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Potentiation of Cell-Mediated Host Defense Using Fruitbodies and Mycelia of Medicinal Mushrooms

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ABSTRACT: Mushrooms have drawn the attention of researchers for their medicinal properties. The culturing of mushrooms presents opportunities and problems for the creation of products best suited for consumers, especially for those with immune deficits. Although polysaccharides have drawn the most attention of researchers in the past, other constituent groups, including glycoproteins and ergosterols, promote immune responses. Additionally, the culturing of mycelium on rice creates a novel constituent family—arabinoxylanes. Arabinoxylanes are formed from the fermentation effects from mycelium on the rice carrier. Yield efficiencies of polysaccharides, glycoproteins, ergosterols, and arabinoxylanes vary according to the species utilized, and are qualitatively different in how they influence immunomodulatory responses. This study compares the effects of 7 higher Basidiomycetes species, individually and in concert, on mouse macrophages and natural killer (NK) activity from human spleen cells when co-cultured with cancer cells and exposed to extracted fractions of these medicinal mushrooms.

KEY WORDS: medicinal mushrooms, ethnomycology, arabinoxylanes, glycoproteins, ergosterols, macrophages, immunomodulatory response, mycelium, natural killer cells

INTRODUCTION

More than 460 million years ago, we shared a common ancestry with fungi. We chose the path of engulfing our nutrients whereas the fungi evolved to digest its nutrients externally. Our evolutionary success is the result of complex strategies for preventing parasitization from other organisms, especially microbes. That we share a common ancestry with fungi provides us with an abundance of fungal-based antibiotics to fight common bacterial enemies. Most microbial diseases that afflict plants do not afflict mammals, whereas the diseases attacking fungi also imperil us. Our understanding of mushrooms has evolved for their use as medicinal foods and adjuvant therapies. The manner in which our bodies interact with mushrooms provides us with novel opportunities for optimizing health.

MATERIALS AND METHODS

Processing of Mushrooms

Proprietary strains, sourced and/or originated by the author, were grown under Class 100 clean room conditions on sterilized, certified organic short grain brown rice, according to methods described by Stamets (2000). The moistened rice was sterilized in high-density polypropylene bags and inoculated with mycelium, which was fermented in liquid culture for several days. Each strain was grown to optimize the number of cell

ABBREVIATIONS

AHCC: active hexose-correlated compound; CFU: colony-forming units; NK: natural killer.

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divisions (CFUs = colony forming units) prior to transfer into grain. Once inoculated, each strain was incubated for a duration to optimize their CFU maxima, and then flash frozen to −18°C. The frozen myceliated rice was then freeze-dried in a negative pressure vacuum of 1500–2000 millibars and then heated to 75°C for 24 hours. The freeze-dried material was then milled to a fineness of 200-800 μ mesh. This freeze-dried powder was then processed through an autoclave at 120°C, at 1 kg/sq cm, for more than 4 hours, and resulted in CFUs under 100 colonies/g. This raw material was then presented for analysis.

Analysis of Primary Constituents

Beta glucans were measured using the glucose oxidase method. To make soluble glucose, a 1-g sample with 5 mg of cellulose was mixed with 0.1 mol of phosphoric acid as a relieving agent, at pH 6.5, and maintained at a temperature of 50°C for 21 hours. Another 1-g sample with 0.1 mg of chitinase was mixed with 5 mL of citric acid as a relieving agent, at pH 6.5, and maintained for 1 hour at 50°C. The liquids, after treatment with enzymes, were kept for 16 hours at 4°C to dialyze, and then were freeze-dried and made into a powder, which was then measured.

The structure of beta glucans was determined using a NMR, Model MY-60 (Nippon Electric Co., Japan) kinds and relative structural percentage of beta glucan by type was measured. For controls, 1-3, 1-4, and 1-6 of Reishi (IFO-1988) were used. The relative structural signatures were calculated from absorption areas of the NMR signatures. Signal absorption allowed the calculation of relative structures.

The method for measuring crude arabinoxylanes was calculated by the following procedure. A 5-g sample with 20 mL of 1 M sodium chloride was treated at 50°C for 2 hours. Then 20 mL of 2 M potassium chloride was added, followed by the addition of 0.3 mol 10 mL of barium hydroxide. After 2 hours at 50°C, the soluble portion was freeze-dried. Mass was determined by measuring mg/g.

The method for measuring glycoprotein was calculated by the following procedure. NaCl (0.05 mol 50 mL) was added to the 5-g sample. The mixture was blended for 48 hours at 35°C and then centrifuged at 10,000 rpm for separation. The precipitant was diluted 500% with 75% ETOH. The precipitate was then treated with acetic acid (pH 4.0) as a relieving agent and centrifuged at 12,000 rpm, with the residue separated from the solution. The precipitant was saturated with 40% ammonium sulfate for 16 hours and then centrifuged at 8,000 rpm and separated from the residue. Ammonium sulfate was added to 60% of mass, and then the precipitant was freeze-dried for measuring glycoproteins.

The method for measuring for ergosterol (mg/g) utilized the addition of 20 mL of ethylene chloride; it was extracted by centrifuging at 10,000 rpm for 30 minutes and the precipitant was then collected. Acetone (500% to mass) was added to the mass of the precipitant and dialyzed with distilled water for 24 hours. The precipitant was then treated with chloroform at minus −4°C. The resulting precipitant was crude ergosterol.

RESULTS AND DISCUSSION

Long ago, people in Europe and Asia recognized Polyporaceae species (wood conks) as rich resources for treating a variety of illnesses. One of the first polypore mushrooms recommended medicinally was *Fomitopsis officinalis* (Vill.:Fr.) Bond. et Singer (Quinine Fungus), by Dioscorides in 65 AD, as a treatment against "coughing illness" (i.e., consumption), later thought to be tuberculosis (see Fig. 1). *F. officinalis* has been known as the source of agaricin, a pharmaceutical compound used as a febrifuge in the treatment of wasting diseases. The fungus was known to the ancient Greek natural philosophers and medieval herbalists as Agarick or Agarikon, and their writings attribute almost miraculous healing properties to Agarick for a wide range of human ailments. The Haida First Peoples of the Queen Charlotte Islands of British Columbia used this mushroom medicinally to stave off diseases from the spirit world (Blanchette et al., 1992). This species is now so rare as to be thought to be extinct in regions of Europe, and it is becoming increasingly scarce around the world as trees decline in numbers and size.

Of the 1 to 2 million species of fungi, only about 10% are mushrooms, of which about 15,000

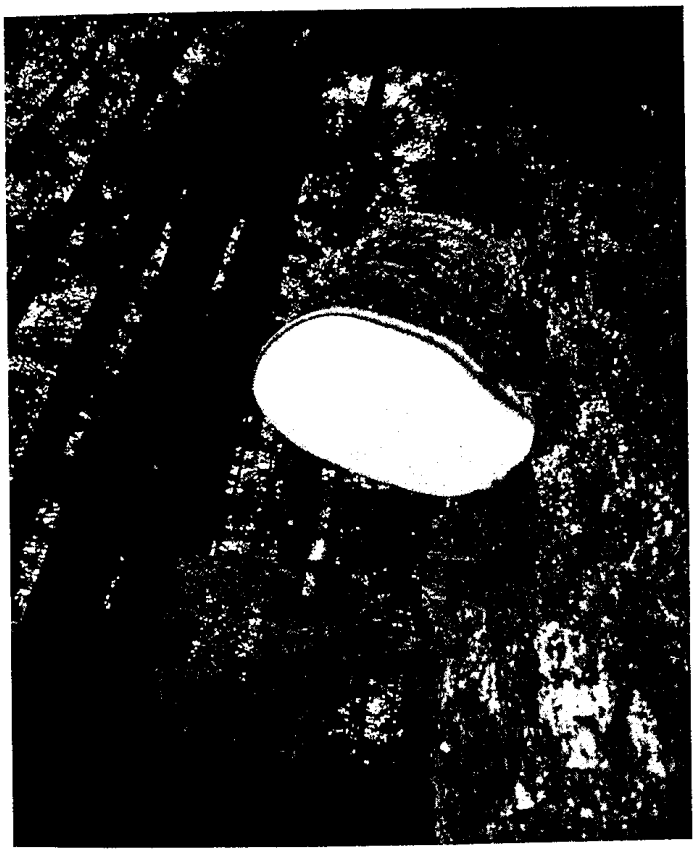

FIGURE 1. A conk of *Fomitopsis officinalis*, approximately 25 years old, growing in the Old Growth forest of the Elwha River valley in the Olympic mountains of Washington State, USA. (Photo by P. Stamets)

are described. A good estimate is that 150,000 species of mushroom exist in the genome, with current calculations showing about 5% having interesting nutritional or medicinal properties. This means that there are roughly 7500 more mushroom species, yet untapped, worthy of pursuit by medicinal mushroom researchers, given our current tools and interests (Hawksworth, 2001). With the sudden loss of unique habitats, from deforestation, pollution, and population stress, the fungal genome appears to be declining, in concert with the loss of other biological communities (Leck, 1991). The rate of decline in biodiversity of fungi, however, is obscured by a lack of knowledge and inability to identify species as rapidly as they are disappearing. In some cases, mushroom species previously thought to be on the brink of extinction have rebounded in habitats left alone to mature naturally (Watling, 1988). As our framework of reference is so short—only 200 years at most—it is not yet possible to know how long the mycelial mats of species remain resident before declining below the threshold for recovery.

Often times, mycelium can grow for many years before producing a single fruitbody. This latent mycelial matrix is the key to the competitive nature of fungi, and the source of many of its most important constituents and metabolites. As the mycelium differentiates into a fruitbody, cell specialization occurs, as more features are expressed. Each species has a unique cellular architecture, primarily composed of cell wall sugar polymers whose degradation through heat, in combination with digestion, results in unique subderivatives, many of which activate immune responses.

Some mushrooms, like *Bridgeoporus nobilissimus* (Cooke) Volk, Burdsall et Ammirati (the Noble Polypore), a giant mushroom notable for its longevity in the harshest of environments, may hold secrets for cell life survival and resistance to disease (see Fig. 2). Reported from the Cascades and Olympics of Washington and Oregon, this mushroom only forms on trees—or stumps of trees—that are hundreds of years old. As old growth habitats recede, this mushroom's options for survival become increasingly limited (Stamets, 2002b).

Annual polypores, such as *Ganoderma lucidum* (Curt.:Fr.) P. Karst. (Reishi mushroom) (see Fig. 3), a centerpiece of the Asian ethnopharmacopoeia, fare better than the perennials, not requiring such large host trees before producing progeny—spores—from their wood-like shelfs. Through tissue culture, many strains can be preserved to protect the genome from further loss, at a time when human health is imperiled from both biological and chemical toxins. By culturing these mushrooms through cloning, we capture a phenotype, a strain for future study. As this and other studies show, mushrooms are powerful, natural medicines, with species-specific properties applicable to diverse targets for disease prevention and cure.

Collecting mushrooms in the wild for medicinal properties is often difficult and unreliable. Even more so, acquiring mushrooms from distributors who purchase mushrooms from multiple, untraceable sources makes medicinal research studies dangerously unrepeatable. As strains vary in potency of various constituents, working with a

FIGURE 2. The mammoth, *Bridgeoporus nobilissimus*, the first mushroom species in the United States to be considered endangered. This species grows exclusively on Old Growth trees, especially Noble Firs (*Abies procera*), and lives for decades, perhaps longer, resisting rot. This species may generate the second largest mushrooms in the world, with *Rigidioporus ulmarius* (Sow.:Fr.) Imazeki, at Kew, Royal Botanic Gardens in England being the largest known thus far. (Photo by Jim Gouin)

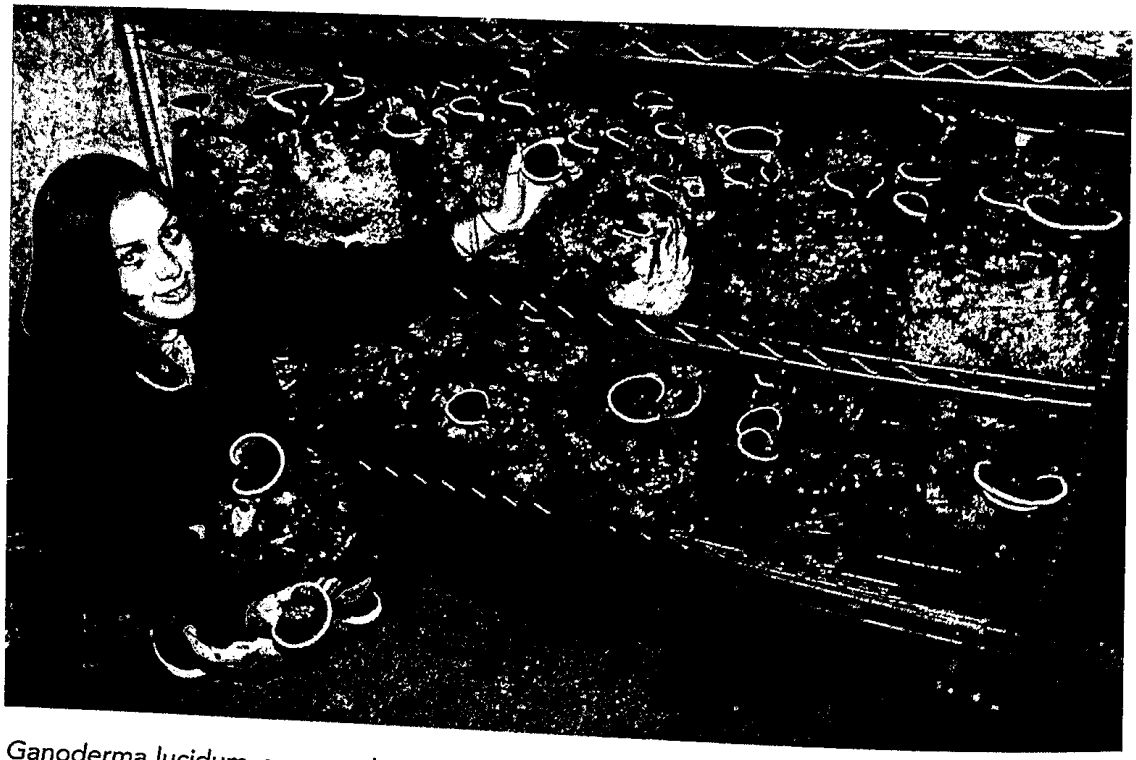

FIGURE 3. *Ganoderma lucidum*, an annual mushroom commercially cultivated at Fungi Perfecti Research Laboratories, Kamilche Pt., Washington State, USA. (Photo by P. Stamets) This strain was used in the current study.

strain of ascertained potency clarifies recommendations for use. Not only do strains differ in their potencies, they are also unique in their rate of decline in vitality. Strains that are over-cultured are millions of cell divisions away from their genetic origins. As most mushrooms are sexual in their reproductive cycles, their mycelia lose vitality with age. One method for preventing premature or natural senescence is to keep the strains closest to their natural origins by using young strain stocks (Stamets, 2000). Limiting the number of cell divisions of master cultures allows for the type of explosive growth as represented in Figure 4.

Although use spans millennia, scant research authenticating their legendary properties has been available until recently. The first credible, scientific studies were conducted in Japan by Ikekawa, an epidemiologist at the National Cancer Research Institute in the late 1960's, which led to the identification of numerous anticancer constituents in mushrooms (Ikekawa, 1969, 1985). In studying 20 years of cancer trends, he determined that mushroom consumption was the causal factor responsible for significantly lowering the background cancer rates in the prefectures where mushroom cultivation centers significantly contributed to the local diet (Ikekawa, 1989).

Mushrooms emerge from the network of cells called mycelium. As the loose mycelium compacts and blossoms into a mushroom, the seemingly uniform mycelium differentiates, developing into unique features, and is collectively recognized as a fruitbody, or mushroom. Both mycelium and mushroom are composed primarily of heavy molecular weight sugar polymers (hemicellulose, polysaccharides, glycoproteins), which, upon ingestion, either activate the immune system or can be directly tumoricidal, while not harming healthy host cells (Ooi and Liu, 2000; Wasser, 2002a,b). In cloning a mushroom, the cell line regrows as mycelium. Just before fruiting, the cells fill with nutrients, become multinucleate, suddenly expanding, allowing for rapid construction of a fruitbody. This potential for fructification defines

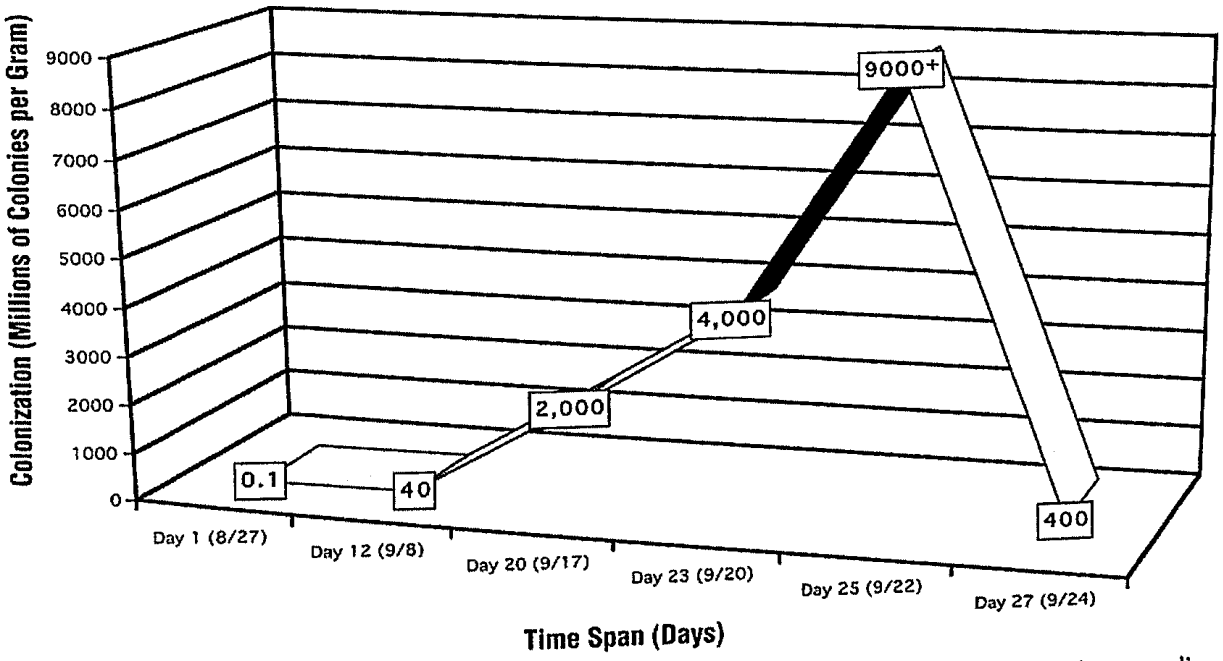

FIGURE 4. Classic growth of mycelium on sterilized grain, charted over time. After peaking, the mycelium quickly declines in vigor, with increasing cell loss and greater susceptibility to bacterial disease. Tracking strains for optimum performance means intimately knowing the nuance of each strain, especially its origins and the numbers of generations through which it has been subsequently re-cultured. Growth performance is limited by senescence, all other factors being equal. Each strain has a growth signature, achieving different optima of CFUs (colony-forming units), a direct reflection of cell mass and, in most cases, potency. Aged mycelium cannot achieve the explosive cell growth depicted here.

the potency of a strain, and it is the fertile mycelium that shows the greatest promise for diverse medicinal products.

Foremost, mushrooms have been used as food and are nutritionally rich with crude protein, ranging from 10–40% (Chang and Hayes, eds., 1978), and with significant amounts of vitamin B_2, niacin, and foliates at 1.8–5.1, 31–65, and 0.36–0.64 mg/100 g, respectively, based on dry weight. Mushrooms are an especially good source of ergosterol, which is activated by sunlight into provitamin D_2. One species in this study, *Cordyceps sinensis* (Berk.) Sacc., had ~7% ergosterol when the mycelium was grown on rice (see Table 1). Ergosterol also imparts cancer-controlling effects in concert with polysaccharides, glycoproteins, triterpenoids, and the nascent antimicrobial properties inherent within mushrooms. For example, in studying *Agaricus bisporus* (J. Lge) Imbach, *Lentinus edodes* (Berk.) Singer, and *Pleurotus ostreatus* (Jacq.:Fr.) Kumm., an average of ~11 g/kg of phosphorus, ~37 g/kg of potassium, and ~70 g/kg zinc are contained within the mushrooms, by dry weight (Mattila et al., 2001). Ubiquitin-like glycoproteins from mushrooms have strong antiviral activities. Wang and Ng (2000) first identified a novel glycoprotein from oyster mushrooms that inhibits HIV-1 reverse transcriptase activity, causing cleavage of transfer RNA and resulting in the failure of viral proliferation. Glycoproteins are abundantly manufactured by the mycelium on rice. For instance, glycoprotein content can constitute up to ~40% of the dry mass of myceliated rice from colonization by *Grifola frondosa* (Dicks.:Fr.) S. F. Gray. However, many nutrients from mushrooms are unavailable until they have been cooked, which softens the tough chitinous-like hyphal structure, increasing overall digestibility of both cell wall constituents and releasing cellular components. Furthermore, heat sterilization destroys errant microbials that may be feeding on the mycelium, a consequence exacerbated by loss of vigor as strains senesce with age.

All strains age. All strains die. Bacteria are ready to exploit the rich nutritional resource mushrooms provide, at the earliest opportunity. Mushrooms have evolved to produce antibacterial agents to stave off infection. Once the fungus has aged, the natural defenses of the mycelium succumb to bacterial predation. This is an important consideration in maintaining cultures at the peak of their growth, and handling the mycelium with as little disturbance as possible. Only through careful preservation of strains, through good laboratory practices, can consistency of production of medicinal mushrooms be assured, and the life force of the mushrooms be protected.

Many constituents in mushrooms have direct medicinal properties as antiviral (Brandt and Piraino, 2000), antiprotozoal (Lovy et al., 1999), antitumor (Mizuno et al., 1995), antibacterial (Suay et al., 2000; Stamets, 2002a), and antioxidant (Mau et al., 2002) agents. Modern medicine is increasingly attracted to medicinal mushrooms as adjuvant and/or preventive therapies. Cancer growth is limited by exposure to mushrooms' tumoricidal protein-bound polysaccharides, which cause apoptosis of diseased cells while activating the host's immune defense cells, particularly natural killer (NK) cells and macrophages. Furthermore, mushrooms contain strong antioxidants, which stimulate the expression of manganese superoxide anions and dismutase-mimicking compounds that mitigate the effects of free radicals, protecting mitochondria from damage due to oxidative stress (Pang et al., 2000).

Exotic anticancer, tumorigenic substances from mushrooms have also been isolated. From the poisonous mushroom, *Omphalotus olearius* (DC.:Fr.) Fay.) (a mushroom that glows in the dark), strong tumor-killing agents show promise as chemotherapeutic medicines. These isolated constituents, called illudins, are cytotoxic to a wide range of cancer cells (lung, colon, ovarian) upon exposure, and this process is enhanced when combined with other chemotherapeutic agents, such as irinotecan (Britten et al., 1999) or mitocyin C (Kelner et al., 2002). Because this mushroom is poisonous, it is more likely to be a source of chemotherapeutic agents for the pharmaceutical rather than for the nutraceutical products industries.

A new heat stable, chemotherapeutic compound inhibiting the expression of aromatase, an enzyme associated with breast cancer tumor development that ameliorates estrogen production, has been isolated from the commercial portobello mushroom, known to science as *Agaricus bisporus* or *A. brunnescens* Peck (Bankhead, 1999; Grube et al., 2001). Many mushrooms are likely to have these aromatase-inhibiting compounds, which could

TABLE 1
Comparative Table of Natural Killer (NK) Cell Activity from Human Spleen Cells in Response to Primary Active Constituent Families Generated from the Mycelia of Medicinal Mushrooms

Species	Crude Arabinoxylane Content (mg/1g)	Glycoprotein Content (mg/1g)	Ergosterol Content (mg/1g)	Beta Glucans				% Increase in NK Activity (100mg/Day for 5 Days)
				Type			Content (mg/1g)	
				1,3	1,4	1,6		
Seven mushroom mycelium blend	148.5	265.1	28.3	++	+	++	20.04	97.6
Maitake mycelium *(Grifola frondosa)*	112.7	399.4	16.7	+	+	+++	25.1	92.9
Himematsutake mycelium *(Agaricus brasiliensis) [=Agaricus blazei*]*	78.0	387.4	19.2	++	-	++	18.3	89.4
Chaga mycelium *(Inonotus obliquus)*	93.1	308.7	18.4	+++	-	++	29.6	82.9
Reishi mycelium *(Ganoderma lucidum)*	109.5	284.5	14.5	+++	-	+	21.9	78.5
Cordyceps mycelium *(Cordyceps sinensis)*	240.1	321.5	67.9	++	-	++	20.5	71.4
Mesima mycelium *(Phellinus linteus)*	196.5	198.4	37.1	++	-	++	27.6	54.0
Yamabushitake mycelium *(Hericium erinaceus)*	238.9	215.7	37.1	++	-	+	12.3	53.0

*See Wasser, et al. (2002)

have important implications for the dietary profiles of postmenopausal women who are at high risk for breast cancer. Studies are currently examining other mushrooms for these estrogen-modulating factors.

Another Agaricus mushroom attracting much attention is from Brazil, commonly called the Royal Sun Agaricus or Himematsutake (see Fig. 5). This mushroom was at first thought to be *Agaricus blazei* Murrill, originally described from Florida (USA). However, recent taxonomic studies show that this Brazilian mushroom is not the same as Murrill's Floridian species and has recently been published as a new species, *Agaricus brasiliensis* Wasser et al. (Wasser et al., 2002). Upon being told of their health-enhancing properties by the local population, specimens of this mushroom were collected by Japanese coffee growers in Brazil and taken back to Japan for study. Both portobello (*Agaricus bisporus* = *A. brunnescens*) and *A. brasiliensis* contain hydrazines, called agaritines, whose subderivatives are highly carcinogenic (Stijve and Amazoṇas, 2001). Hydrazines are primary cancer-causing agents in tobacco smoke. Agaritines are more heat stable than most hydrazines, which are classically volatile, and cause free radical damage to contacted tissues. Agaritines are denatured when cooked at high temperatures using a technique that, concurrently, does not adversely affect their beneficial medicinal properties.

Protein-bound mushroom polysaccharides behave as superoxide dismutase-mimicking compounds, helping to remodulate immune function by restoring the function of NK cells (Nakamura and Matsunaga, 1998). Perhaps the best studied mushroom for use as an adjuvant to conventional therapies is *Trametes versicolor* (L.:Fr.) Pil. (= *Coriolus versicolor* (L.:Fr.) Quél.) (see Fig. 6). In clinical studies of 224 patients (Sugimachi et al., 1997) and 262 patients (Nakazato et al., 1994) afflicted with gastric cancer and treated with chemotherapy, followed by a regimen using the protein-bound polysaccharide (PSK) from *T. versicolor*, the results showed a significant decrease in recurrence and increase in the 5-year disease-free survival rate of the patients by ~22%. Studies also show that serial dilutions of a cold water/ethanol extract from the mycelium of Fungi Perfecti's *T. versicolor* strain induced profound apoptosis in human carcinoma cell lines in a time- and dose-dependent manner (Fisher et al., 2003).

Dried fruitbodies and freeze-dried mycelium-fermented ("myceliated") rice of the same strain of

FIGURE 5. *Agaricus brasiliensis* (grown by David Sumerlin and the author).

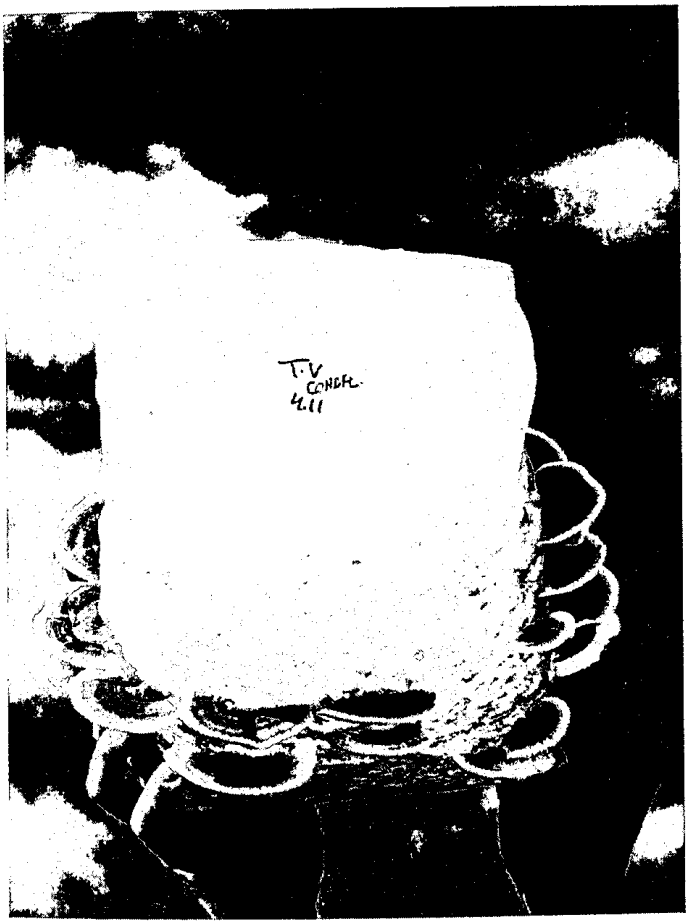

FIGURE 6. *Trametes versicolor* grown on sterilized sawdust. This is the same strain reported herein and also used in the carcinoma studies by Fisher et al. (2003). (Photo by P. Stamets.)

Ganoderma lucidum from Fungi Perfecti Laboratories was presented for analysis. Dried *G. lucidum* fruitbodies contained 40% beta glucans, whereas the myceliated rice contained only 2–3%. Arabinose and xylose content of brown rice, bound within hemicellulose, constitutes only 0.41% and 0.35% of the dry mass, respectively (Juliano, 1985). Notably, species differ in their modification of rice and the production of active constituent groups. After mycelial fermentation with *G. lucidum*, the content of crude arabinoxylanes escalates to 11%. Of the 7 species tested, *Agaricus brasiliensis* modified the rice, creating 7.8% arabinoxylanes, whereas *Cordyceps sinensis*, on the same carrier, generated 24% arabinoxylanes. The extremes with glycoprotein production ranged from ~20% with *Phellinus linteus* (Berk. et Curt.) Teng to ~40% with *Grifola frondosa*. Interestingly, the species producing the least amount of ergosterol, at ~1.5%, was *Ganoderma lucidum*, whereas *Cordyceps sinensis* produced the most, at ~7% (see Table 1). These differences in mycofermentation yields illustrate the uniqueness of each species' biological activities and will be determining factors in the future for designing target-specific mycomedicinals.

The use of mushroom mycelia to enzymatically modify arabinoxylanes, the key ingredient in active hexose-correlated compound (AHCC), was extensively studied in Japan and then in the United States (Ghoneum et al., 1995; Ghoneum, 1998a), showing significant augmentation of human immune function. Most manufacturers of mushroom-induced arabinoxylane products use a single species, *Lentinus edodes*, although early work by Ghoneum reported the use of three, including *L. edodes*, *Schizophyllum commune* Fr.:Fr., and *Trametes versicolor* (Ghoneum, 1998b). The study presented in this article clearly shows that other species are better modifiers than those previously reported.

Ethanol/water extracts from *Ganoderma lucidum* fruitbody potentiated macrophage activity, as measured from the expression of superoxide anions, to 3000%, whereas the *G. lucidum* myceliated rice elicited a 5400% response (see Table 2). With NK cells, extracts of the *G. lucidum* fruitbodies enhanced activity by 321%, whereas the myceliated rice caused NK enhancement by 253%. In comparing the results of using a 7-species blend, the macrophage activity increased by 3267% from the myceliated rice, compared to a mean average of 2962% for the activity of each of the 7 species individually. The greatest macrophage activity from the species surveyed, 5800%, came from *Phellinus linteus*, a medicinal mushroom increasingly popular with mycomedicinalists in Korea.

When mycelium is grown beyond its optimum, not only is there a decrease in vitality of the cells, as depicted in Figure 4, but there is also a decline in activity as an immunomodulator. For instance, when *Ph. linteus* was grown out on sterilized rice for 60 vs. 150 days, beta glucans increased marginally from 2.3% to 2.7% and, in response, NK activity declined from 237% to 174% and macrophage activity declined from 5800% to 3200%, underscoring that the age of mycelium, a function of both duration of incubation and the ability of the mycelium to generate downstream cell populations, pro-

TABLE 2
Mouse Macrophage Activity from Implanted Sarcoma180 in Response to Medicinal Mushrooms

Mushrooms Tested	Dosage / Mouse (µg/g)	Macrophage Expression of SOA			
	Hours	0	50	100	150
Saline Control	100	2	34	40	41
Seven mushroom mycelium blend	50	3	49	87	91
	%increase	100%	1633%	2900%	3033%
	100	3	67	92	98
	%increase	100%	2233%	3067%	3267%
Himematsutake mycelium *(Agaricus brasiliensis) [=Agaricus blazei*]*	50	2	40	63	76
	%increase	100%	2000%	3150%	3800%
	100	3	47	75	88
	%increase	100%	1567%	2500%	2933%
Maitake mycelium *(Grifola frondosa)*	50	3	27	58	79
	%increase	100%	900%	1933%	2633%
	100	3	39	69	83
	%increase	100%	1300%	2300%	2767%
Cordyceps mycelium *(Cordyceps sinensis)*	50	2	46	80	89
	%increase	100%	2300%	4000%	4450%
	100	3	51	85	92
	%increase	100%	1700%	2833%	3067%
Chaga mycelium *(Inonotus obliquus)*	50	2	19	33	61
	%increase	100%	950%	1650%	3050%
	100	2	27	41	73
	%increase	100%	1350%	2050%	3650%
Yamabushitake mycelium *(Hericium erinaceus)*	50	2	16	28	39
	%increase	100%	800%	1400%	1950%
	100	2	19	32	46
	%increase	100%	950%	1600%	2300%
Meshima mycelium *(Phellinus linteus)*	50	1	19	33	58
	%increase	100%	1900%	3300%	5800%
	100	2	24	47	67
	%increase	100%	1200%	2350%	3350%
Reishi mycelium *(Ganoderma lucidum)*	50	1	18	29	43
	%increase	100%	1800%	2900%	4300%
	100	1	21	36	54
	%increase	100%	2100%	3600%	5400%
Reishi fruitbody *(Ganoderma lucidum)*	50	3	37	79	89
	%increase	100%	1233%	2633%	2967%
	100	3	41	83	90
	%increase	100%	1367%	2767%	3000%

*See Wasser, et al. (2002)

TABLE 3
Comparative Table of Natural Killer (NK) Cell Activity from Human Spleen Cells in Response to Medicinal Mushrooms

Species Administered	#mg/kg per Day for 5 Days	NK Activity (%) From Human Spleen Cells Co-Cultured With Cancer Cells	NK Activity (% Versus Control)
Control (saline)	100	31.0 ± 2.8	100
Seven mushroom mycelium blend	50	95.8 ± 4.8	309
	100	97.6 ± 5.1	315
Himematsutake mycelium *(Agaricus brasiliensis) [=Agaricus blazei*]*	50	78.6 ± 1.9	254
	100	84.9 ± 2.8	274
Cordyceps mycelium *(Cordyceps sinensis)*	50	65.4 ± 3.9	211
	100	71.4 ± 3.8	230
Reishi mycelium *(Ganoderma lucidum)*	50	64.4 ± 3.3	208
	100	78.5 ± 1.8	253
Reishi fruitbody *(Ganoderma lucidum)*	50	93.4 ± 4.5	301
	100	99.4 ± 5.3	321
Maitake mycelium *(Grifola frondosa)*	50	82.4 ± 2.6	266
	100	92.9 ± 3.1	300
Yamabushitake mycelium *(Hericium erinaceus)*	50	42.4 ± 1.8	137
	100	53.0 ± 2.3	171
Chaga mycelium *(Inonotus obliquus)*	50	72.4 ± 1.6	234
	100	82.9 ± 2.3	267
Mesima mycelium *(Phellinus linteus)*	50	41.8 ± 1.9	135
	100	54.0 ± 2.5	174

*See Wasser, et al. (2002)

foundly influences quantifiable immune markers (see Tables 2 and 3). Maintaining cell lines closest to their genetic origins, combined with knowing growth curve optima, is critically important for designing the best medicinal mushroom products. Work in progress will analyze the activity of increasingly complex combinations of species of medicinal mushrooms in comparison to their activities individually.

Constellations of active constituents from mushrooms offer a powerful means of potentiating host defense. Mushrooms are a rich source for novel medicines that can afford a broad defense shield against immunological disorders and, in some cases, be used target-specifically to a particular illness. That unique agents are specific to individual mushrooms underscores the complexity of the fungal genome and its potential use for developing new treatment regimens. Despite dramatic differences in beta glucan and arabinoxylane contents between mushrooms and their mycelium-on-grain, immune function is similarly enhanced. These results suggest that immune response is further augmented when multiple mushrooms are used in concert. Synergistic combinations of mushroom species appear to activate collective subfields of receptor sites, eliciting a wide range of positive responses that result in improved immunological health. The future for medicinal mushrooms leads toward optimizing proportionalities with vigorous strains, customized to the host defense profile of the subject, with consistency in the production of cellular constituents and by-products.

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