintained between 23 °C and 25 °C. The whole period of mushroom growing from inoculation to fruit body formation lasted between 15 and 35 days, depending on each fungal

species used in experiments. From all these fungal species tested in our experiments, *Pleurotus ostreatus* was registered as the fastest mushroom culture (15–20 days), in comparison with the fungal strains of *Lentinus edodes* (25–30 days).

As control samples for each variant of culture composts used for experimental growth of all these fungal species, we used wood chips of the oak that were kept in water 3 days before the experiments and were then steam sterilized for disinfection. Because of their high content of carbohydrates and nitrogen, the variants of culture composts supplemented with wheat grains at a ratio of 1:10 and rice grains at the ratio 1:5 as well as a water content of 60% were optimal for the fruit body production of *Pleurotus ostreatus* and, respectively, *Lentinus edodes*. In addition, some strains of this fungal species were grown in our experiments on culture substrates made of winery wastes and rye grains at a ratio of 1:7 and a water content of 50%. A higher ratio of rye grains might lead to an increase of total dry weight of the fruit body, but also could induce the formation of smaller fruit bodies than those of the control samples.

The results achieved by using propylene-bag cultures revealed that the composition of culture substrate had significant effects on fruit body production as well as on the characteristic shape of the fruit bodies. The final fruit body production of these fungal species used in experiments was registered between 7 and 10 kg, relative to 100 kg of compost.

Medicinal Mushrooms: A New Source for Breast Cancer Therapeutics

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It is known that advanced breast cancers do not respond well to chemotherapy, and their gene expression arouses uncontrolled growth. Although ER-positive breast cancers respond to hormonal therapy, the treatment of ER-negative cancers is more complicated. Despite the positive results of most of the chemotherapeutic regimes, cellular adaptations have enabled tumor cells to evade many of the chemotherapeutic drugs. One of these cellular chemoresistance factors is the transcription factor nuclear factor-kappa B (NF-κB). NF-κB can be such a target in breast cancer treatment, and its removal by an inhibitor can reverse the specific antiapoptosis of cancer cells. Therefore, NF-κB was selected as the main target in the present study.

Recently, the application of low-molecular-weight compounds with fungal origin as natural therapeutics in cancer treatment has been gaining more attention. There are studies demonstrating that these biologically active fungal substances could be used as a novel pharmaceutical source in breast cancer therapy (Petrova et al., 2005). For

instance, the caffeic acid phenethyl ester (CAPE), which specifically inhibits DNA binding of NF-κB and showed some promising results in human breast cancer MCF-7 cells, is found to be produced by *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinus edodes* (Berk.) Singer, and *Phellinus linteus* (Berk. et M.A. Curtis) Teng (Mattila et al., 2001; Nakamura et al., 2003). Low-molecular-weight compounds isolated from fruit bodies and spore extracts of *Ganoderma lucidum* (W.Curt.: Fr.) P. Karst. also exhibited breast cancer and, more specifically, NF-κB inhibitory activity (Sliva et al., 2002; Sliva, 2003; Jiang et al., 2004).

Following these perspectives, we started a screening program of 75 strains of 67 species, kept in the Culture Collection of the Institute of Evolution, University of Haifa (HAI), belonging to different taxonomical and ecological groups. Mycelia were grown in submerged conditions for biomass production. Three organic solvents with different polarities (ethyl alcohol, ethyl acetate, and diethyl ether) were used for dry biomass extraction in order to isolate fungal secondary metabolites. Moreover, the culture broth, left after the mycelia filtration, was also extracted with ethyl acetate in order to receive bioactive compounds produced in the media during fungal growth.

Our aim was to isolate some bioactive low-molecular-weight compounds from mycelia and culture broth in order to investigate their potential NF-κB inhibitory effect in human breast cancer. For this purpose in the present study, different types of breast cancer cell lines that express a reporter gene (luciferase) under the control of NF-κB responsive element, were examined. Using these cell lines we tested the ability of our fungal extracts to affect the reporter activity. Positive extracts were further evaluated for their effect on the expression and function of NF-κB using Western blotting.

In order to determine the chemical structure of the active substances, all extracts that show desired activity were subjected to chemical fractionation.

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Poria cocos (Schwein.) F.A. Wolf in Japanese Traditional Herbal Medicines: Insights from Kampo Case Studies and Implications for Contemporary Research

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The mushroom *Poria cocos* (Polyporaceae) is used in approximately 30% of all Kampo (traditional Japanese herbal medicine) formulas. It consists of several pharmacologically active ingredients, including the vitamin D precursor ergosterol and the triterpene pachymic acid.

In Kampo, *P. cocos* is known as bukuryo and is only used in combination with other herbs. To date, little research has been done on this mushroom in isolation. However, extracts have demonstrated the inhibition of tumor promotion, inflammation, induction, and red cell lysis by reactive oxygen species. In clinical use, poria is part of the multi-herb formulas used for enhancing immunocompetence; in treating *suidoku* (traditional Kampo medicine diagnosis, which roughly translates into "water overload")and various women's health issues, and has many more indications. Currently, poria is part of a United States Food and Drug Administration (FDA)-approved trial of a non-estrogenic multi-herb formula for the management of menopausal hot flashes.

In Japan, 148 Kampo formulas are prescribed by physicians on a regular basis. These are pharmaceutical grade, government-regulated prescription medicines that medical students are taught to prescribe and that are covered by the National Health Service. Some poria-containing formulas are more than 1800 years old. Significant clinical experience and preclinical research exists on the dozens of *Poria*-containing formulas that are prescribed today.

Poria cocos, as a formula ingredient, is not from a fruiting body but is actually cut from the mycelial mass that grows in the ground under certain species of pine in Japan and China. The harvested mycelial mass is wet and soft; the form used in Kampo herbal medicines is dry and hard. Traditionally, poria was cut into small (<1 cm^3) cubes and set in the sun to dry. This treatment, by necessity, would expose the large surface area of the mushroom mycelia to solar UV-B radiation, which in turn would transform the ergosterol to ergocalciferol—vitamin D_2.

One of Kampo's most famous practitioners, Keisetsu Otsuka, in 1965 published *Kampo Shinryou San Ju Nen,* which summarized 374 representative cases from his practice in the years 1931–1959. This presentation will share two translated case studies in which two *Poria cocos*-containing formulas (*yoku-kan-san* and *hachi-miji-ou-gan*) were prescribed with remarkable success. These cases will be analyzed from the perspective of contemporary scientific and medical knowledge to argue that vitamin D from sun-dried poria was the active ingredient most responsible for the patients' recovery. Implications for both the interpretation of historical use and the generation of contemporary research will be discussed.

This presentation will argue that *Poria cocos* deserves greater attention as an agent for promoting human health. Certainly *P. cocos*, like other medicinal mushrooms, can be a significant source of vitamin D. In addition, many mushroom triterpenes are also pharmacologically active, and *P. cocos* documented actions are not all attributable to vitamin D.

Furthermore, *P. cocos* is certainly safe for human ingestion. Single-dose toxicity testing demonstrated that the LD_{50} of *P. cocos* was more than 50 g/kg, p.o. in mice. Repeated-dose toxicity testing, 5 g/kg or 10 g/kg orally administered to rats once daily for 14 days, demonstrated no changes in general symptoms, food consumption, water ingestion, and body weight. Spontaneous locomotor activity was suppressed on the 9th and 10th days. In the hematological tests, BUN was affected at 10 g/kg (*Tox Sci,* 1998, **23**, 229–233). In addition, *P. cocos* appears to be quite safe for long-term use. The Ames test as well as micronucleus and chromosomal aberration assays, used for carcinogenicity testing, were all negative (Yin X. J. et al., 1991. *Mut Res*, **260**, 73–82).

The Light Factor in Biotechnology Cultivation of Medicinal Mushrooms

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It has been proven that light is one of the main morphogenetic factors for growth and development of many cultivated mushrooms. However, the mechanism of photoreception in mycelium and fruiting bodies has not been sufficiently studied. Today the light of the visible part of the spectrum is used in technologies of submerged cultivation of filamentous fungi. It has been shown that light with wavelengths of 650 nm and 530 nm has essential influence on the growth regulators and activity on growth processes of these mushrooms and has modified the lipid and carbohydrate spore composition. The light-induced modifications had prolonged action and were transmitted to the following ontogenetic stages, from spores to mycelium. Infrared rays in definite doses cause the intensification of growth in culture of medicinal mushrooms *Coriolus vaporarius* (Pers.:Fr.) Bond. et Singer and *Serpula lacrimans* (Wulf. apud. Jacq.:Fr.) Schroet. The absence or existence of light influence during the period of vegetative mycelial growth affects the character of further bearing.

Laser techniques have also found practical application in biotechnology and are one of the most dynamically developing fields of investigation. The possible directed influence of laser radiation on intracellular processes and biosynthesis regulation is due to the selective influence of monochromatic light on the photosensitive structures, photoreceptors, and intracellular processes in microorganisms. One advantage of laser radiation is the chance to create high light brightness in a narrow range of wavelengths, not achievable with usual noncoherent light sources. Such properties allow us to investigate the possible realization of highly effective biotechnologies for obtaining microbial cultures with high biological activity. At the same time, the practical use of monochromatic light in biotechnological

processes is limited by the absence of information precisely determining mechanisms of light action, effective wavelengths, and irradiation modes.

Our preliminary investigations proved the stimulating effect of light in a visual part of the spectrum on the growth and development of some species of medicinal mushrooms (Poyedinok et al., 2002, 2003, 2004). We investigated the effect of helium-neon and argon laser irradiation on *Pleurotus ostreatus* (Jacq.:Fr.) P.Kumm., *Lentinus edodes* (Berk.) Singer, and *Hericium erinaceus* (Bull.:Fr.) Pers. production and determined that laser treatment accelerated mycelial growth, shortened the phases of mushroom development, produced more vigorous mycelium, and increased fruit body yields (36–51%). The activation of sowing mycelium by means of red light irradiation makes it possible to reduce the dose necessary for substrate inoculation by twofold. It was shown that laser irradiation also stimulated the mycelial growth in submerged culture and enlarged the accumulation of biomass twofold.

The influence of low-intensity laser light on antibiotic activity of *Pleurotus ostreatus* under submerged cultivation was investigated. The irradiation was carried out in two regimens: as continuous and intermittent light. Exposure doses were identical in both variants of experience. The irradiated mycelium was used as an inoculum in submerged cultivation. The antibiotic activity of cultural liquid was analyzed with the method of diffusion into agar medium. Eleven test organisms were used: *Staphyloccocus aureus, Bacillus mycoides, B. pumilis, Leuconostoc mesenteroides, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Commamonas terrigena, Saccharomyces cerevisiae, Candida ablicans,* and *Aspergillus niger.* The antibiotic activity of mycelial and culture broth extracts against *Micrococcus luteus, Staphyloccocus aureus,* and *Bacillus mycoides* was increased by 10–20%.

Modern Taxonomy and Medicinal Value of the *Flammulina* Mushrooms

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Flammulina mushrooms have an important place among Basidiomycetes as well as being useful in the production of medicines. In China, Japan, and other oriental countries these mushrooms (trivial name: enoki-take) have been cultivated traditionally since ancient times because of their nutritional and therapeutic value. Nowadays, they are among the six most actively cultivated mushroom species in the world. During the last decade of the last century, world production of *Flammulina* constantly increased. By the end of the 20th century, its production was over 300,000 tons per year.

In nature *Flammulina* is a cosmopolite inhabiting deciduous forests during cold seasons. It can survive even light freezing temperatures and, therefore, it has been named "winter mushroom." These mushrooms can be easily cultured on various media. Good growth and fruiting in culture as well as the capacity for biosynthesis of different biologically active metabolites make *Flammulina* cultures very popular for study.

Before the 1980s this genus was represented by only one species—*Flammulina velutipes* (W.Curt.: Fr.) Singer—although many authors pointed out varieties and forms of the species. In 1983, Bas made the first taxonomical record of *Flammulina* for Western Europe (Bas, 1983). He distinguished three species: *F. velutipes*, including two varieties—var. *velutipes* with f. *velutipes* and f. *longispora* Bas, and var. *lacteal* (Quél.) Bas; *F. ononidis* Arnolds; and *F. fennae* Bas. The validity of the species was later supported by the mating study of monokaryon isolates (Lamoure, 1989).

Nevertheless, study of numerous *Flammulina* specimens from different geographical regions showed that this genus needed more detailed investigation. In the late 1990s, a Mexican species, *F. mexicana* Redhead et al. (Redhead et al., 2000), and a New Zealand species, *F. stratosa* Redhead et al. (Redhead et al., 1998), were described. The taxonomy, biology, and distribution of taxa in *Flammulina* were investigated by Redhead and Petersen (1999). They showed the complexity of relationships among the taxa. After detailed examination of many specimens, the authors proposed two new species epithets and one varietal name—*F. populicola* Redhead et Petersen, *F. rossica* Redhead et Petersen, and *F. velutipes* var. *lupinicola* Redhead et Petersen—as well as two new combinations, *F. elastica* (Lasch) Redhead et Petersen and *F. elastica* f. *longispora* (Bas) Redhead et Petersen.

Genetic and molecular studies were carried out to support the described taxa. The mating study showed that *F. mexicana, F. stratosa, F. populicola,* and *F. fennae* were genetically isolated taxa. *F. velutipes* could be separated from the other species, but its intraspecific taxa (vars. *velutipes, lactea,* and *lupinicola*) could not be distinguished. They were also partially compatible with those of *F. ononidis*. Similarly, monokaryon isolates of *F. rossica* and *F. elastica* were partially compatible with one another but incompatible with those of other taxa. Low levels of interspecific hybridization were noted between *F. velutipes* and *F. populicola* and between *F. velutipes* and *F. rossica/elastica* (Petersen et al., 1999). These data were supported by the parsimony analysis of rDNA (ITS1-5.8S-ITS2 region) sequences, wherein the main clades were in agreement with defined mating groups and showed the phylogenetic relationships between the species (Hughes et al., 1999). Isozyme analysis also could help to separate *F. velutipes* cultures from *F. rossica/elastica*, *F. ononidid,* and *F. fennae* (Alekhina et al., 2001).

Using these means, the modern molecular and genetic methods allow us to distinguish not only *Flammulina* specimens, but also cultures maintained in culture collections. This is most important considering the possibility of using *Flammulina* cultures in medicine. Much physiological and biochemical research has been carried out on *Flammulina* cultures all over the world since 1950s. Before this, only one species, *F. velutipes,* was found in the literature.

During recent years field trips, several new cultures of *F. velutipes* and *F. fennae* were isolated. Mating study and DNA analysis confirmed species names. The new strains were included in the LE (BIN) Culture Collection.

Much physiological and biochemical research has been carried out all over the world since 1950. Research had mainly been done on one species: *F. velutipes*. Literary data showed that *F. velutipes* obtained various biologically active compounds. Antibiotic properties and different enzymes such as proteinase, ribonuclease, phosphodiesterase, chitinase, acyl-KoA-synthetase, laccase, hydrolase complexes, hypocholesterol, and blood sugar had decreasing effects. Polysaccharides with antitumor activity were studied using *Flammulina* cultures. In Japan these mushrooms were used in making healthful beverage preparations (Psurtseva, 1987).

Study of *Flammulina* cultures from the Komarov Botanical Institute Basidiomycetes Culture Collection showed that the biosynthesis of proteolytic enzymes with thrombolytic and fibrinolytic activity was one of the specific characteristics. Strain producers of proteinase were selected. Methods of surface and submerged fermentations for exoenzymes were developed (Psurtseva and Mnoukhina, 1996 a,b).

Cultural study using morphological, physiological, and biochemical methods helped to verify *Flammulina* cultures from the LE (BIN) Culture Collection as *F. velutipes* var. *velutipes* f. *velutipes* and f. *longispora, F. velutipes* var. *lactea*, *F. ononidis* and *F. fennae* (Psurtseva and Mnoukhina, 1998). For biological species determination, the cultures were cultivated for fruit bodies and monokaryons were obtained. The

mating study allowed the distinguishing of *F. velutipes* strains from *F. rossica/elastica* complex (Petersen et al., 1999; Psurtseva and Petersen, 2000). Taxonomic verification following up-to-date nomenclature was done on *Flammulina* strains maintained in the LE (BIN) Culture Collection. These strains could be of considerable interest for medicinal investigation.

ACKNOWLEDGMENT

This research was supported by Hesler Endowment Fund (The University of Tennessee, USA) in 1996, RFFI (Russian Fund of Fundamental Researchers) grant 03-04-49604 and INTAS grant 03-51-5889.

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Antimicrobial Azaphilones from the Xylariaceous Inedible Mushrooms

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In the course of our investigation of biologically active substances from inedible mushrooms, we studied the chemical constituents of six species belonging to the Xylariaceae family (Sardariomycetideae) and isolated various types of azaphilone derivatives, which showed moderate to strong antimicrobial activity, such as entonaemins A–C (1–3), together with (+)-mitorubrinol acetate (4), (+)-mitorubrin (5), (+)-mitorubrinol (6), and mitorubrinic acid (7) from *Entonaema splendens* (Hashimoto T. and Asakawa, 1998), daldinin C (8), daldinin E, F (9, 10) from *Hypoxylon fuscum* (Quang et al., 2004a), cohaerins A,B (11, 12) from *H. cohaerens,* rubiginosin A–C (13–15) from *H. rubiginosum* (Quang et al., 2004b), multiformins A–D (16–19) from *H. multiforme,* and sassafrins A–D (20–23) from *Creospharea sassafras.* Their absolute structures were elucidated by 2D NMR, MS, IR, UV, CD spectra and chemical reaction (see figures on following page).

The in vitro antimicrobial activities of isolated azaphilones (1–23) at a dose of 50 μg per paper disc were tested against a panel of laboratory control strains belonging to the American Type Culture Collection, Maryland, USA: *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enteritidis, Escherichia coli* 95, and fungal organisms *Aspergillus niger* and *Candida albicans.* The disc diffusion method, according to the NCCLS (National Committee for Clinical Laboratory Standards—Performance standards for antimicrobial disk susceptibility testing; 6th International Supplement, Wayne Pa. 1997: M2-A6), was employed for the determination of the antimicrobial activity of the compounds. Table 1 (see next page) summarizes the antimicrobial properties of these azaphilones.

Accordingly, moderate to strong activity was observed against all tested strains. These effects, however, appear to be nonselective, because neither fungi nor bacteria remained unaffected by any of the azaphilone. They confirm previously reported bioactivities for other azaphilones and point toward the role of azaphilones as defense metabolites that may protect the stromata of Xylariaceae against feeding enemies or colonizing microbes.

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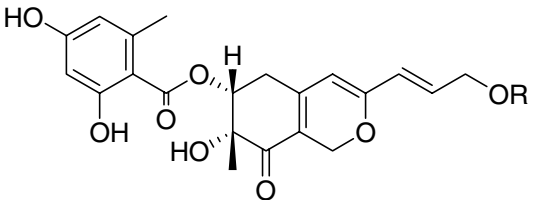

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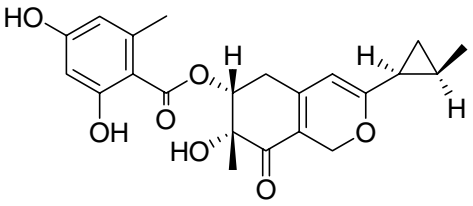

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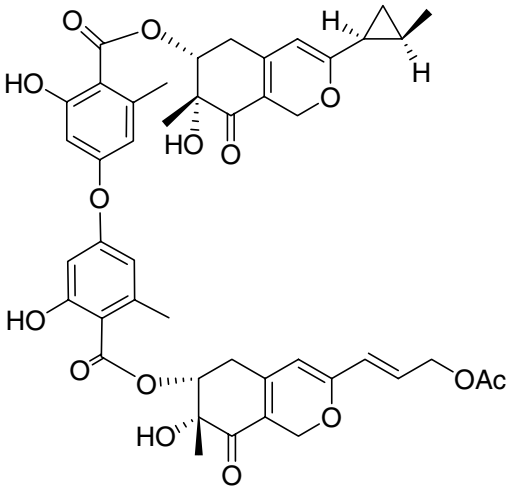

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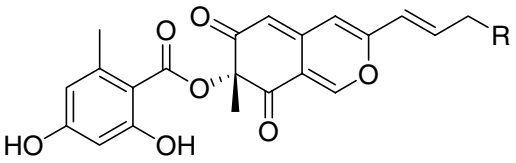

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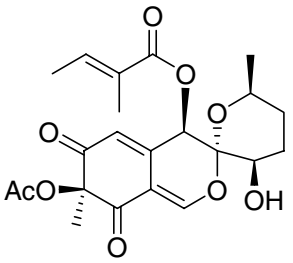
7. R = COOH



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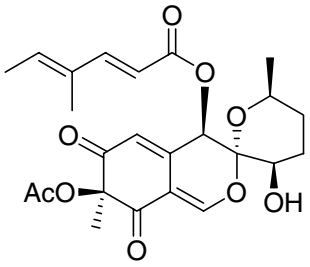

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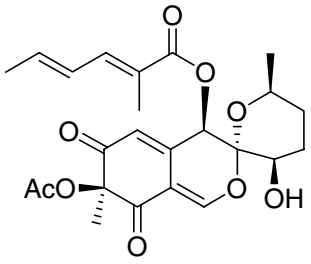

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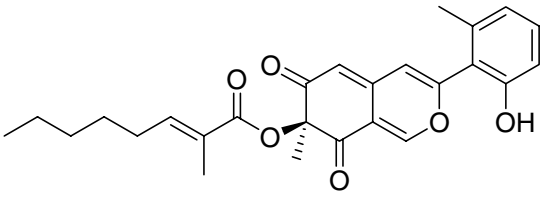

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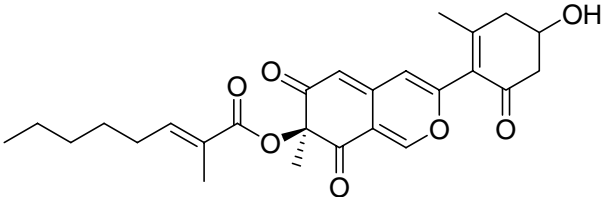

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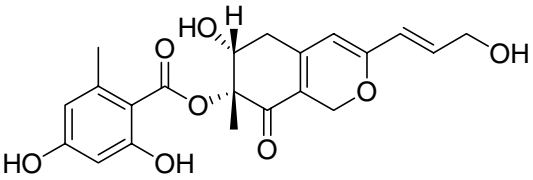

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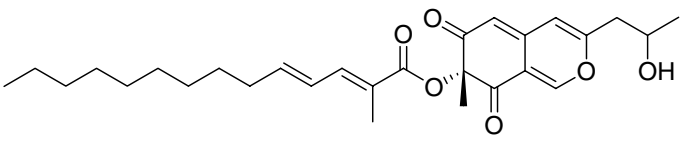

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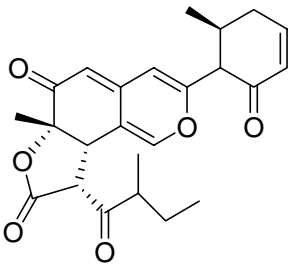

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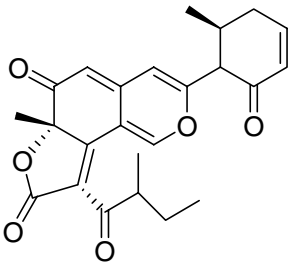

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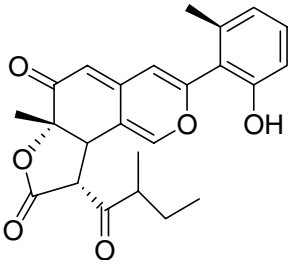

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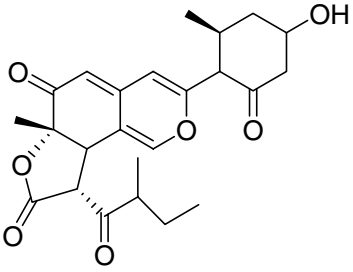

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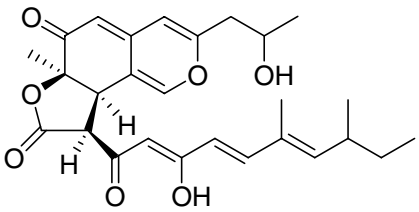

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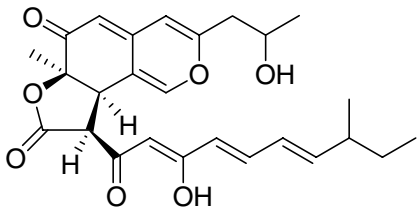

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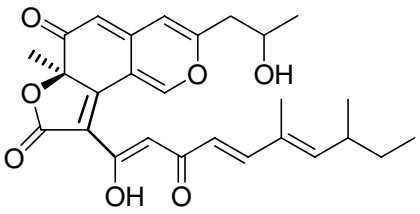

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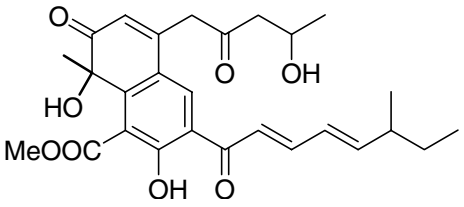

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Table 1. Antimicrobial Activity of Selected Azaphilones (1-23) (Diameter of the Zone of Growth Inhibition, Bactericidal, or Fungicidal Zone in mm).

Microorganism / Sample	*S. aureus*	*P. aeruginosa*	*K. pneumoniae*	*S. enteritidis*	*E. coli*	*A. niger*	*C. albicans*
Entonaemins A (1)	18	7	13	7	8	14	14
DaldininC (8)	7	7	8	8	7	14	15
Daldinin E (9)	8	13	13	8	13	17	16
Daldinin F (10)	14	7	7	7	7	16	15
Rubiginosin A (13)	7	7	9	8	13	15	15
Rubiginosin B (14)	13	16	15	15	19	16	16
Rubiginosin C (15)	17	17	20	19	18	20	18
Multiformin A (16)	17	0 (+14)	18	0	0	0 (+19)	0 (+16)
Multiformin B (17)	18	19	20	19	19	18	19
Multiformin C (18)	20	18	18	16	18	17	17
Multiformin D (19)	18	0 (+17)	16	0 (+16)	0 (+15)	0	0 (+20)
Sassafrin A (20)	0 (+15)	18	20	20	20	20	19
Sassafrin B (21)	19	19	20	21	14	20	19
Sassafrin C (22)	22	22	22	20	22	19	18
Sassafrin D (23)	17	19	17	17	19	18	17

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Dissemination of Case Studies on Ganotherapy

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The treatment of various ailments through Ganotherapy has been initiated very recently in India and is now under experiment. However, the experiment has found some advances in the treatment of various ailments, which has been revealed through observations of different patients' ages ranging from 5 to 65 years. The time span of the ailments from which the patients suffered was 1.5 to 13 years. The purpose of the observation was to evaluate the effectiveness of the prescribed *Ganoderma* spp. capsules. The efforts were intended for promoting alternative medication for curing and maintaining health and at the same time creating social acceptance, adaptation, and affordability of this treatment among a greater segment of people.

During the study period, there were 20 different patients closely monitored after ingesting 500 mg capsules in various doses. All the cases were documented in a systematic way. The patients were suffering from low blood pressure (1), asthma (4), liver and stomach problem (1), obesity and health maintaining (1), prostate cancer (1), diabetes (1), paralysis (1), uterus tumor (1), gangrene (1), blood dysentery (1), respiratory and weakness problem (1), gastritis and gout (1), sinusitis and teeth problem (1), piles (1), rheumatic fever and brain disorder (1), kidney problem (1), and gynecological unwanted pains (1). According to our observations, it has been found that 80% of the patients were cured and are now leading a better quality of daily life.

It may be urged and stressed that a wider scope of clinical research work on Ganotherapy in the country is needed. International collaborative research and development activity is encouraged and welcomed on an urgent basis to continue clinical research.

Biotechnological Potential of Mushrooms: Drugs and Dye Production

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Mushrooms are higher basidiomycetous fungi of immense biotechnological importance. Most mushrooms represent an unlimited source of secondary metabolites of high medicinal value. Since ancient times, mushrooms have been valued as an edible and medicinal resource by tribal and local people of India. The large numbers of biologically active molecules have been identified in many mushroom species in India and all over the world. The search for new medicinal antibiotic substances from higher Basiodimycetes and the study of the medicinal value of these wild edible and medicinal mushrooms has become a matter of great interest in India. These naturally occurring biologically active antibiotic substances can be obtained from the mushrooms.

There is a wide range of bioactivity in mushrooms, which include antitumor activity of polysaccharides; antimicrobial and antiparasitic effects; antibiotics; antidiabetic, anti-hyperlipidemic, and antiaging benefits. They also can be used in the treatment of hepatitis B. We evaluated 17 mushrooms for antimicrobial activity. Most of them belonged to the group Polyporales, which showed remarkable activity against human pathogenic bacteria and fungi. The tests were carried out with hot water extracts of the mushrooms. The disc diffusion technique was used for the detection of activity. Results indicated that these mushrooms could be used for the preparation of antimicrobial agents.

Natural and ecofriendly organic dyes are very useful as coloring agents. These dyes are found in naturally collected or artificially growing fruit bodies, pure culture mycelia, and culture filtrate of mushrooms. There is now renewed demand for natural organic dyes from national and international markets because they provide better quality dyes for food, medicines, and cosmetics. The textile industry has also accepted natural dyes because they produce permanent color without damaging the fiber, thereby yielding more profitable product. These dyes are also used for making paints. And the remains of mushroom are used to make paper.

We have screened mushrooms from Amravati and its suburbs for dye production. The main aim of the present study was to screen the mushrooms of the Amravati region for antimicrobial activity against human pathogenic bacteria and fungi and also for dye production, which can be used as natural and ecofriendly dyes.

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Edible Tribal Mushroom Resources of Central India and Their Ethnological Aspects

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Madhya Pradesh and Chhattisgarh, two Central Indian states, have an area of 443,400 sq km, the bulk of which lies between latitudes 21° and 25° N and longitudes 74° and 84° E with a forest area of 15447 km^2—i.e., about 37.84% of the total geographical area of the states. The region has the largest concentration of tribal population and forest dwellers, forming about one-fourth of the population of the states. Tribal members subsist to a great extent on nontraditional food sources, especially wild edible mushrooms, which grow abundantly during monsoon and post-monsoon seasons and are sometimes collected in large quantities for sale in urban markets.

A study conducted during 1997–2000 in tribal markets of 120 tribal localities of only five districts of two states yielded 53 edible mushrooms belonging to four orders, 11 families, and 18 genera of Basidiomycetes. They are mostly symbiotic (ectomycorrhizal), and few are saprotrophic or parasitic. Family Russulaceae dominated the mushroom flora represented by two genera—*Russula* with 23 species and *Lactarius* with nine species. Other prominent mushroom genera include *Cantharellus* (2 spp.), *Termitomyces* (2 spp.), and *Pleurotus*, *Lentinus*, *Calvatia*, *Scleroderma, Lycoperdon, Astraeus, Clitocybe*, each with one species. Proximate analysis with reference to protein, fat, carbohydrate, crude fiber, ash and moisture content, and energy value of 22 common edible mushrooms have been determined. Most of them have been found to be on a par with common edible mushrooms in nutritional content, but few, including *Pleurotus florida* Eger (nom. nudum), *Lentinus cladopus* Lev., *Cantharellus* sp., *Clitocybe gibba* (Pers.:Fr.) P.Kumm., etc., showed higher protein and lower fat content. Inquiries regarding ethnological aspects of edible tribal mushrooms were made mainly from tribal heads (*Vaidyas* and *Guniyas*, etc.) through personal contacts and interviews. Information obtained had revealed that many mushrooms, apart from being used for edible purpose, are also used by tribes for different ailments. These aspects will be discussed in the present communication.

Use of Agro-Industrial Waste for Production of Laccase and Manganese Peroxidase from White-Rot Basidiomycetes

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Ligninolytic enzymes have significant potential applications in various industries, including pulp and paper, bioremediation, textile, and laundry. Because the biotechnological applications require large amounts of low-cost enzymes, it is essential to search for powerful producers of enzymes as well as for abundant and inexpensive lignocelluloses wastes. Some of these wastes may contain significant concentrations of soluble carbohydrates and inducers of enzyme synthesis, enabling an efficient production of lignocellulolytic enzymes.

Various agroindustrial byproducts have been used for submerged fermentation of *Cerrena unicolor* (Bull.: Fr.) Murrill and *C. maxima* (Fr.) Ryvarden for ligninolytic enzyme production. Six agroindustrial byproducts were tested in shake flask trails. Wheat bran appeared to be the best growth substrate for fermentation of *C. unicolor,* enabling a very high accumulation of laccase (87.450 IU/L on day 7) in culture liquid. Kiwi, as a growth substrate, supported remarkable secretion of manganese peroxidase (2016 IU/L on day 7). Ethanol production wastes also provided a high yield of laccase, whereas other substrates (banana peels, peanut shells, and cotton stalks) appeared to be rather poor growth substrates for laccase and manganese peroxidase production.

Testing of *C. maxima* as a ligninolytic enzyme producer showed that this fungus is a weaker producer of laccase and peroxidase than *C. unicolor.* However, the supplementation of wheat bran, ethanol production wastes, and kiwi highly stimulated laccase production, which reached 6247 IU/L, 6141 IU/L, and 5569 IU/L, respectively. In contrast to *C. unicolor,* the second tested fungus, *C. maxima*, produced very low titres of manganese peroxidase (9.2–43.7 IU/L) in fermentation of five lignocellulosic substrates, whereas no manganese peroxidase was detected in submerged fermentation of kiwi. The data prove that the composition of lignocellulose substrates appear to determine the type and amount of enzyme produced by the wood-rotting Basidiomycetes.

Subsequently, *C. unicolor* cultivation was performed in a 10-liter fermentor using ethanol production wastes as a growth substrate. In order to maximize fungus growth and enzyme production, the pH was automatically controlled at 5.5 during the fermentation process. Laccase activity began on the second day and gradually increased, peaking on the 11th day (233.015 IU/L). Manganese peroxidase showed two peaks, on day 6 (107 IU/L) and on day 10 (142 IU/L).

Fermentation of *C. unicolor* on wheat bran supported lower laccase levels (185.640 IU/L, 12th day) but a much higher manganese peroxidase yield (6.300 IU/L, 11th day).

Similar to the shake flask trial, alcohol production waste supported quicker laccase production. On the 7th day, alcohol production waste and wheat bran reached 81% and 57% of their maximal laccase concentration, respectively.

We plan to elucidate the specific components in the substrates that affect the kinetics and regulate laccase synthesis. This aspect as well as enhancement of laccase synthesis is crucial for large-scale production. Continuation of yield increase will also include supplementation of inducers as well as further concentration of substrates via batch and fed-batch regimes.

Antimicrobial and Antagonistic Properties of *Ganoderma lucidum* (W.Curt.: Fr.) Lloyd

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Ganoderma lucidum is a Basidiomycetes fungus belonging to the family Ganodermataceae. Several studies have shown that *Ganoderma* spp. are selected as potent sources of antimicrobial compounds. To prove this potential and to show the antagonistic properties of *G. lucidum* against a number of bacteria and fungi, this study was conducted.

Antimicrobial test were carried out of crude extracts recovered from dried powdered *G. lucidum* fruit bodies using solvents with different concentration (25%, 50%, 75%, and 100%) of butanol (BuOH), ethanol (EtOH), methanol (MeOH), and water against bacteria (*Bacillus subtilis* 010 and *Escherichia coli* 002) and fungi (*Aspergillus niger* 3029 and *Trichoderma viride* 4012). Results were obtained after 24 hours for bacterial test organisms and 5 days for fungal test organisms.

The dual culture method was adapted in *in vitro* antagonism of *G. lucidum*. *G. lucidum* against isolates of fungi such as *A. niger* 3029, *Penicillium chrysogenum* 5533, *Rhizopus* oryzae 5011, and *T. viride* were grown in potato dextrose agar (PDA).

Results on antimicrobial test showed that *G. lucidum* extracts recovered using various solvents exhibited a static (inhibitory) effect on both bacteria and fungi. All crude extracts showed strong inhibition against *B. subtilis, E. coli,* and *A. niger.* On the other hand, the least static effect was obtained against *T. viride* by *G. lucidum* extracted using 25% EtOH. Extracts at 100% BuOH yielded the greatest zone of inhibition against test microorganisms. Water, the universal solvent, was shown to be an equally good extractant—*G. lucidum* extracts using water also exhibited antimicrobial activity against all test organisms.

Evaluation of *in vitro* organism of *G. lucidum* against *P. chrysogenum, R. oryzae,* and *T. viride* resulted in the test organisms' inability to produce spores, showing that spore formation was destabilized. On the other hand, *A. niger* and *G. lucidum* were shown to be able to live harmoniously in the same plate.

Further studies on the antimicrobial property of *G. lucidum* on other pathogenic microorganisms and viruses are recommended. Studies on antagonistic property against other fungi, specifically those with a longer mycelial stage—e.g. *Pencillium* spp.—are also recommended. Likewise, purification of β-D-glucans, a water-soluble polyssacharide, to which the antimicrobial porerty is attributed, should also be considered. And, because *G. lucidum* extract is water-soluble, the use of *G. lucidum* as tea can also be recommended.

Evaluation of Growth and Yield of Indian Oyster Mushroom *Pleurotus pulmonarius* (Fr.) Quél. on Three Agricultural Wastes Supplemented with Cottonseed Hulls (CSH) in Uganda

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The main economic activity in Uganda is agriculture, which provides livelihood for over 80% of the country's population. However, the ever rising population on static land resources has led to decline in agricultural productivity. Consequently, there is a decline in household incomes, thus compromising government efforts to fight poverty and food insecurity. To avert this scenario, Ugandan farmers and the Ugandan Government are keen to explore and develop alternative livelihood strategies.

Mushroom cultivation offers a viable solution because currently there is a strong demand for mushrooms, locally and internationally. In order to sustain production to meet this demand, improvement of cultivation practices is paramount. Locally, cottonseed hulls (CSH) is the major substrate commonly used for cultivation, especially in urban areas, where most farmers are located. Supply of this substrate is limited as a result of decline in cotton productivity. There is scarcity of the substrate (CSH) mainly because of its multipurpose use in agricultural communities coupled with high costs of transportation from the remote cotton producing areas. Therefore, it became necessary to evaluate other alternative abundant substrates in whole or in combination with CSH in mushroom production.

Whole CSH (100%), sawdust, soybean husks, and coffee hulls were supplemented with CSH at rates of 0, 10, 20, 30, 40, and 50% were the substrate combinations tested. The substrates were separately characterized physically and chemically to determine their nutritional contents and suitability to support mushroom growth. All substrate combinations were pasteurized by steaming for 3 hours and inoculated with *Pleurotus pulmonarius*. Mycelial colonization rates, mushroom yields, and biological efficiency were determined. Sawdust formulations colonized fastest with the longest cropping cycles, whereas soy-cotton seed (in a ratio of 50:50%) substrate had the highest mushroom yields, amounting to 624.5 g per kg of substrate (weight of substrate computed on an air dry basis) and biological efficiency (BE) of 62.44%. Pure coffee produced the lowest yield of 121.3 g per kg of substrate and a BE of 13.16%.

Through the use of such alternative formulations, Ugandan farmers will maximize mushroom production by use of substrates abundant in their localities. In addition, the amount of CSH used in mushroom production will decrease, offsetting transportation costs and the possibility of pest and disease transmission from one locality to another.

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Immunomodulating Activity of *Bjerkandera* sp.

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Medicinal mushrooms are widely used now as traditional medicinal ingredients for the treatment of various diseases and related health problems. Our studies were devoted to the biological activity of submerged mycelium of wood-rooting mushroom *Bjerkandera* sp. (Hapalopilaceae, Polyporales). Fruit bodies of the mushroom were collected in the forest of the Leningrad region of Russia and introduced into culture. Mycelium was cultivated on the semi-synthetic media containing glucose as a carbon source and peptone as a source of nitrogen.

Immunomodulating activity of aqueous extracts from fruit bodies and submerged mycelium was preliminarily studied in a test for generation of reactive forms of oxygen by the cells of human peripheral blood, using the method of luminal-dependent chemiluminescence. The test was selected on the basis of the supposition about stimulating effect of extracts on phagocytes, because it is known that compounds of a polysaccharide nature boost phagocytosis. Obtained results demonstrated pronounced activity of the extracts from fruit bodies. Mycelia extracts also possess significant activity, but the level of chemiluminescence detected for mycelium was lower than for fruit bodies.

The mitogenic activity of mycelium extracts was studied in the reaction of blast transformation of spleen and thymus cells of CBA strain mice. Fruit body extracts demonstrated high proliferation activity on spleen cells, whereas mycelium extracts were active only in relatively high concentrations. Thymus cells were not influenced by fruit body extracts or by mycelium. From the obtained results we can conclude that the studied preparations from fruit bodies and submerged mycelium of *Bjerkandera* sp. generally stimulate B-lymphocytes and macrophages, causing a significantly lower influence on the functional activity of T-lymphocytes.

The ability of extracts to participate in the synthesis by human blood cells of immunoregulating proteins—cytokines, particularly interleukine-1β and interleukine-8, a key mediator of inflammatory and immune responses of an organism—was studied. The results display a stimulating effect on both proinflammatory cytokines production by peripheral blood cells. On the contrary, there was no influence of extracts detected for the production of interleukine-2.

Interesting results, which were not detected for the extracts of other Basidiomycetes, were obtained during the studies of the influence of extracts of submerged mycelium of *Bjerkandera* sp. on the proliferation of spleen and thymus cells, preliminarily stimulated by concanavaline A. It appears that the addition of high concentrations of extracts was suppressing the proliferation of overactivated T and B cells. The resembling effect was detected during the studies of the influence of preparations on

the production of interleukine-2 by concanavaline A stimulated spleen cells. Low concentrations of extract (less than 6 μg/mL) did not influence the activity of stimulated cells, whereas higher concentrations of extracts sharply decreased the levels of interleukine-2.

The detected ability of extracts of *Bjerkandera* sp. to suppress the activity of overstimulated immunocompetent cells can be useful in the development of preparations for treatment of various disorders of the immune system, such as allergies and autoimmune diseases.

In Vitro Studies on Interactions Between Strains of *Trichoderma* spp. and *Lentinus edodes* (Berk.) Singer Mycelium

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The popularity of shiitake, *Lentinus edodes*, is steadily increasing as more and more people learn about the nutritional value, unique taste, and the medicinal merits of this most valuable edible mushroom.

Although in the Ukraine the establishment of commercial *L. edodes* growing is in its beginning stages, its production is often restricted or hindered by infections through anamorphic fungi of the genus *Trichoderma*, which caused the so-called "green mold epidemics." This green mold disease has resulted in severe crop losses (30–100% in cultures of *Agaricus bisporus* (J.Lge) Imbach) on mushroom farms worldwide in recent years. This is increasingly forcing scientists to elucidate the particular mechanism through which the phenomenon of mycoparasitism and specific host responses might be explained. But data on *Lentinus*/*Trichoderma* interactions are still rare. Among other reasons concerning culture conditions, it became obvious that the degree of crop damage mainly depends on the species of the pathogenic molds and the varying agressivity of their strains (biotypes) as well as on the individual resistance of the breaded shiitake strain.

Therefore, the purpose of this work was to study the antagonistic actions between different *L. edodes* breeding strains and strains of *Trichoderma* spp., which originated from six shiitake farms in the Ukraine, in dual cultures on solid media. The aim was to observe whether there were characteristic patterns of interactions caused by the individual *Trichoderma* isolates and to identify the latter by morphological and molecular methods.

For the characterization of interaction types, each of six *Lentinus edodes* breeding strains from the Ukrainian culture collections have been confronted with 23 *Trichoderma* strains isolated from sawdust blocks showing green mold symptoms. Dual cultures were prepared by transferring a 5 mm disc of *L. edodes* mycelium from an 8-day-old culture to one side of a 90-mm Petri dish containing 15 mL 2% MEA. Plates were incubated at 25 ± 1 °C. After 5 days, a 5 mm disc of *Trichoderma* mycelium from the margin of a 4-day-old culture was placed in an adverse position, 4 cm apart from the *L. edodes* disc. Then, incubation at 25 °C was continued for 28 days.

The same experimental design was used to study the impact of temperature on interaction patterns. Three replicates were made for each combination of the six *L. edodes* and 23 *Trichoderma* strains. As controls, the growth behavior of each of the *L. edodes* and *Trichoderma* strains was examined in single cultures under the conditions mentioned above (three replicates each).

Based on morphological observations and the results of BLAST searches for the ITS1-5.8S-ITS2 rDNA region, 14 out of the 23 *Trichoderma* strains could be identified as *Trichoderma harzianum* Rifai, which represents the anamorphic state of *Hypocrea lixii* Pat. The similarity to strains recorded in GenBank was between 96 and 100%. Four of the isolates could be assigned to *Trichoderma koningii* Oudem. (GenBank similarity 97–99%). One of the isolates belongs to *T. citrinoviride* Bissett (GenBank similarity 100%) or with a similarity of 99% to *T. reesei* E.G. Simmons. The sequence data for the remaining isolate did not allow a closer determination. All identified *Trichoderma* species are known to be associated with the green mold epidemic of commercially grown *Agaricus bisporus* and/or *Lentinus edodes*.

Reproducible antagonistic interaction types exhibiting a relatively constant appearance of colonies were categorized into three main types (IT 1–3) according to the level of their interaction or the aggressivity of the individual *Trichoderma* species:

- IT 1: a high antagonistic action (mycoparasitism) was characterized by strong growth suppression of the shiitake colony immediately after contact with the opponent, leading to complete overgrowth within 4–5 days in the most unfavourable cases. This antagonistic action finally lead to the lysis of host mycelia. Ninety percent of the tested *Trichoderma* strains caused this type of interaction.

- IT 2: medium antagonistic action (passive antagonism) was characterized by restricted growth of shiitake with a distinct, reddish-brown, on average 6 mm broad inhibition zone between the two colonies.

- IT 3: a low antagonistic action was defined by suspended growth of the shiitake colony within the first days. But after 3-4 days the mycelium of *L. edodes* overgrew the mycelia of the *Trichoderma* strains.

These interaction types strongly varied with the applied incubation temperature: growth rates of the opponents or the aggressivity of individual *Trichoderma* strains distinctly changed at lower and higher temperatures (15 °C and 30 °C). This shows that much more knowledge about antagonistic properties of the involved biotypes (different strains with distinguishing interaction behavior belonging to the same species) of both *L. edodes* and *Trichoderma* spp. is needed.

The results clearly suggest that harvested losses in commercially grown shiitake depend on both the production strain used and the individual properties of *Trichoderma* biotypes. Considering the exploding market for shiitake and other cultivable mushrooms, it is a promising aim to elucidate the biodiversity of potential pathogens and to uncover their mode of action. But reliable and easily applicable tools for an accurate identification of *Trichoderma* species or their biotypes are still missing. Moreover, the establishment of an international databank on green mold epidemics would be highly appreciated by commercial mushroom growers and scientists as well.

Antimicrobial Activity of Two Wild Mushrooms, *Clitocybe alexandri* (Gill.) Konrad and *Rhizopogon roseolus* (Corda) T.M. Fries, Collected in Turkey

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During the last decades, mushrooms have been shown to be able to exhibit antimicrobial activity. Two wild mushrooms, *Clitocybe alexandri* (Tricholomataceae) and *Rhizopogon roseolus* (Rhizopogonaceae), collected from the southwest of Turkey and consumed as edible mushrooms by the villagers, were analyzed for their antimicrobial activity by using the disc diffusion method. Alcohol, methanol, ether, water, ethyl acetate, and *n*-hexan extracts from fruit bodies of mushrooms were assayed against twelve microorganisms.

The test microorganisms selected for the antimicrobial activity study were *Bacillus cereus* CM 99, *B. subtilis* ATCC 6683, *Escherichia coli* ATCC 11230, *Proteus vulgaris* ATCC 6997, *Klebsiella pneumoniae* CCM 2318, *Saccharomyces cerevisiae* ATCC 9763, *Pseudomonas fluorescens*, *Micrococcus luteus* ATTC 9341, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimidium* CCM 5445, *Serratia marcescens* CCM 583, and *Staphylococcus aureus* ATCC 6538-P. Disks were saturated with about 20 µL of extracts and placed on an agar surface. Differences in the microbial activity of extracts were observed. Methanol extract obtained from two mushrooms presented significant activity against bacteria *E. coli*, *B. subtilis*, and *E. aerogenes,* as compared with test antibiotics, Novobiocin, Nalidixic acid, and Ampicillin. However, the best antifungal activity was recorded in ethyl acetate extract from *Clitocybe alexandri* against *Candida albicans* (14 mm) and *Saccharomyces cerevisiae* (12 mm). No antimicrobial activity against any microorganisms was recorded in water, ether, and *n*-hexan extract from *Rhizopogon roseolus* in this experimental study. This research has shown that different extracts obtained from two macromycetes have been used *in vitro* to inhibit the growth of some important bacteria and fungi.

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The Selection of Alternative Substrates for Medicinal Mushroom Cultivation

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For the present only *Agaricus bisporus* (J.Lge) Imbach and *Pleurotus ostreatus* (Jacq.: Fr.) P.Kumm. are widely cultivated in the Ukraine. However, interest in *Lentinus* spp., *Ganoderma* spp., and other mushrooms with known medicinal properties has increased. In our investigations, special attention was paid to mushroom strains that performed well on non-wood substrates from agricultural waste.

Using a complex of physiological characteristics, including growth rate of mycelia on agar and liquid media, growth and fructification on different lignocellulosic substrates were selected from some strains of such medicinal mushrooms as *Agrocybe aegerita* (Brig.) Singer, *Auricularia* spp., *Flammulina velutipes* (W. Curt.: Fr.) P. Karst., *Ganoderma lucidum* (W.Curt.: Fr.) P. Karst., *Grifola frodosa* (Dicks.: Fr.) S.F. Gray, *Hericium erinaceus* (Bull.: Fr.) Pers., *Hypsizigus marmoreus* (Peck) Bigel., and *Lentinus edodes* (Berk) Singer. Suitable agar culture media were used, and generation spawn media were found. The maximal growth rate of mycelium (12 mm/day) was observed for a selected strain of *Ganoderma lucidum* on wheat agar medium (pH 6.0) at 28 °C. The mycelium growth rates of other mushrooms did not rise above 8 mm/day.

It is important that optimum-value pH media for growth mycelium of *Hypsizygus marmoreus, Auricularia polytricha,* and *Grifola frondosa* appeared above than 6.5. It should be noted that substrates for cultivation should include calcium carbonate to effectively raise pH. Investigations showed that inoculation of sterilized grain or sawdust with submerged liquid mycelium is the most efficient path of spawn production, especially for slowly growing medicinal mushrooms such as *Grifola frondosa, Hypsizygus marmoreus,* and *Lentinus edodes.*

For small-scale cultivation, different formulas of sawdust and alternative substrates from agricultural wastes, including wheat straw, cornstalks, shive of flax, buck-wheat shell, and husks of sunflower seed (HSS), were used. In accordance with the obtained results, the HSS substrate was one of the most suitable for the cultivation of *Agrocybe aegerita, Auricularia polytricha, Flammulina velutipes, Ganoderma lucidum, Hericium erinaceus, Lentinus edodes,* and *Hypsizygus marmoreus* selected strains as well as for the gourmet Oyster mushrooms, including *P. ostreatus, P. citrinopileatus* Singer, *P. sajorcaju* (Fr.) Singer (=*P. pulmonarius* (Fr.) Quèl.), and *P. djamor* (Fr.) Boedjin. For various combinations of HSS substrate, we received a heavier yield of fruit bodies in comparison with supplemented sawdust (beech) substrates. Our results reveal new perspectives on the development cultivation technology for new Ukrainian mushroom species, not only on sawdust but also on alternative substrates.

Bioconversion of Plant Raw Materials in Value-Added Products by *Lentinus edodes* (Berk.) Singer and *Pleurotus* spp.

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Lentinus edodes and *Pleurotus* spp. were compared for their ability to produce extracellular hydrolytic and oxidative enzymes in solid-state fermentation (SSF) and submerged fermentation (SF) of different lignocellulosic agroindustrial wastes. From received data the following conclusions were drawn.

First, fungi cultivation in identical culture conditions reveals wide differences among both species and strains of the same species. For example, in SSF of tree leaves by fungi of genus *Pleurotus,* endoglucanase activity varied from 0.5 U mL^{-1} (*P. ostreatus* IBB 8) to 26.0 U mL^{-1} (*P. ostreatus* (Jacq.: Fr.)P. Kumm. IBB 2191), whereas xylanase activity varied from 3.1 U mL^{-1} (*P. tuberregium* (Rumph.: Fr.) Singer IBB 624) to 43.7 U mL^{-1} (*P. ostreatus* 2175).

Second, the nature and chemical composition of lignocellulosic material is one of the main factors determining the expression of fungi lignocellulolytic potential as well as the ratio of individual enzymes in lignocellulolytic system. For example, *P. dryinus* (Pers.:Fr.) P.Kumm. IBB 903 maximal laccase activity was equal to 119, 195, 794, and 4103 U/L^{-1} in SF of milled tree leaves, banana, apple, and mandarin peels, respectively.

Third, the effect of lignocellulosic substrate concentration on enzyme synthesis varies in dependence on fungi peculiarities. Whereas *P. dryinus* IBB 903 hydrolytic and oxidative enzyme activity increased by 3–8 times with the elevation of mandarin peels or tree leaves concentration from 1 to 4–6%, in the case of *P. tuberregium* IBB 624 the lowest substrate concentration was sufficient to ensure the maximal production of laccase, cellulase, and xylanase by this mushroom.

Fourth, the data proved that most plant raw materials tested contained sufficient nitrogen concentrations to ensure both mushroom growth and enzyme synthesis. However, depending on fungi and lignocellulosic substrate, supplementation of additional nitrogen sources in the medium effects the yield of lignocellulolytic enzymes. It is worth noting that in most cases only increased fungi growth and biomass in the presence of additional nitrogen accounts for the higher level of laccase activity.

Fifth, supplementing the culture medium by stimulators (xylidine, copper, or manganese) increased the yield of *P. dryinus* IBB 903 ligninolytic enzymes or caused their early and rapid accumulation in culture liquid.

The investigation of fruiting body yield and hydrolytic and oxidative enzyme accumulation during bioconversion of different lignocellulosic wastes by *Pleurotus* spp. and *Lentinus edodes* showed that bio-

logical efficiency reached 86–118% and 81–116%, depending on growth substrate and medium used to soak the substrate.

Cultivation of two strains of *P. ostreatus* on wheat straw and tree leaves as growth substrates clearly showed physiological and biochemical changes taking place during mushroom growth and development on plant raw materials. Mushroom cellulases and xylanase activity appeared to be very low during the vegetative phase of substrate colonization by mycelia. Their activity sharply rose with the appearance of primordia and reached their maximum at a stage of matured fruiting bodies. After fruit bodies were harvested, when the culture was in vegetative phase of development, the activity of these enzymes decreased again. In contrast, mushroom cultures were distinguished by high laccase and MnP activity during substrate colonization. The activity of both enzymes decreased at stages of primordia appearance and fruiting bodies maturation. However, when the culture enters the second vegetative phase (5 days after the fruit bodies were harvested), laccase and MnP activities increased again.

The study of lignocellulolytic enzyme activity of *L. edodes* in mushroom cultivation on wheat straw and tree leaves showed the same regularities that were revealed in cultivation of *Pleurotus* spp. with insignificant variations. The vegetative growth of these edible mushrooms is characterized by comparatively low activity of polysaccharases and high activity of ligninolytic enzymes. During the period of fruiting body formation, when the mushroom growth requires large quantities of plastic materials and energy, the secretion of cellulases and xylanase sharply increases to accelerate polysaccharide hydrolysis. In contrast, ligninolytic enzyme production appears, to some extent, is repressed to prevent the expenditure of constructive materials and energy.

Finally, the data on mushroom enzyme activity show that residual spent substrate may become a cheap source of lignocellulolytic enzymes for several biotechnological applications.

Ability of Selenium Absorption by Mycelia of *Pleurotus eryngii* (DC.:Fr.) Quél., Depending on Selenium Source in Medium

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Pleurotus eryngii is an important edible and medicinal species. Selenium (Se) is a trace element, which at nutritional levels has numerous anticarcinogenic or preventive effects against carcinogen-induced breast, colon, liver, and skin cancer in animals, but at higher levels is known to be toxic.

The aim of this investigation was to study how three different Se sources added to the synthetic medium in specific concentrations influenced the ability of the mycelia to absorb this microelement.

Eight investigated strains of *P. eryngii* var. *eryngii* (code numbers 193; 201; 356; 507; 616; 711; 716; 728) and one *P. eryngii* var. *tingitanus* (code number 555) were taken from the Culture Collection of the Institute of Evolution, University of Haifa, Israel (HAI). Se was used in forms of sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), and selenium dioxide (SeO_2) in the following concentrations: 0.3 mg/L, 0.7 mg/L, 1 mg/L, and 1.3 mg/L. Se concentration in mycelia was measured by graphite furnace Atomic Absorption Spectrometer (VARIAN, Australia).

Na_2SeO_3 was a good Se source for the absorption by mycelia and for the incorporation in Se compounds in the cell in all investigated strains. The increase of Se concentration in the medium led to increase of its content in mycelia also, except in strain HAI 201.

In investigated strains of *P. eryngii* var. *eryngii,* Se concentration in mycelia increased with its addition to the medium, when Se was added in the form of Na_2SeO_4, except in HAI 201 and 507, where Se content in mycelia decreased compared to the control in Se concentration of 0.3 mg/L, whereas in the presence of higher Se concentrations it increased. Strain HAI 711 proved to be the best Se absorber (725 μg/g of dry weight at 1 mg/L, and 575 μg/g of dry weight at 1.3 mg/L of Se concentration in the medium). In *P. eryngii* var. *tingitanus,* not only was decrease of Se concentration in mycelia noted compared to the control, but also its total absence, when Se was present in medium in concentration of 0.7 mg/L.

SeO_2 as well as Na_2SeO_3 were shown as good Se sources for its absorption and retention by mycelia of investigated strains. They easily absorbed Se from medium where it was presented in concentration of 1.3 mg/L, except strain HAI 201 where the highest concentration of absorbed Se was in concentration of 0.7 mg/L in the medium.

Among investigated Se sources, Na_2SeO_3 appeared to be the most favorable, while Na_2SeO_4 was the least favorable source for Se absorption by mycelium in majority of studied strains.

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Influence of Selected Microelements on the Laccase and Peroxidases Production by *Pleurotus eryngii* (DC.: Fr.) Quél. in Submerged Cultures

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Pleurotus eryngii var. *eryngii* is a white-rot fungus because of its ability to produce extracellular ligninolytic enzymes laccase (Lac), two peroxidases (Mn dependent peroxidase, MnP, and versatile peroxidase, VP), and aryl-alcohol oxidase (AAO). Microelements copper (Cu), manganese (Mn), zinc (Zn), iron (Fe), and selenium (Se) influenced the Lac and peroxidase production by this species.

The aim of this investigation was to study the effect of Cu, Mn, Fe, Zn, and Se under conditions of submerged fermentation (SF) of dry ground mandarin peels on the production of Lac and peroxidases in the selected *P. eryngii* var. *eryngii* strain.

The culture of *P. eryngii* var. *eryngii*, strain No. 616, is preserved in the Culture Collection of the Institute of Evolution, University of Haifa (HAI). The investigated *P. eryngii* strain was grown under SF conditions of dry ground mandarin peels (as a carbon source) and $(NH_4)_2SO_4$ (as a nitrogen source). Different combinations and concentrations of Cu, Mn, Fe, Zn, and Se in the forms of: $CuSO_4 \times 7H_2O$, $MnSO_4 \times H_2O$, $FeSO_4 \times 7H_2O$, $ZnSO_4 \times 7H_2O$, Na_2SeO_3, Na_2SeO_4, and SeO_2 were analyzed. Ligninolytic enzyme activities were measured after 5 and 7 days of cultivation. Lac and peroxidase activities were determined using syringaldasine and phenol red, respectively. An UV-160A Spectrophotometer (Shimaden) was used for these assays.

The optimum Cu^{2+} concentration for Lac production was 1 mM (300.12 U/L after 7 days of cultivation), while for peroxidases 10 mM Mn^{2+} concentration of 5 mM led to peaks of Lac (322.62 U/L, after 7 days of cultivation) and peroxidase activities. However, peroxidase production was significantly lower at Mn^{2+} concentrations of 1 mM and 3 mM compared to the control. The addition of Zn^{2+} and Fe^{2+} to the medium in a concentration of 1 mM led to the significantly high increase of Lac, slight increase of VP, and decrease of MnP activity. Se presented in the concentration of 1 mM, in all investigated forms, caused a decrease of Lac activity, especially when it was added in the form of SeO_2, while the lowest decrease was shown in the presence of Na_2SeO_4. In the presence of all forms of Se, MnP activity decreased after 5 days and increased after 7 days of cultivation in comparison with the control, while VP activity increased, especially when Se was presented in the form of SeO_2.

Anticancer Medicinal Mushrooms Can Provide Significant Vitamin D_2 (Ergocalciferol)

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Studies since 1966 have documented antitumor activities by the β-glucan and triterpene components of numerous Basidiomycetes mushrooms. However, these active ingredients may not explain all of the observed pharmacologic effects. To date, the potential pharmacologic role of vitamin D_2 (ergosterol) in medicinal mushrooms has not been examined. Vitamin D_2, when transformed by the liver into the potent vitamin D_3, is a seco-steroid that, like thyroid hormone, binds to a specific receptor within the superfamily of nuclear receptors for steroid hormones. The vitamin D nuclear receptor is found not only on intestine, bone, liver, and kidney tissues but also on lymphocytes, monocytes, and macrophages as well as hematopoietic, skin, muscle, heart, pancreas, adrenal, brain, reproductive, lung, pituitary, thyroid, and cartilage tissues. Vitamin D_3 regulates gene expression for multiple physiological functions, including those for the central nervous system and immune system. This study tested the hypothesis that in mushroom species with documented anticancer activity, mushroom ergosterol exposure to UV-B energy from sun drying significantly increases vitamin D_2 content.

Three specific medicinal mushroom species with strain-specific documented chains of custody were grown under standard conditions indoors. Fruiting bodies were harvested and dried indoors by commercial dryers or outside under the summer sun for 6–8 hours. The products were then subjected to standardized HPLC analysis in conformity with the Official Methods of Analysis of AOAC International (2000) 17th Ed., AOAC International, Gaithersburg, MD, USA, Official Method 982.29 (Modified).

Ganoderma lucidum (W.Curt.:Fr.)Lloyd (Reishi), *Lentinus edodes* (Berk.) Singer (Shiitake), and *Grifola frondosa* (Dicks.:Fr.) S.F.Gray (Maitake) dried indoors demonstrated D_2 content of 6, 134, and 460 IU per 100 grams, respectively. When dried by sunlight outdoors, the D_2 content increased to 2760, 21,400, and 31,900 IU per 100 grams, respectively.

These data have three important implications. First, the potential vitamin D_2 content of antitumor medicinal mushrooms must be considered as a confounding factor in mushroom research. Vitamin D_3 stabilizes chromosomal structure and prevents DNA double-strand breaks induced by either endogenous or exogenous factors. Vitamin D_3 induces cell cycle arrest, promotes differentiation, and induces apoptosis. Vitamin D_3 acts as an antiproliferation agent against many cancers, including breast, prostate, colon, and bladder. In addition, vitamin D_3 inhibits both tumor invasion and tumor angiogenesis.

Second, sun-exposed mushrooms may be an excellent dietary response for addressing the significant world-wide incidence of vitamin D deficiency. Inadequate vitamin D status not only places people at risk for osteoporosis, but appears to be a significant risk factor for development of cancer. For many cancers, a significant inverse correlation exists between mortality rates and UV-B radiation exposure. For example, the risk of fatal breast cancer in the major

urban areas of the United States is inversely proportional to the intensity of local sunlight ($r = -0.80$, $p = 0.0001$), and such inverse correlations also exist for prostate, colon, bladder, ovary, non-Hodgkin's lymphoma, esophageal, kidney, lung, pancreatic, rectal, stomach, and corpus uteri cancers.

Third, this study challenges the USDA claim that the average edible mushroom contains 76 IU (1.9 μg) of vitamin D per 100 grams and that shiitake mushrooms contain 1550 IU per 100 grams. For retrospective studies of diet and nutrition, use of these standard references may undermine the scientific legitimacy of both the data and its analysis.

Vitamin D_2 is not as potent as vitamin D_3. For many years, vitamin D_2 was considered much safer than vitamin D_3 because of the additional metabolism required for activation. Thus, although the current RDA for vitamin D is 400 IU per day, prescription doses of ergocalciferol at 50,000 IU twice a week only slowly increase vitamin D levels in deficient adults. However, vitamin D in excessive doses can be toxic and even fatal. This is particularly true in persons with cancers and other diseases that predispose them to hypercalcemia. Further understanding of vitamin D_2's pharmacokinetics and metabolite biologic activities is necessary.

Distribution of Medicinally Important Mushrooms of Northern Mountainous Areas of Pakistan

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Pakistan has very significant mountain ranges; the Hindukush, Karakurm, and Himalaya. Hindukush mountain ranges further extend from the northeast to the southeast to the southwest up to Koh-Safed. These mountain ranges contain some of the highest peaks in the world—i.e., K2 (9861 m), Nanga Parbat (8126 m), Rakaposhi (7788 m), Trich Mir (7690 m), and Deosai Plateau (4333–5333 m). The following important areas with natural vegetation of northern Pakistan have been surveyed during the rainy season: North Chitral, North Swat, Kaghan Valley, Gilgit, and Skardu including the Deosai Plateau. The natural lakes of these areas, such as Saif ul Maluk, Lulusar, Sadpapra, Kachura, and Shoezal, were also surveyed. The vegetation of these places of natural forests mostly consists of *Pinus wallichiana, P. roxburghi, Abies pindrow, Quercus incana, Juglan regia, Juniper bushes, Betula utillis*, shrubs, herbs, and grasses. They grow at various altitudes and in ecozones, such as the subtropical pine zone (12 species), temperate zone (24 species), subalpine (20 species), and the alpine zone (10 species). The common genera were *Agaricus, Clitocybe, Calvatia, Coprinus, Coriolus, Fomes, Ganoderma, Morchella,* and *Podaxis.* The terrestrial environment under these forests was rich in organic matter, which was derived by the activity of a number of organisms (fungi, bacteria, and invertebrate, etc). More than fifty species of medicinally important mushrooms belonging to 40 genera have been recorded such as mycorrhizal, parasitic, saprophytic, and terrestrial/coprophillous in their habitat.

GACOCA Formulation of East African Wild Mushrooms Show Promise in Combating Kaposi's Sarcoma and HIV/AIDS

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Preliminary studies involving the use of a formulation comprising a mixture of powdered fruit bodies of several wild medicinal East African mushrooms in the treatment of patients with Kaposi's sarcoma (an opportunistic skin cancer affecting patients afflicted with HIV/AIDS) show promising therapeutic results. The same product has also benefited patients with HIV/AIDS.

The product, GACOCA, has been used in Tanzania since 2001. Initially it was used to treat patients with HIV/AIDS in Ifakara Morogoro in central Tanzania under the supervision of Dr. Henry Moshi of St. Francis Hospital, who found that patients achieved noticeable improvement in health. Later, trials in Dare s-Salaam on patients with Kaposi's sarcoma revealed encouraging responses with complete resolution of the condition in a fraction of these patients. GACOCA has also demonstrated promising effectiveness in other conditions including diabetes, high blood pressure, libido problems, and bedwetting. These encouraging medicinal values of the GACOCA mushroom warrant further evaluation in properly designed clinical trials.

GACOCA contains several mushrooms of established medicinal value, which are abundantly available in the East African woodlands. I list them below according to their botanical names.

- *Ganoderma* species include *G. lucidum* (W.Curt.: Fr.) Lloyd, *G. applanatum* (Pers.:Wallr.) Pat., and *G. pfeifferi* Bres. *Ganoderma* species have been extensively researched. Their antitumor, antiviral, cholestrol reducing, and anti-fatigue effects have been demonstrated. Many of the vast numbers of its polysaccharides and triterpernoids studied so far show immunomodulatory properties. Triterpernoids inhibit cholesterol synthesis, allergenic responses, and histamines. In addition, anti-inflammatory, antibiotic, antifungal, anti-aging, and stamina-enhancing effects have been mentioned. Extracts of this mushroom prevented the death of lymphocytes infected with HIV and inhibited the replication of the virus within the mother and daughter cells.

- *Trametes versicolor* (L.:Fr.) Lloyd (=*Coriolus versicolor*) (Turkey tail) has immune enhancement, antitumor, antiviral, antibacterial, and antioxidant properties. PSK, a derivative of this mushroom, is an approved anticancer drug in Asia. The drug is popularly used in the treatment of stomach cancer, among others. Another form of extract, PSP, is an antiviral agent inhibiting HIV replication based on *in vitro* studies. This is probably the most well-documented medicinal mushroom according to authorities in medicinal mushrooms.

- *Cantharellus* species include *C. cibarius* Fr.:Fr., *C. cornucopioides* (L.) Fr., and *C. isabellinus* Heinem. These mushrooms are abundantly available in Tanzania and contain essential amino acids as

well as vitamin A. Consumption of these mushrooms is beneficial in preventing night blindness, inflammation of the eyes, and dry skin, according to traditional Chinese medicine. It also helps to tonify the mucus membranes and may increase resistance against certain infectious diseases of the respiratory tract. Studies in mice show that ethanol extract of *C. cibarius* sporophore can inhibit growth of sarcoma 180 (a type of experimental animal cancer).

Some mushrooms such as *Schizophyllum commune* Fr.:Fr., *Phellinus igniarus* (L.) Quèl., and *Fomes fomentarius* (L.) J.J. Kickx are mixed in GACOCA. These species of the woody mushroom are common in East Africa. Traditional healers in the regions use the mushroom as medication. It is claimed, that mixed in various proportions, they could be effective in treating epilepsy and general chest pain. The treatment is performed by having patient inhale smoke from a burning substance under a cloth hood, by inhaling vapor from boiling substances, and sometimes by taking the liquid in a cup of tea. These mixtures are also used locally for the treatment of mental disorders (claimed in the traditional medicine setting to remove demons).

The literature reports that these mushrooms can heal the inflammation of the brain (as in Alzheimer's disease) at early stages. *Phellinus linteus* (Berk. et M.A. Curt.) Teng is a more familiar product in the medical literature. It has antitumor and immunomodulating properties, especially in the enhancement of activity of B lymphocytes. Studies reported in 2000 found that this mushroom had the highest rate of inhibition against implanted sarcoma cancer 180 in mice, resulting in 96.7% inhibition. A report in 2001 explored the antimutagenic properties of this mushroom, showing activity in limiting or preventing tumor development. Research in Korea led to the establishment of *Ph. linteus* as a standard treatment for cancer.

The mushroom species described above have been identified in large quantities in Tanzania and East Africa, and we are working with hospital clinicians, natural therapists, and traditional healers to study their medicinal value in our settings. It is my intention to make this information available so that the GACOCA mushroom will interest scientists, medical professionals, environmentalists, the business community, educators, and any other stakeholders in the region and the world. I believe there is big potential here, especially now when pollution is already a concern elsewhere in the world.

I was introduced to this mushroom back in 1967. At that time I was staying in Sigulu Island on lake Victoria, Uganda, with my grandmother Veronica Ajiambo, who adopted me after the death of my father in 1963 at Jipe, Mwanga in Kilimanjaro, Tanzania. She taught me the art of identifying and picking mushrooms.

In 1980 I learned about mushroom cultivation. In 1993 I started collecting mushroom literature from the best world authorities. I read all I could about mushrooms, cultivated them, collected them, identified them, and worked with local consultants to design clinical trials.

Today in Tanzania the GACOCA mushroom is being used as an immune booster by patients with HIV^+, Kaposi's sarcoma, and arthritis, among other illnesses. Through HBCPs (home based care providers) the GACOCA mushroom is distributed to the community in the Kibaha coast region. I work with KIDEFO (Kibaha Development Foundation) to support the community healthwise. We intend to expand the service countrywide, and expect to make GACOCA extracts in early 2005. The center for production of this mushroom is in Kibaha Maili moja coast region in Tanzania. It is collected from different regions in the country and processed for community. Some is sold to pharmaceutical shops and supermarkets to sustain the GACOCA project.

ACKNOWLEDGMENTS

COSTECH (Tanzania Commission for Science and Technology), Dare s-Salaam. Prof. Dr. Jeff Luande, Ocean Road Cancer Research Institute, Dar es Salaam, Tanzania. Dr. Flora Fabian, Holistic Family Clinic, India and Morogoro Road Junction, Dar es Salaam, Tanzania. Dr. Henry Moshi, Watani Dispensary, Ifakara Morogoro, Tanzania.

Mating System and Genetic Variations of *Tricholoma crassum* (Berk.) Sacc. in Some Area of Thailand by Isozyme Electrophoresis and PCR-RFLP Method

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Tricholoma crassum (Tricholomataceae, Agaricales) is a large, good edible species. It has nutritional quality, having 10.02 gm carbohydrate, 0.287 gm fats, 18.58 gm protein, 2.71 mg calcium, and 3.35 mg iron in every 100 gm of fresh weight. It is found growing abundantly throughout Thailand.

The mating system of *T. crassum* was studied. The monokaryon isolates were selected from ten sporocarp collections in five provinces in Thailand—Ubonratchathani, Sakonnakorn, Mahasarakham, Srisaket, and Nakornratcha-sima. Suitable growth conditions for the mycelial cultivation were PDYA medium pH 7 and 25 °C incubation. Twelve fast-growing monokaryotic strains were selected for mating system studies. The mating system was determined by pairing the monokaryotic mycelia of each collection in all pairwise combinations. The presence of clamp connections after mating indicated sexual compatibility. The ratio of compatible crosses to all combination crosses were 1:4, which indicated tetrapolar heterothallism. In addition, multiple alleles among the monokaryons for each sporocarp of the five provinces were examined on the basis of their mating interactions, and the results were two factors (A and B), and each possessed 16 different alleles.

One hundred and thirty-eight monokarytic isolates of *T. crassum* from the above five provinces were cultured on growth media. The suitable conditions for the mycelial growth were PDYB medium at 25 °C for 21 days. All samples were analyzed for isozyme variability on polyacrylamide gel electrophoresis with 11 enzyme systems: isocitrate dehydrogenase, leucine aminopeptidase, acid phosphatase, phosphogluconate dehydrogenase, alkaline phosphatase, alcohol dehydrogenase, glucose–6–phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, laccase, and esterase. Eight of the systems showed polymorphism. Cluster analysis based on isozyme variability using the NTSYSpc 2.00 and UPGMA methods revealed two clusters at a similarity coefficient of 0.67. The first cluster consisted of monokaryotic isolates from Nakornratchasima, Mahasalakham, and eight samples of Ubonratchathani. The second cluster consisted of the isolates from Sakonnakhorn, Srisaket, and 30 samples of Ubonratchathani.

The genetic variations of nine additional samples of *T. crassum* from four additional provinces—Roiyed, Burerum, Patumthani, and Nakhon Pathom—were studied by the technique of PCR-RFLP. Two pairs of primers, ITS1- ITS4 and O1-LR12R, were used respectively for PCR amplification on ITS (internal transcribed spacer) and IGS (intergenic spacer) regions of the nuclear ribosomal gene, followed by digestion with *Hind*III, *Dde*I, *Hae*III, *Eco*RI, and *Hinf*I. Data analyses of the PCR-RFLP products based on the similarity index and the UPGMA method in the WinBoot program revealed three clusters that were related to their geographic origins, except the samples from Burirum, which showed genetic variation from the same areas at a similarity coefficient of 0.8 and were grouped into the third cluster.

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On-Farm Study of Small-Scale Mushroom Cultivation as a Means of Creating a High-Quality Soil Amendment from Wood Products

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Small-scale agriculture faces an altogether different set of issues than conventional agriculture; one such issue is fertility management. In low overhead market gardens, the expense of a tractor is often not justifiable and compost management can become labor intensive. Thermo-composting requires moving large volumes of material in a time sensitive manner. In addition, compost ingredients such as manure and food scraps can be difficult to handle and odiferous and must be carefully managed so as not to become a health hazard. Alternatively, by cropping mushrooms on woody products, the labor of compost production can be pelletized. Mushroom compost offers a safe and environmentally benign alternative to composted manures and food scraps, while reducing physical strain.

Worldwide, mushroom cultivation has been used to manage agronomic waste products while simultaneously obtaining food and profit. This feasibility study examines the logistics of small-scale oyster mushroom production on the diversified farm.

For the purposes of this on-farm study, two model systems are being examined, one using pasteurized rice straw and the other pasteurized alder (chips/sawdust/supplemented). For each of these systems the hours of labor, operating expenses, yields, biological efficiency, duration of incubation, duration of fruiting, and amount of compost produced (kg spent mushroom substrate) will be documented.

Spent mushroom substrate will be applied directly to the field after cropping. Spent mushroom substrate will be tested for nutrient composition over a 6-month period. Samples will be taken once a month for straw and woodchip substrates.

Due to factors such as mushroom strain, substrate, environment, and technique, results will vary from system to system. However, this study will provide some indication of the amount of labor, finances, and facilities required for mushroom cultivation. Findings will be synthesized into a poster presentation. This presentation will encompass the feasibility study results and a discussion of alternative applications of spent mushroom substrate on the farm. The discussion will include results from an on-farm trial using mycelium as a dietary supplement in pastured poultry.

Minicultivation of Medical Mushrooms

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Most medical mushrooms are grown on specialized farms. These farms are often located far from the end-user living in big cities. The time needed for transportation and sale can be shortened only to a certain extent. Even observing all the necessary requirements, transportation and storage may result in mushrooms' losing taste and medicinal properties.

It is proposed to use a method of minicultivation for growing medical mushrooms under home conditions. Minicultivation of mushrooms is usable as well in laboratory practices to select high-productive mushroom strains and to examine the quality of spawn.

The main parameters for this method can be seen in the example of growing oyster mushrooms (*Pleurotus pulmonarius* (Fr.:Fr.) Quél. or *P. ostreatus* (Jacq.: Fr.) P. Kumm.). For preparing a nutrient medium, rye powder is mixed with vermiculite and water (3:12:4 v/v). The received mixture is thoroughly stirred and transferred to 200–250 mL autoclavable jars, leaving a 1–2 cm space at the rim. The jars should have tapered sides and lack shoulders. For this purpose, regular glasses are appropriate. Then dry vermiculite is put into the jar, the jar is closed and sterilized under high pressure for 1 hour. The medium can be inoculated either in spores and/or suspended mycelium using a syringe with a long needle, or with spawn plugs which are stuck into the substrate. Inoculated jars are kept at the optimal temperature for each strain. When the substrate is completely covered with mycelium, the jars are opened, the top layer of dry vermiculite is removed, and the mushroom block is shaken out and hung in a cultivation chamber by plastic holders. The cultivation chamber should be transparent, provide high air humidity (approximately 90%), and inflow of fresh air. For a cultivation chamber, one can use an aquarium or transparent plastic box. To maintain high humidity, one should spray the chamber walls twice a day. Normal fruit formation requires adequate illumination. In 7–10 days following the transfer of the block to the cultivation chamber, oyster mushrooms are supposed to appear first, and then in the following 3 weeks three waves of fruiting are achieved, just as in industrial cultivation. Each block usually bears 20–25 grams of mushrooms.

This method, with some modification, is used for growing *Lentinus edodes* (Berk.) Singer and nonwoodland mushrooms *Psilocybe cubensis* (Earle) Singer and *Stropharia melanosperma* (Bull.:Fr.) Bres.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Cytotoxic Activities of *Funalia trogii* (Berk.) Bond. Et. Singer ATCC 200800 Bioactive Extract on HeLa Cells and Fibroblast Cells

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This study demonstrated the cytotoxic and cell growth inhibitory effect of a bioactive extract of *Funalia trogii* (Berk.) Bond. et Singer, which was grown in solid-state fermentation at room temperature, on He-La and fibroblast and the mitotic index of lymphocytes. The effect demonstrated by the bioactive extract of *F. trogii* on laccase, peroxidase, SOD, ascorbate peroxidase, catalase, and glutation peroxidase is shown in Table 1.

A study on the possible cytotoxic and cell growth inhibitory effects of the bioactive extracts of two white-rot fungi showed that these extracts had significant cytotoxic and antiproliferative properties on the HeLa cancer cell line. Hence, the aim of this study was to examine the *in vitro* cytotoxic activities of standardized aqueous bioactive extracts prepared from *Funalia trogii* on HeLa and fibroblast cell lines using a MTT (3-[4,5-Dimetiltiazol-2-]-2-5-difeniltetrazolium bromide) cytotoxicity assay. *F. trogii* solutions were found to exhibit cytotoxic effects on HeLa cell lines. Based on the data, it was found that toxicity ratios of 0.05 µL of *F. trogii* extract solutions were 71.5%. Furthermore, *F. trogii* extract solutions were also cytotoxic on fibroblast cell lines.

Analysis of the data indicated that the toxicity ratio of 0.05 µL of *F. trogii* solutions was 51.3%. These results showed that the extracts had a substantial cytotoxic action on HeLa cell lines but less on fibroblast cells. In separate experiments the mitotic index reached nearly the same value at 4 µg/mL MMC, 75 µL concentrations of non-heated fungal extract. Therefore, in order to compare the mutagenic potential of fungal extract and MMC, we used concentration values in SCE analysis. Table 2 represents the SCE frequency of cultures treated with non-heated fungal extract, positive control, and negative controls. A significant induction of SCE was observed in cultured lymphocytes treated with MMC (4 µg/mL) compared with non-heated fungal extract and negative control ($p < 0.001$). There was no significant difference between negative control and non-heated fungal extract ($p > 0.05$, $p = 0.73$). In conclusion, we did not observe any genotoxic effect.

In bioactive extracts of fungi, we revealed the determination of enzyme or enzymes responsible for cytotoxic effect on HeLa cell line. As a result, antitumor activitiy was shown by two enzymes—laccase and peroxidase—produced by fungi. Bioactive extracts have natural quinone substances from lignin by production of peroxidase and laccase. These enzymes acted more selectively on HeLa cells, arresting the cell in the G-phase of the cell cycle and inducing apoptosis. The basis of our work

TABLE 1. Effect of Bioactive Extract of *Funalia trogii* on Mitotic Index Values in Cultured Human Blood Lymphocytes and Bioactive Extract Enzyme Activities

Bioactive extracts	Normal mitotic index	*F. trogii* extract		
		Untreated	Treated	
	Negative control	Room temperature	65 °C	85 °C
Blood sample 1	7.8	3.70	3.20	4.80
Blood sample 2	8.2	3.33	3.40	5.20
Average	8.0	3.50	3.30	5.00
Laccase Activity (IU)	—	704.24 (8.5 CU)	674.23 (7.8 CU)	84.62 (0.43 CU)
Peroxidase Activity (IU)	—	780.32 (8.8 CU)	695.67 (6.0 CU)	50.76 (0.19 CU)
SOD (IU)	—	59.0	57.0	56.0
Ascorbate peroxidase (IU)	—	19.0	8.0	4.0
Catalase (nmol/min)	—	2.72	1.57	0.22
Glutathion reductase (IU)	—	3.3	3.1	2.2

was that bioreductive activation was also a highly specific delivery mechanism for targeting a variety of processes and was important for tumor growth. Thus, bioreduction of a quinone in the hypoxic region of a tumor would result in the formation of an intermediate semiquinone or hydroquinone (depending on 1-e or 2-e reduction). Hence, we proved that the covalent bond in quinone is metabolically stable, the effector quinone substance is only released within the hypoxic regions of tumors, and the desired large differential between the effects of the quinone is attained. We are able to produce the quinones naturally, which can act as excellent subtrates for NAD(P) H-quinone oxidoreductase (DT-diaphorase) and thus target tumors rich in this enzyme, thereby providing cytotoxic activation by a hypoxia independent mechanism.

This study provides evidence for *in vitro* antitumor activity of a bioactive extract from *F. trogii*. Therefore, upon *in vivo* data, which will follow this study, it may become a promising cytotoxic product for treatment of various types of cancer.

TABLE 2. SCE Frequency in Cultured Human Blood Lymphocytes Treated with Bioactive Extract and Negative and Positive Controls

Groups	Examined second metaphases	SCE/metaphase mean ± SE
Bioactive Extract (0.015 µL)	50	3.98 ± 0.10*
PC (MMC 4 µg/mL)	50	10.41 ± 0.01 **
NC	50	3.71 ± 0.065

** $p < 0.001$, * $p > 0.05$ compared with negative control, NC: negative control, PC: positive control, MMC: mitomycin C

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

The Structure of the Polysaccharides Produced by Higher Basidiomyces *Tremella mesenterica* Ritz.: Fr. and *Inonotus levis* P. Karst.

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Polysaccharides are the best known and most potent mushroom-derived substances, with various positive health effects including immunostimulation, lowering glucose level in blood, anticancer activity, and many others. Biologically active polysaccharides are widerspread among higher Basidiomycetes mushrooms, and most of them have unique structures in different species. There is still no clear correlation between biological activity and structural features of the polysaccharides. This situation results in part from poor knowledge of polysaccharide structures. Herein, we present the results from analyzing two polymers of *Tremella mesenterica* and *Inonotus levis.*

Tremella mesenterica (Yellow Brain mushroom) possesses a wide spectrum of medicinal properties, including immunostimulating, protecting against radiation, antidiabetic, anti-inflammatory, hypocholesterolemic, hepatoprotective, and antiallergic effects. Glucuronoxylomannan (GXM), an extracellular polysaccharide produced by single cell culture of *T. mesenterica*, has a number of beneficial health effects. We developed a new strain of *T. mesenterica*, CBS 101939, which grows in submerged culture and offers superior yields of one-cell biomass rich in exocellular heteropolysaccharide GXM.

NMR and chemical analysis showed that it has a defined structure of repeating unit, which is *O*-acetylated at several points, stoichometrically at *O*-6 of two mannose residues, and partially at GlcA residue, which is present in non-acetylated or acetylated at positions 3 or 4 forms (see Fig. 1).

These results differ from existing data on the structures of mushroom mannan-based polysaccharides, where mannan backbone was believed to be

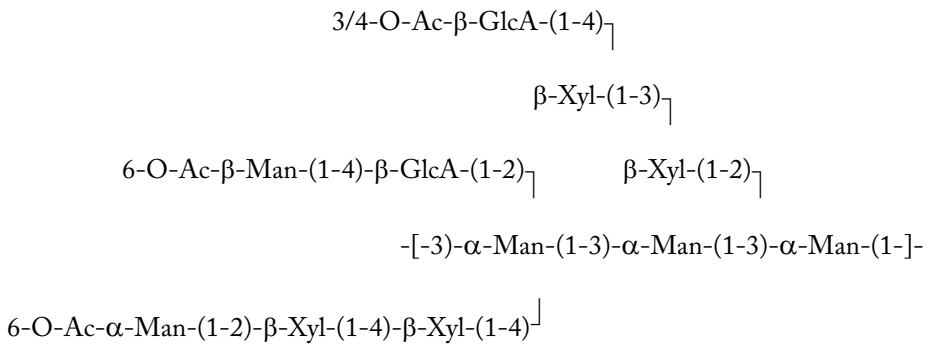

FIGURE 1. Structure of *Tremella mesenterica* polysaccharide.

β-GlcA-(1-2)-α-Gal-(1-6)-α-Gal3OMe-(1-[-6)-α-Gal-(1-6)-α-Gal3OMe-(1-]$_{5-10}$-

FIGURE 2. Structure of *Inonotus levis* polysaccharide.

randomly glycosylated with xylan chains of different lengths.

Inonotus levis polysaccharide had the structure shown in Figure 2, where terminal glucuronic acid residue is present in about half of the molecules, thus making some of the chains acidic and others neutral. We believe that these short polymeric chains were originally attached to some protein via serine or threonine residue and were cleaved off due to alkaline conditions of extraction. Another polymer, co-extracted with this galactan, was a branched, phosphorylated mannan with a structure similar to that of the mannan from yeast *Saccharomyces cerevisiae* yeast.

Cordyceps sinensis (Berk.) Sacc.: Economy, Ecology, and Ethno-Mycology of Yartsa Gunbu, a Medicinal Fungus Endemic for the Tibetan Plateau

Daniel Winkler

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Collection, trade, and use of Yartsa Gunbu (dbYar rTswa dGun 'Bu), "summer grass-winter worm" as the *Cordyceps sinensis* (Caterpillar fungus) is known to Tibetans, has a long-standing history in Tibetan medicine and culture. Although ancient local traditions warn that digging of Yartsa would provoke local spirits, its collection dates back centuries. It has been, and is now more than ever, one of the most important sources of income for rural Tibetans, especially nomadic communities, who often derive over 50% of their annual cash income from its collection in spring and early summer.

The 15th century scholar and doctor Zurkhar Nyamnyi Dorje [1439–1475] mentions Yartsa Gunbu in his text "Oral Instructions on a Myriad of Medicines [*sic*]" (Man nGag bYe Ba Ring bSrel). However, according to some Tibetan doctors, it might have been recorded under a different name in the "four Tantras" (rGyud bZhi) by Yuthok Yontan Gonpo (8th to 11th century). In Tibetan materia medica, *Cordyceps sinensis* is placed in the category of "medicinal essences" (rTsi sMan), which includes several tonics. It is used for general strengthening, boosting the immune system, and virility and is prescribed for kidney and heart problems. It is also used for treatment of hepatitis B. In Tibetan medicine Yartsa is prescribed mostly in compound remedies, which contain a variety of ingredients to balance each other, thus optimizing their efficiency and minimizing side effects.

Field studies in Ganzi Tibetan Autonomous Prefecture, Sichuan (1999–2004), and Nyingchi (Linzhi) prefecture, Tibet AR (2005), analyze collection techniques and trends, local markets and their participants, and the quantity and value of the harvest. Individual specimens are sold by collectors

for 1–5 Yuan (1999) to 4–10 Yuan (2004). Results are contrasted to Chengdu and Lhasa market prices, which peaked at 40,000 Yuan/kg ($4,900) in early 2004. The market is driven by demand in lowland China, where Yartsa Gunbu is known in Mandarin as "dong chong xia cao," a verbatim translation of its original Tibetan name.

The mycology/biology of *Cordyceps* and Thitarodes as well as the geo-ecology of their habitat are presented. *Cordyceps sinensis* is a fungus parasitizing on a range of ghost moth larvae of the Thitarodes (Hepialus) genus, which live in alpine grassland ecosystems of the Tibetan Plateau. *C. sinensis* is distributed on grasslands that receive a minimum of 350 mm average annual precipitation. It is to be found in an altitude of 3300–5000 m rising from the east to the west of the plateau. Locally it occurs within an altitudinal range of 500 m around the potential treeline. Tibetan's reliance on livestock herding has increased the habitat of *Cordyceps* substantially through continuous expansion of its pastoral areas at the expense of forest ecosystems.

While collectors complain about increased competition and intrusion of outsiders during collection season, some researchers and government agencies worry about the sustainability of present harvesting and favor regulation beyond current collection fees and licenses. Inspecting freshly dug specimens in Kangding and Lithang, it was apparent that at the onset of the collection season sporocarps are collected before they have had the chance to mature their asci cells and release spores.

In this context, it needs to be taken into account that semi-artificial production of *Cordyceps sinensis,* whereby caterpillars are bred and exposed to *Cordyceps* propagules, be it indoors or outdoors, is being carried out successfully on an experimental basis in the Tibetan areas. Overall, the current pressure on natural populations of *Cordyceps sinensis* seems not to have seriously endangered the resource. However, ever increasing harvest pressure and the absence of reliable baseline data clearly necessitates more research to formulate sound management strategies to secure the long-term survival of *C. sinensis,* a valuable resource especially for marginalized Tibetan families and mankind in general.

The HPLC Separation and Bioactivity Evaluation of the Polysaccharopeptide (PSP) of *Coriolus* (*Trametes*) *versicolor* Fermentative Mycelia

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Polysaccharopeptide (PSP, commercial name I'm-Yunity™) is isolated from the deep-layer cultivated mycelia of *Coriolus versicolor* Cov-1™ strain, using patented manufacturing processes. It is a covalently linked protein/polysaccharide complex with a molecular size reported as 100 kD. The polysaccharide moiety consists of 56.1% β-(1, 3) glycan. Its peptide moiety contains 18 kinds of amino acids in which

the glutamic acid and aspartic acid are the most abundant.

The pharmacological study and clinical trials have proved that PSP possesses immunomodulatory effects that can benefit cancer patients by improving their immunity and life quality. It also can antagonize the side-effects caused by radio- or chemotherapy.

Previous studies have shown that PSP has several fractions. Electrophoresis results showed that PSP had three bands, and similar results were obtained using column chromatography. Yang MMP and colleagues separated PSP into two fractions by using reverse phase HPLC and detected the first eluant possessing direct antitumor activity. However, the contributions of all PSP fractions to its bioactivities are still not well understood. Therefore, in this study, we applied an anion ion-exchange HPLC method to separate PSP and evaluated the bioactivity of its fractions.

The commercial PSP product was dissolved in 95 °C water and centrifuged to remove auxiliary materials and precipitants. It was further desalted and freeze-dried prior to HPLC separation. The freeze-dried powder was dissolved in an initial buffer and injected into a preparative anion ion-exchange HPLC column. The column was eluted with initial buffer following an increased NaCl gradient. After that, PSP was separated into four fractions: Fr1, without a negative charge; Fr2, with a weak negative charge; Fr3, with a moderate negative charge; and Fr4 with a strong negative charge. Chemical analysis reveals that the polysaccharide content of Fr2 is the highest (92.5%), while the Fr4 is the lowest (35.2%). However, the β-(1, 3) glucan contents in polysaccharides are reversed, and the Fr4 has the highest content (51.0%), while Fr1 and Fr2 have almost no β-(1, 3) glucan detected. Fr1 and Fr2 also have roughly half-protein contents compared to Fr4 and Fr3, and the highest content is in Fr3 (22.6%).

The mouse splenocyte proliferation assay reveals that the Fr4 and Fr3 have stronger immunostimulatory effects than Fr2 and Fr1. The stimulatory index of Fr4, Fr3, Fr2, and Fr1 are 1.29, 1.27, 1.12, and 1.08, respectively. Fr3 and Fr4 also elevate mouse splenocyte $CD4^+/CD8^+$ ratio, while Fr1 and 2 retained or slightly decreased compared to unfractionized PSP. All four fractions strongly stimulate the production of IL-6 and TNF-α from human PBMN cells. The IL-6 production was increased up to around 8.1 fold by Fr 2 and 4, and TNF-α level was even heightened to 12.7 folds by Fr 2 and Fr 3.

In conclusion, PSP probably contains several compounds, and these compounds can be fractionized using an anion ion-exchange HPLC method. Different fractions showed diverse pharmacological activities as well as different physical and chemical properties. To understand the relation of PSP fractions to their bioactivities, it may help us to set up specific quality control methods to monitor and to improve PSP quality and to develop the product with higher efficacy.

AKNOWLEDGMENTS

The authors appreciate Hong Kong Association for Health Care Ltd. and Hong Kong Winsor Ltd. for funding the research and lending support.

International Journal of Medicinal Mushrooms, Vol. 7 (2005)

Mineral Uptake by First Flush Mushrooms (*Pleurotus* spp.) Cultivated on Various Agro-Processing Waste

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The use of agro-processing waste as raw materials for mushroom production was investigated to define a strategy for bioconverting these wastes into edible basidiomata and, hence, retrieve some of the lost nutrients. Sampled wastes (corn cobs and maize bran, cocoa husk and shells, oil palm fiber, empty bunch and kernel cake, spent malt, pito mash, yam, cassava, cocoyam, potato and plantain peelings, rice husk and bran) from both industrial and traditional agro-processors were characterized chemically and formulated into media. In all, 10 formulations were derived. Two species of *Pleurotus*—*P. ostreatus* (strain EM_1) and *P. eous* (strain OT_3)—were used for substrate evaluation. During this cultivation process the ability or lack of these species to take up minerals (Ca, Cu, Fe, Mg, Mn, P, K, and Na) from the formulated media was investigated.

Mineral absorption by the first flush mushrooms during growth on the various media was determined and compared to the amount present in the original and spent media. Ca, Cu, Fe, Mg, Mn, and P were determined using a Perkin Elmer 3110 Atomic Absorption Spectrometer. K and Na were determined using a Jenway PFP7 Flame Photometer. Analysis of variance between the various determinations was done. The correlation between concentration of elements and total yield was also investigated. There was uptake of all the minerals determined, as present in the media, by the first flush mushrooms. Intake, however, of elements by mushrooms differed considerably among the various minerals to the extent that, although mushrooms might be observed to behave almost as a filter for some elements, they accumulate others. The data suggested that K and P had the highest concentrations in the mushrooms, while Mn and Ca had the lowest. Mg, Ca, Mn, and K concentrations were lower in the mushrooms compared to the initial and exhausted media, indicating that there was no appreciable accumulation after their infiltration into the mushrooms. There was appreciable accumulation of Cu, as the concentration in the mushrooms was higher than in the initial media. The concentration of P in mushrooms was comparable to that of the initial media. There was accumulation of Na in the mushrooms and the concentration was comparable to that in the exhausted media. The concentration of K was generally high compared to the other minerals. Calcium was not significantly present in the mushrooms analyzed despite its high concentration in the formulated media.

The highest colonization rates and sporophore yields were achieved from corn cob and cocoa husk-based substrates, whereas lesser yields were produced from rice husk-based media. Yield from tuber-based (cassava, yam, and cocoyam peelings) media could not be relied upon due to contamination that resulted in incomplete spawn run. There was generally no correlation between mineral uptake and yield.

The study generally demonstrated the ability of *Pleurotus* to absorb these elements during cultivation, albeit to varying extents. Hence, through controlled addition of specific minerals to substrates on which mushrooms are cultivated, the elements can be absorbed by the growing mycelium, and translocated to the sporophores. The mushrooms then could become good or even excellent sources of these minerals.

Secondary Metabolites from Edible and Medicinal Mushrooms as Molecular Therapy for Prostate Cancer

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Prostate cancer (PCa) is the second leading cause of death for Western men. Primary PCa is hormone dependent—that is, it is manageable by hormonal therapy. However, it rapidly develops to hormone-refractory tumors because of 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 some Asian countries. Several reports linked Eastern diets and cancer occurrence, especially for PCa. The implication of several mushrooms in the PCa prevention in Asia is of special interest. Over the last two to three decades, scientific and medical studies have been carried out in Japan, China, Korea and, more recently, the US that have increasingly demonstrated the potent and unique health enhancing properties of compounds extracted from a range of medicinal mushrooms.

It is well established that medicinal mushrooms possess a variety of health-promoting qualities and represent a potential source of pharmaceuticals for a number of diseases, including cancer. Although most of the attention to their anticancer activity revolved around the activity of high-molecular-weight polysaccharides, their mechanism of action is yet to be determined.

Our focus is in low-molecular-weight secondary metabolites with a well defined mechanism of action targeting the expression and function of the AR as a potential therapy for PCa. These modulators might interfere with ligand binding, DNA binding, AR cross-talk with co-activators, and the cell transcription machinery. Reducing AR expression or function to a critical level would not only slow the growth of PCa cells, but also result in apoptosis. We hypothesize that many mushroom secondary metabolites are excellent candidates for AR modulators. This suggestion is supported by four decades of research on anticancer activities of mushroom extracts and isolated compounds. A possibility exists that the pharmacophoric structural features of some of these compounds are consistent with quantitative structure-activity relationship (QSAR) models and AR binding studies.

Our proliferation and transcriptional assay initial results also indicated the presence of such active metabolites. A total of 220 mushroom extracts were screened. We used the stable cell line, MDA-kb2, carrying the luciferase gene fused to MMTV promoter, for screening of androgen agonists and antagonists. Using this system, one can measure anti-androgenic activity by measuring the decrease in reporter activity, caused by the appropriate compounds on DHT-induced luciferase activity. Eight extracts showed anti-androgenic activity, reducing DHT-induced luciferase activity by up to 64.8%, including extracts from *Coprinus comatus* (O.F.Müll.) S.F.Gray. Of special interest are those extracts that can also inhibit androgen-dependent LNCaP cell proliferation. Cell viability assays demonstrated that ethanol extracts from C. comatus selectively inhibit LNCaP cells.

Data revealing the effect of selected active mushroom extracts on the activity and expression of AR and PSA will be presented. Furthermore, the effects of selected mushroom extracts on apoptosis and cell cycle progression will be demonstrated. In addition, we will use chemical separation to obtain active fractions aiming isolation, identification, and structural elucidation of active moieties.

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Cultivation of the King-Oyster Mushroom *Pleurotus eryngii* (DC.:Fr.) Quél. on Substrates Deriving from the Olive-Oil Industry

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The cultivation of edible fungi is a controlled bioconversion of agro-industrial lignocellulosic wastes and residues into value-added products. The development of mushroom production methodologies on nonconventional substrates is one of the main targets of applied research in this domain. In the Mediterranean region, olive-oil mill wastes and byproducts are generated in huge quantities, and their handling or treatment is problematic mainly because of their high organic content, composition, and physicochemical properties. In contrast to the dominant centrifugal olive-oil extraction systems (where, in addition to olive oil, waste water and pomace are generated), the recently introduced two-phase decanters function by separating the malaxed olive paste from the oily phase without requiring any addition of water, thus producing very limited quantities of liquid wastes and a moister pomace, which resembles a semisolid sludge ("alpeorujo").

Until now, very limited research has been conducted on methods of treating wastes from the two-phase oil extraction system; it seems to improve olive oil quality, while resulting in an elevation of environmental problems associated with the generation of large quantities of waste water. In preliminary experiments, *Pleurotus eryngii* strains of various origins were screened for their efficacy to colonize media composed of alpeorujo and composted mixtures with olive press cake. For all fourteen strains tested, mycelium grew faster through the composted substrate than through the raw medium. The pretreatment-composting process partly elevated the toxicity of alpeorujo, while at the same time provided readily available nutrients from the action of the thermophilic microorganisms. It also ensured the presence of inducer compounds, which in the case of the white-rot fungi enhance the activity of their lignin-degrading enzymes.

Qualified strains were subsequently tested for their ability to grow and produce basidiomata on composted mixtures of alpeorujo and olive press cake; their performance was evaluated in comparison to data obtained from their cultivation on wheat straw supplemented by wheat bran. After evaluating several cultivation characters (earliness, total yield, yield per flush, biological efficiency, and basidioma mean size), it was found that earliness values, total yield, and basidioma mean size values were not statistically different between the two substrates. On the other hand, biological efficiency was slightly higher on wheat straw; for this particular substrate over 50% of the total mushroom yield was produced from the first flush, while the yield on alpeorujo was rather evenly distributed among the three flushes harvested.

It should be noted that the quality and organoleptic value of the mushrooms produced were exceptionally good in both media for all strains examined. To further enhance the productivity of the two substrates, a *P. eryngii* cultivation methodology was developed based on the application of a casing layer prior to primordia formation. In general, the use of olive by-products as alternative substrates provided promising results, as compared to wheat-straw based

substrates, for the cultivation of *P. eryngii.* These data confer significance to environmentally hazardous and/or low-economic-value agricultural wastes, which could be combined with the diversification of the mushroom market and the introduction of highly prized mushroom species.

Bioactive Components of *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd Can Induce Apoptosis of Tumor Cells

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The antitumor activity of Ling Zhi or Reishi mushroom *Ganoderma lucidum* has been studied widely *in vivo* and *in vitro,* and many reports show that polysaccharides are the main antitumor active substances in *G. lucidum,* acting by improvement of the host immunity. In addition, it was also reported that an extract of *G. lucidum* killed the tumor cells *in vitro.* In our experiment, it was also found that crude extracts of *G. lucidum* (named LZ) and their fractions significantly inhibited the proliferation of different tumor cells, and the fractions induced SW620 cell apoptosis *in vitro.*

The antiproliferative capacity of LZ was tested using 11 tumor cell lines. LZ very strongly inhibited the growth of Jurkat, K562, SW620, LS180, and QGP-1 cell lines, and the IC_{50} of LZ to them was measured as 180, 200, 180, 320, and 360 μg/mL, respectively. Moreover, LZ showed the capacity to inhibit the growth of S180 cell lines, the IC_{50} is 420 μg/mL, and meanwhile it obviously inhibits the growth of MCF7 and Caco-2 only at 1000 μg/mL. However, LZ does not possess inhibitory capacity to BON, Panc-1, and HUH7 cell lines in the range of experimental concentrations.

In order to find out the bioactive components from crude extracts, LZ were divided into two parts by dialysis, and then they were further fractionated using different chromatographic procedures. All fractions obtained were tested for their antiproliferative capacity to SW620 cells. LZ-2-2 and LZ-DW-2-a-3 were found to be active components of LZ. After incubation with LZ and LZ-2-2, SW620 cells were found to form apoptotic bodies *in situ* checked by light microscopy—their apoptosis activity was confirmed by staining with Annexin V-FITC conjugate. The apoptotic percentage of SW620 was quantified by flow cytometry. At the most effective concentration of 600 μg/mL, LZ induced 28% of cells to undergo apoptosis, 39.5% for LZ-2-2, and, at 1000 μg/mL, 51% for LZ-DW-2-a-3. The influence of LZ-2-2 and LZ-DW-2-a-3 on the cell cycle of SW620 cells during their apoptotic processes was analyzed by flow cytometry. Experimental results suggested that SW620 cells were arrested in the G0 phase, and they could not transit from the G2/M phase to the G1 phase after treatment by LZ-2-2 or LZ-DW-2-a-3.

Until now, there has been no final answer as to how the extract of *G. lucidum* inhibited and killed the tumor cells. Our results revealed that some fractions from *G. lucidum* induced apoptosis of tumor cells, which means that *G. lucidum* exerts its antitumor function by the apoptosis pathway as well as by the immune pathway, which is accepted widely as a new pathway.

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A Comparison Study of the Anticancerous Activity and Mechanism of Ethanolic Extracts from Different *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd Strains

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Ganoderma lucidum (Ling Zhi or Reishi), a traditional Chinese medicine, has been used to promote health and prolong the life span in China for thousands of years. The evidence shows that triterpenes are the main effective components of *G. lucidum.* Some triterpenes extracted from *G. lucidum* fruiting body can effectively inhibit the proliferation of tumor cells in vitro, such as human hepatoma cells, cervix uteri tumor cells, breast cancer cells, and promyelocytic leukemia cells.

Compared to the cultivation of *G. lucidum* fruiting body, the liquid fermentation technique can achieve an industrial scale production with rigidly controlled manufacture conditions. It may be the future for producing high-quality Ganoderma products. However, there has not been much research addressed to the antitumor activity of triterpenes from mycelia. Only a few showed that triterpenes from mycelia could inhibit the proliferation of human hepatoma cells.

In this study, we used liquid fermentation to culture eight different *G. lucidum* strains and screened the strains producing high-anticancerous activity triterpenes against human acute promyelocytic leukemia HL-60 cell line.

It is known that triterpenes can be extracted with organic solvents such as ethanol, methanol, and chloroform. Therefore, we used ethanol to do the extraction. After that, a colorimetric method was used to detect the total triterpene contents of the extracts. MTT assay was applied to compare the antiproliferation effects. Morphological observation, flow cytometric cell cycle analysis, and Annexin-V/PI bivariate assay were employed to identify the mechanism of antitumor activity.

The results showed that mycelia from all eight strains possessed inhibition effects against HL-60 proliferation. Among eight strains, ethanolic extract from strain L5 was identified to have the highest anticancerous activity. Its percentage of inhibition was 91.4 0.9% at a concentration of 125 μ/mL (72 hours). At the 48th hour, 13.3% of cells underwent early apoptosis after treatment. Cell cycle analysis revealed that in comparison with the control, the percentage of G_0/G_1 cells were elevated 15.9%, whereas that of S cells decreased 8.4% and G_2/M cells decreased 7.6%. It is the first time that the antitumorous effect of *G. lucidum* mycelial triterpene containing extract was related to the cycle arrest in HL-60 cells. The results also clearly demonstrate that in addition to *G. lucidum* fruiting body, the mycelia can also produce anticancerous substances. *G. lucidum* could be the resource to provide candidates for antitumorous substance screening.

Finally, the statistics showed total triterpene content did not have a close relation to the antitumor effect. Statistical results revealed that the total content can influence the antitumor activity, but the exact influence has not been elucidated. For example, the total triterpene content of L5 is 12.9%, just close to the mean of eight strains, but its percent of inhibition is as high as 91.4%, much higher than

the rest. This might be due to the content of the particular anticancerous triterpene(s) not being able to be detected when performing the total triterpene measurement. Therefore, it is necessary to detect the antitumor activity in addition to the total triterpene contents before the anticancerous triterpene(s) is identified and becomes measurable.

AKNOWLEDGMENTS

The work was partially funded by Hong Kong Association for Hearth Care Ltd. The authors also appreciate the support from Shanghai Yang's Herb Institute (R.P. China) and Professor S.C. Jong (ATCC, USA).

Distribution, Morphology, and Taxonomy of *Haploporus suaveolens* (L.: Fr.) Fr., a Rare Polypore Revealing Medicinal Properties

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The search for active producers of metabolites having medicinal importance has been an urgent task for mycologists and biotechnologists for several decades. For successful performance of this task, the knowledge of the biology and ecology of the fungi, as well as the importance of their evolutionary development and natural taxonomical relationships, is necessary.

Being a rare and interesting species, *Haploporus suaveolens* (should not be confused with *Trametes suaveolens* (Fr.) Fr.) has received much attention by mycologists over the two last centuries. There are indications of diverse household and medicinal uses of this fungus within North American indigenous peoples (Blanchette, 1997); however, both in America and Europe the fungus has still not been investigated for medicinal and biochemical aspects. The main reason is its scarce distribution and small number of culture strains. Hence, the study of biological features and revealing the natural taxonomical position of this species could have a great importance.

SHORT DESCRIPTION

Haploporus suaveolens (L.: Fr.) Donk, Proc. Koninkl. Nederl. Akad. Wetenschappen (C) 47: 20, 1971. – *Boletus suaveolens* L., Sp. Plant., 1177, 1753. – *Agaricopulpa suaveolens* (L.) Paul., 1793. – *Polyporus suaveolens* L.: Fr., 1821 (misappl.); *Polyporus odorus* Sommerf., 1826: Fr., 1828. – *Trametes odora* (Sommerf.: Fr.) Fr., 1838. – *Fomitopsis odora* (Sommerf.: Fr.) P. Karst., 1881 (nom. invalid.). – *Haploporus odorus* (Sommerf.: Fr.) Singer, 1944; *Ungulina fraxinea* var. *albida* Bourdot, 1932; *Fomitopsis odoratissima* Bond., 1950.

BASIDIOCARPS: perennial, sessile, corky, applanate-ungulate to bracket-shaped, 5–15 × 3–7 × 3–6 cm; surface whitish or cream then covered by thin grayish to brownish crust; margin obtuse, pores 3–4(–5) per mm; context corky, soft, zonate and fibrillose, whitish, yellowish or cream, odor very strong, pleasant, aniseed or almond, taste bitter. HYPHAL SYSTEM trimitic: skeletal hyphae (2–)2.5–3.5(–5.2) µm, in tube trama weakly dextrinoid and cyanophilous, without these reactions in medullar tissue. Binding hyphae 1.7–2.5 µm wide, IKI and CB–. Generative hyphae (1.5–)1.7–2.2(–3.5) µm, clamped. CYSTIDIA none. BASIDIA (13.5–)15–20(–22) × 5–5.8(–6.3) µm, clavate, four-spored, fibulate at the base. SPORES (5.2–)5.5–6(–7.2) × (3.5–)4–5(–5.3) µm, ovoid to short-cylindrical, thick-walled, often guttulate.

ECOLOGY AND DISTRIBUTION

The fungus grows mainly on drying *Salix caprea* in old taiga forests but may occur also on *Syringa, Padus, Cerasus, Ulmus,* and *Tilia* (these data were available from herbarium labels; they seem to be correct). It produces slowly developing white rot reminiscent superficially of *Fomes fomentarius*. The main habitats of *Haploporus suaveolens* are moist herb-rich biotopes where the old trees of *Salix caprea* are abundant. The species is widely distributed in the boreal zone of the northern hemisphere and known from North America, Nordic countries, boreal Russia, and East Asia (Bondartsev, 1953; Eriksson, 1958; Bondartseva, 1961; Niemelä, 1971; Gilbertson and Ryvarden, 1986) but everywhere is rare. Most of known records are from the boreal zone of Europe.

IDENTIFICATION

Characteristic features of *H. suaveolens* are moderately projecting, light-weight rounded caps with obtuse margin and slightly oblique base, obscurely stratified tubular hymenophore with 3–5 pores per mm, and a strong aniseed-almond smell of fresh specimens persisting up to 40 years in herbarium. Pileal form is varying from bracket- or console-shaped to irregular, stalactite-like. *Trametes suaveolens* (Fr.) Fr. is another fungus often growing on *Salix* spp. and having strong aniseed smell; this latter differs by its larger pores (1–3 per mm) with opaque thick dissepiments, and more or less tomentose upper surface. In general, there is no difficulty in identifying *H. suaveolens* in both nature and herbarium.

NOMENCLATURE

The name *Haploporus suaveolens* (L.: Fr.) Donk is seemingly more correct than *H. odorus* (Sommerf.) Bond. et Singer but is avoided by most polyporologists due to its Friesian (1821) misinterpretation and application to another species, known nowadays as *Trametes suaveolens* (Fr.) Fr. However, the Linnaean (1737) description is undoubtedly intended for the first species (Donk, 1971), and Fries' "Systema mycologicum" is start-pointed mycological work due to an automatically sanctioned *name* but not a *species concept*. Another argument to use the name *Polyporus suaveolens* L.: Fr. is the ambiguous status of *Polyporus odorus* Sommerf.: Fr. (1828), which was also misapplied by Fries to his *Trametes suaveolens* (Fr.) Fr. (Fries, 1874: 584, see remark to *Trametes inodora* Fr.). No authentic material for either species exists in his herbarium. Therefore, Bondartsev (1950) proposed a new epithet *Fomitopsis odoratissima* Bond. for *Polyporus suaveolens* L.: Fr.; later Niemelä (1971) selected a neotype for *Polyporus odorus* Sommerf. to use this name (as *Haploporus odorus* (Sommerf.) Bond. et Singer). Both operations remain a nomenclatural discussion to be open because Linnaeus' name formally has a priority. Therefore, Donk's (1971) solution seems to be more logical, especially after neotypification of *Trametes suaveolens* (Fr. non L.) Fr. (Ryvarden, 1991).

TAXONOMICAL POSITION

There are two main concepts on the taxonomical position of the species: 1) this is a trametoid fungus (Haploporaceae, Fomitopsidales – Jülich, 1982); and 2) this is closely related to ganodermoid fungi (Perenniporiaceae, Knudsen, 1995). We have studied the fine structure of the sporoderm of *H. suaveolens* (×1500 magnification, oil immersion) and have received the following results. IKI reaction shows subverrucose to almost smooth outline ("amylose"(+) to amylodextrines+). CB reaction bares the heterogeneity of a spore wall, which contains the acyanophilous fibrillar-crystalline and cyanophilous granular matter. Granules (probably homologous to the pilars of *Fayodia* and *Ganoderma*) compose an exine (up to 1 μm thick) freely fitted by thin uniformly cyanophilous perine. Therefore, a spore surface seems to be granulose, resembling many *Ganoderma* species (SEM of *Haploporus* spores by Niemelä, 1971 are practically identical to those of *Ganoderma* species); then the spores of *H. suaveolens* must be considered as peculiar ganodermoid *hemitectospores*.

Therefore, we disagree with the relationships of *Haploporus* with Ganodermatales, the group con-

taining many resourceful medicinal mushrooms. The species under consideration could be considered as a possible perspective object for biochemical and medicinal research.

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