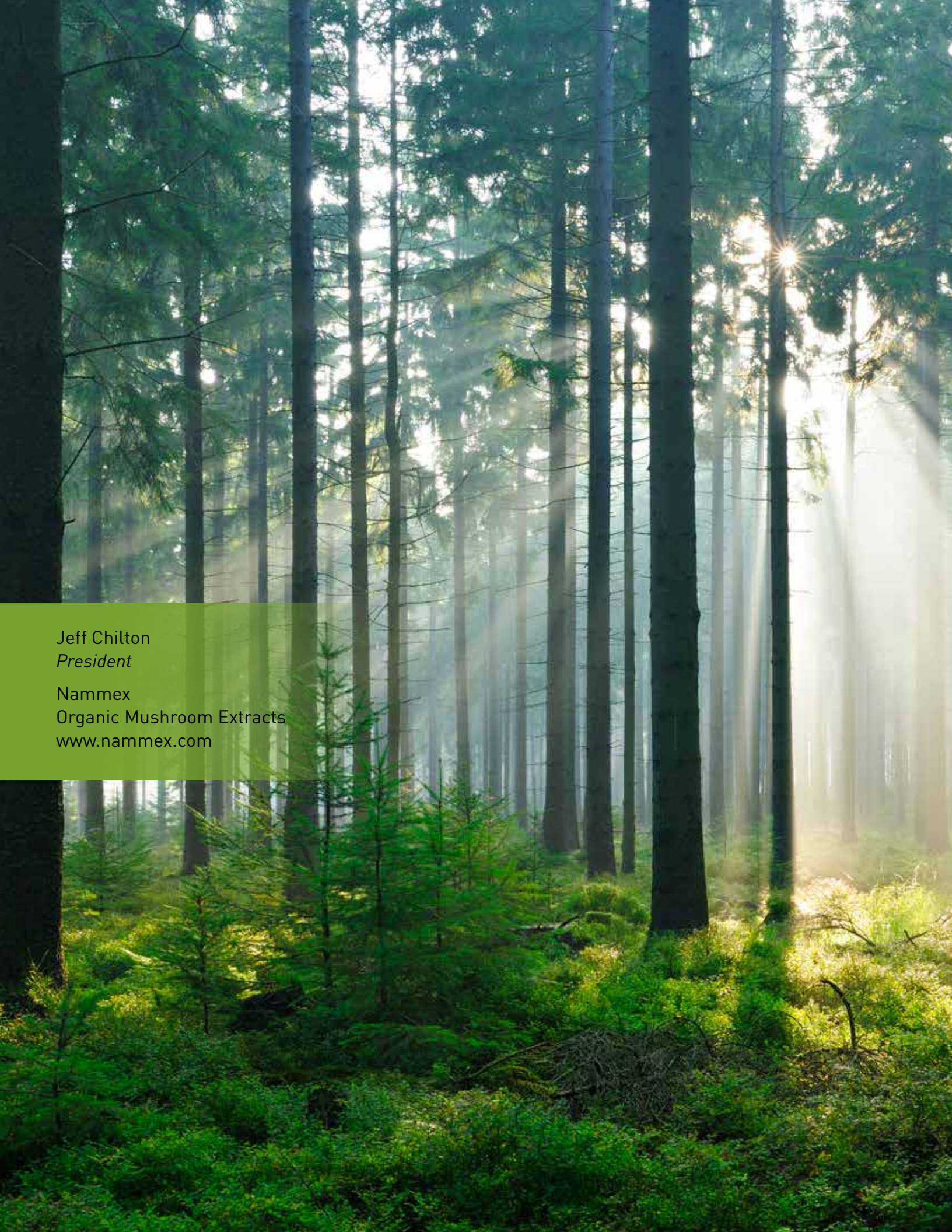
Redefining Medicinal Mushrooms

A new scientific screening program for active compounds

nammex
Organic Mushroom Extracts



Jeff Chilton
President

Nammex
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www.nammex.com

Executive Summary

There is significant concern within the regulatory community regarding health claims made about nutritional supplements as well as about the identity and purity of the natural products themselves. Medicinal mushrooms are a category that has experienced high growth but few actual quality control standards. This analytical program provides an opportunity for manufacturers to realize a higher level of product efficacy and benefit.

The proper identification and delineation of "plant part" is clarified. A basidiomycete organism has 3 main parts: a mycelium, mushroom and spore.

Key active compounds are identified as beta-D-glucans, triterpenoids and ergosterol. Starch is utilized as an indicator of adulteration. Analytical methods which can quantify the key active compounds are identified and used to test approximately 100 mushroom and mycelium samples.

Results of the analyses demonstrate that mushrooms are high in beta-D-glucans and very low in starch. Mycelium produced on cereal grains is low in beta-D-glucans and high in starch. Ergosterol analysis shows the actual amount of fungal material in the products.

Mushrooms grown on natural substrates have the precursors to produce important secondary metabolites such as triterpenoids whereas mycelium produced on cereal grains lack these precursors.

Reishi mushrooms growing on buried wood logs in a greenhouse.

Introduction

Medicinal mushrooms are fungal organisms that are considered health foods, nutritional supplements and nutraceuticals. They are part of a very extensive natural health products category in Asia where Traditional Chinese Medicine has utilized herbal preparations for thousands of years. Asia is also the historical site of mushroom cultivation. Shiitake cultivation is reported to have originated in China in the 12th century. Today China is responsible for 70% of the world's mushroom production.

It is estimated that the worldwide market value of medicinal mushrooms was US $6.0 billion in 1999, and $18.0 billion in 2014 [1,2]. Over the last 25 years the market for these products has expanded greatly in North America. In 1990 there were only a few nutritional supplement companies offering mushroom products whereas in 2015 just about every company has one or more mushroom preparations in their product line. One might say that medicinal mushrooms have arrived and in view of their growth trajectory, are destined to become a much bigger market in North America.

As more medicinal mushroom products come to the market, systematic scientific verification of the active compounds will become more important. This has been true of all medicinal herbs sold as nutritional supplements and is a critical component of the regulatory framework that is so important to public health and safety. Verification of active compounds is also consistent with the need to keep the marketplace relatively free from products that are devoid of medicinal value, which can damage the credibility of the category. It is in every company's interest to do whatever possible to guarantee the activity of the products it sells.

If we are able to evaluate mushroom products on the basis of two or more of the primary active compounds, this would bring a level of consistency and uniformity to an otherwise uncertain and unknown area of product quality [3].To this end, the most important outcome would be the ability to prescribe or at least be more accurate in the recommendations of an efficacious and therapeutic dose. The end result would be greater consumer confidence in the product as well as a higher level of product integrity.

Defining Medicinal Mushrooms

As simple as this may seem, medicinal mushrooms as a category encompass more than just mushrooms. This is due to the fact that a mushroom is just one stage or "plant part" of a fungal organism. This particular fungal organism that we generally speak of as a medicinal mushroom belongs to a taxonomic grouping called basidiomycetes [4]. An understanding of the life cycle of a basidiomycete helps to assess the medicinal activities of the respective stages, since all stages are currently utilized for their medicinal properties.

It is fitting to start with the spore, which is a beginning and an end of the basidiomycete life cycle. Microscopic spores are generated in the billions, even by a single mushroom. Present in large numbers in all soils, spores have also been counted at up to 10,000 in a cubic meter of air. Similar in function to a seed, fungal spores begin the life cycle

by germinating when environmental conditions are favorable. The germinating spore produces a hypha, a threadlike tube that spreads and branches in every direction. When multiple spores germinate and their hyphae grow together, a network called a mycelium is formed [4].

Mycelium is the vegetative state and the assimilative stage of a fungal organism [5]. Simply put, mycelium is the part of a basidiomycete that grows into its surroundings amassing nutrients that allow it to propagate and also produce a mushroom. The mushroom is defined as a specialized reproductive structure of the fungus.

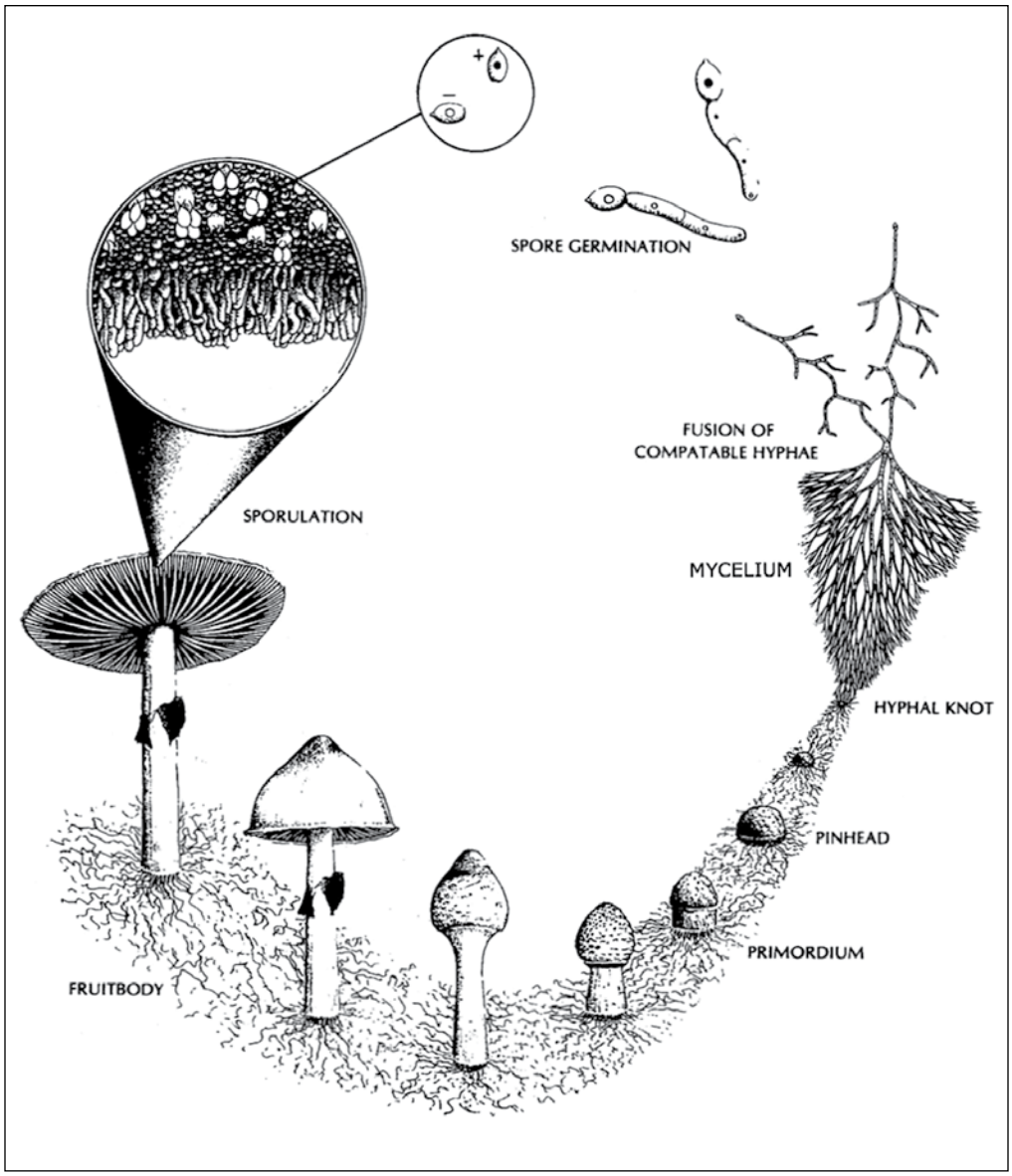


Figure 1. The basidiomycete life cycle. [51]

Mycelium is the "body" of a fungal organism whereas the mushroom is considered the "fruiting body"[6]. Mushroom and mycelium are not synonymous.

In nature one rarely sees the mycelium because it is naturally embedded in its food source, what is often referred to as the "substrate." Using the secretion of enzymes to digest its substrate, mycelium grows in a constellation of mostly dead organic matter such as dead trees, woody debris, fallen leaves, and annual plants of all kinds. Fungal mycelium is one of nature's premier recyclers. At times mycelium is exposed and can be seen, but rarely and only when environmental conditions are compatible with the mycelium's need for moisture.

When environmental conditions are conducive, a fertile mycelium will produce a mushroom. Like many organisms, this life cycle generally follows a predictable pattern. The warmer temperatures of spring and summer stimulate vegetative mycelium growth and the assimilation of nutrients via enzymatic digestion. Cooler temperatures and change of season to autumn signal fruit body formation. Whereas the mycelium is a rather simple structural unit with a design that favors the broadest possible growth pattern, the mushroom is a more complex and complicated form that has the ability to produce a plethora of interesting natural compounds.

In summary, the mycelium "body" accumulates food and energy which enables it to spread and to ultimately produce a mushroom. The mushroom is the "fruiting body" and produces the spores or "seed." The mushroom produced spores will germinate into threadlike tubes called "hyphae." These hyphae grow together in a mass of filaments to form the mycelium [7]. Now the life cycle of the fungal organism is completed. And although the mushroom and the mycelium are similar in that both are composed of hyphae, they are meaningfully different in structure, composition and function.

What is commonly referred to as a medicinal mushroom is actually a fungal organism with three distinct plant parts: Mushroom, Mycelium and Spore.

It is therefore important to make this distinction. One can correctly say Reishi mycelium or Ganoderma mycelium. But it is incorrect to say Reishi mushroom mycelium since these are separate entities. It follows that one cannot have a product label that says Reishi mushroom when the ingredients are primarily Reishi mycelium or Reishi spores.

Active Medicinal Compounds

The primary medicinal compounds in basidiomycetes have been identified and characterized and therefore can be targeted for analysis.

Beta-D-glucans

Extensive scientific research has shown that there are a number of different chemical compounds in mushrooms and mycelium that are responsible for their medicinal properties. A significant amount of this initial research comes from the development of basidiomycete-based approved drugs in Japan and China: Lentinan, a pure (1→3)beta-D-glucan [e] extracted from shiitake mushroom, and PSK/PSP, protein-bound beta-glucans from the fermentation of *Trametes* [c] mycelium. Beta-glucans and protein-bound beta-glucans have been identified as primary sources of medicinal basidiomycete activity. The research involved in this drug development initiated a flood of further investigation that has produced a compelling body of evidence to support the use of basidiomycetes as nutritional supplements. In essence, this mushroom-based drug research has given us a direct path to marker compounds that can be utilized for quality control.

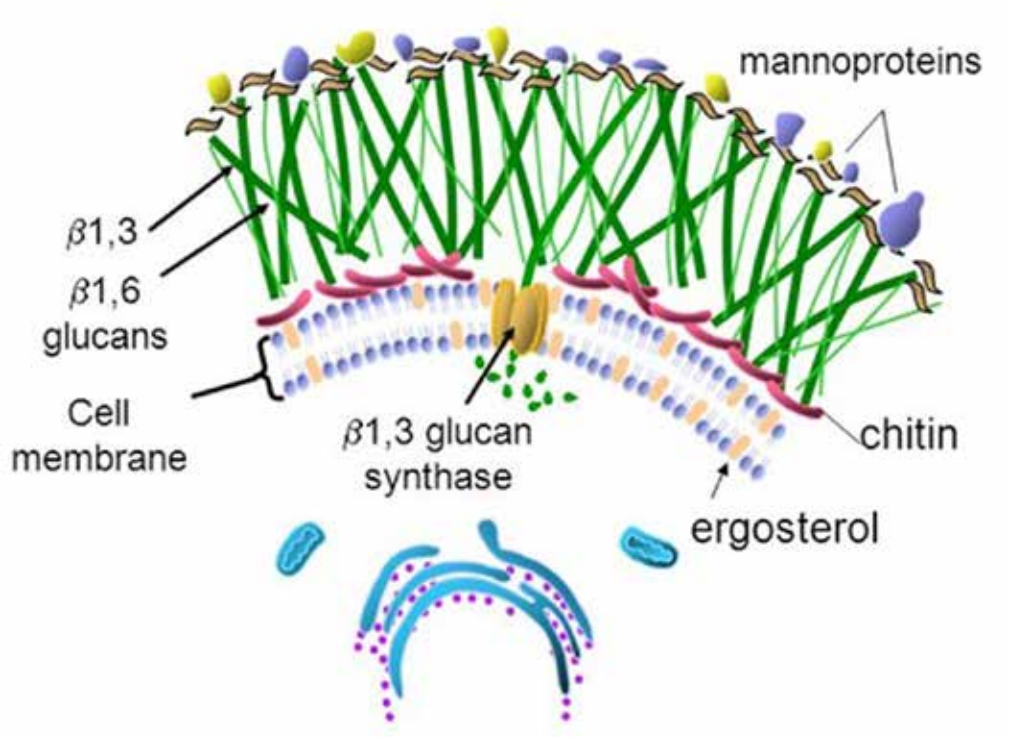


Figure 2. Fungal cell wall. [52]

Further research into the active polysaccharides in basidiomycetes have identified them as beta-D-glucans. Beta-D-glucans are a naturally occurring structural component of the cell walls of mushrooms, mycelium, yeast, and certain bacteria. They are also abundant in the endosperm cell walls of cereal grains. Fully half the mass of the fungal cell wall is made up of beta-glucans. Fungal beta-glucans primarily consist of a (1→3)-beta-linked main chain with single D-glucosyl residues linked (1→6)-beta- to every third D-glucosyl residue in the main chain. Other structures containing (1→3)- and (1→6)-beta-linkages are also present.

In contrast, cereal endosperm beta-glucans are linear polysaccharides composed mainly of cellotriosyl- and cellotetraosyl- units linked (1→3)-beta. Lesser amounts of cello-oligosaccharides of higher degree of polymerisation also linked (1→3)-beta- are also present in these polysaccharides. Side chains contain (1→4)-beta-linkages.

The structural complexity of the fungal beta-glucans varies and is considered a primary determinant of activity. This is also the main accepted theory of why some basidiomycetes are more active than others and why basidiomycete beta-glucans are more immunologically active than cereal beta-glucans [8,9].

The properties of these beta-glucans indicate that they activate or potentiate both innate and adaptive immunity. Dr. Goro Chihara, the Japanese scientist responsible for developing Lentinan, has termed these compounds Biological Response Modifiers: BRMs [10,11,48]. Another term utilized for this activity is Host Defense Potentiation. Generally speaking, when beta-glucans activate our immune system, the numbers of macrophages, NK cells, and subsets of T-cells are increased and their functions are enhanced. The mechanism for this activation is the presence of specific beta-glucan receptor sites in our small intestine [12]. Since beta-glucans are not degraded by digestive enzymes, they pass intact into the intestine. (Many scientists believe that there are various types of receptors because of the functional diversity of beta-glucans [13].)

Medicinal mushrooms are a traditional remedy in cancer therapy (mycelium was not available for traditional use) [8]. Today purified beta-glucans and BRMs are primarily utilized in cancer therapy, often in conjunction with anti-cancer drugs [13a]. It should be noted however that the immunological potentiation not only shields us against cancers, but also increases our protection against viral, bacterial, fungal and parasitic infections. As such, beta-glucans are considered antibiotic and antiviral [14].

Triterpenoids

A second category of active compounds in basidiomycetes that contribute to their overall effect are lipids known as triterpenoids. Research ascribes primary activities as liver protective, lipid lowering, antioxidant, inhibition of histamine release and anti-inflammatory [15,16]. Triterpenoids also play a complementary role with beta-glucans in immune system activation. The triterpenoids are responsible for the bitter taste of reishi and this bitterness can be used as a quick method of determining the quality of a reishi product.

Triterpenoids have been extensively researched and occur in significant amounts in a few important medicinal mushrooms, those being reishi, chaga, and *Antrodia*. For these mushrooms, triterpenoid analysis using HPLC can provide a means to quantify these compounds as well as provide a definitive fingerprint [17,47]. (See figure 3.)

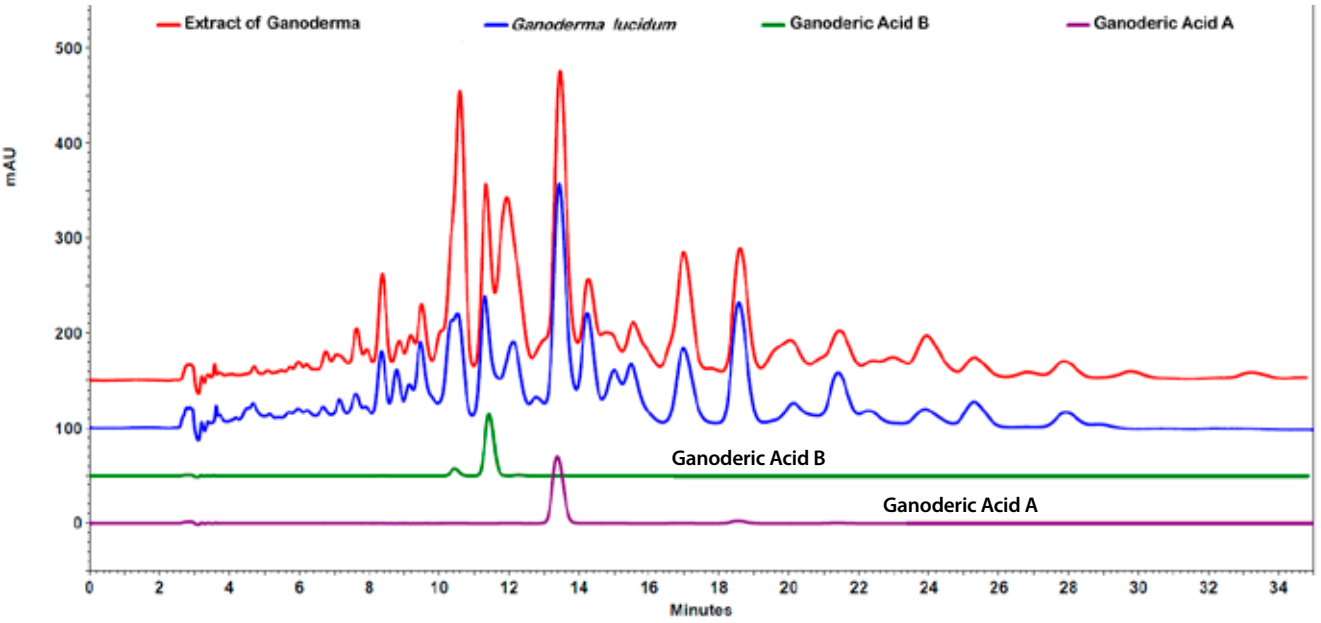


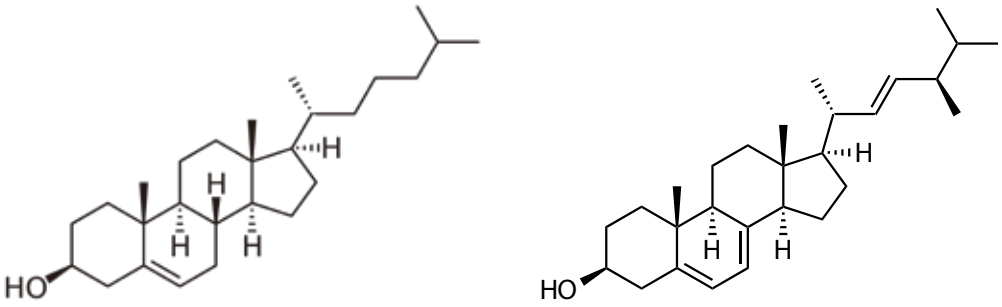
Figure 3. HPLC chromatogram showing the conformation of a reishi mushroom extract with the mushroom raw material. Ganoderic acid A and B standards are overlaid. [47]

Sterols

Another compound that is also considered a triterpenoid is ergosterol and its analogues (I will not include ergosterol when I speak about triterpenoids in general) [d]. Ergosterol is present in all fungi and is a corollary to cholesterol in humans. (See figure 4.) It is a definitive marker for fungal presence and has been utilized for years by the grain industry to test for fungal contamination [18]. Recently, ergosterol was even discovered to have antitumor and antioxidant properties [12]. Ergosterol testing can provide an important marker for quantifying the amount of fungal material in a given product [19].

It should also be noted that ergosterol is a precursor to vitamin D. Exposure to UV light converts ergosterol to vitamin D2. High ergosterol or high vitamin D content may become a medicinal choice and therefore protecting mushrooms from UV or exposing them to UV would become important for the desired effects of the final product.

Figure 4. Chemical structure of ergosterol and cholesterol.



Cholesterol **Ergosterol**

Statins

Over the years researchers have discovered that certain mushrooms, *Pleurotus* in particular, have the ability to lower cholesterol. More detailed work has discovered statins as the most likely compound responsible. Lovastatin (mevinolin) is a secondary metabolite that occurs in many fungi and most of the medicinal basidiomycetes. It is a potent inhibitor of HMG-CoA reductase, the main rate-limiting enzyme in cholesterol production [14,20]. Lovastatin analysis may provide another marker for species with demonstrably high levels of this compound. This should, however, be tempered with the fact that quite high amounts of mushroom powder were necessary to affect an action. It has also been observed that mushroom powder and edible mushrooms have a significant content of dietary fiber, which would also influence cholesterol formation. The responsible fiber compounds are beta-glucans, chitin, and heteropolysaccharides (pectinous substances, hemicellulose) [21,22].

Nucleosides

Nucleosides such as adenosine, guanosine, uridine, cytidine, etc. occur in varying amounts in most of the basidiomycetes [23]. It is possible to utilize them as markers and some researchers have created fingerprints using up to 10 of these compounds [49]. A recent study of nucleosides in *Antrodia* mycelium demonstrated that not only was *Antrodia* pure mycelium quite different from two commercial mycelium samples, but also significantly different from the *Antrodia* fruiting body. Notably, the mycelium was devoid of triterpenes for which this basidiomycete is famous. Even the nucleosides showed a significantly different profile [24]. But nucleosides are common in many fungi and the amounts present may not be sufficient to provide benefits. In this study, nucleosides in mycelium and mushroom did not correspond closely.

One nucleoside in particular, cordycepin (3'-deoxyadenosine), is commonly analyzed for *Cordyceps* species. *Cordyceps militaris* has measurable amounts, whereas *Ophiocordyceps sinensis* [a] has only traces. This is one method of differentiating the two species when confronted with a powder. Additionally, given the uncertainties of *Cordyceps anamorphs* (mycelium) [b] and the probabilities that *C. militaris* mycelium may be the primary species being cultivated, this test has some merit [24a].

It should be noted that Cordycepic acid is a false marker compound. It is more properly called mannitol. Mannitol is present in almost all plants and in most basidiomycetes, and would have little significant activity given the amounts present. It therefore has no genuine role in quality control and should be dropped as a marker compound [25].

Secondary Metabolites

Basidiomycetes are well known for their production of secondary metabolites.

A secondary metabolite is a compound that is produced by the fungal organism from precursor compounds generated during primary metabolism.

Secondary metabolites are not necessary for the actual growth or life of the organism. They will accumulate during growth of the fungus and generally speaking do not degrade easily. Some of these metabolites are biologically active [26]. A prime example of a well-known fungal secondary metabolite is penicillin. In the book, The *Alkaloids*, a chapter on basidiomycetes lists hundreds of alkaloids from dozens of different mushrooms [27]. So just how important are the secondary metabolites in medicinal basidiomycetes? It is these compounds that give some mushrooms a significantly more complex activity fingerprint.

Mushrooms and mycelium both produce secondary metabolites but it is the mushroom that excels.

A prime example is reishi mushroom. This mushroom is a factory for producing triterpenoids. These are primarily called ganoderic acids and the overwhelming majority come from the mushroom and in significant quantities. There are obscure ganoderic acids produced by the mycelium but they have a different structure and are produced in minute quantities. Mycelial triterpenoids are very difficult to detect and are not subject to the same level of research as mushroom triterpenoids, which are being extensively investigated. (See figure 12.)

But that is not the only example of this distinction. Chaga "mushroom" is a sterile conk that is actually a solid hard mass of mycelium, what is termed a sclerotium. Chaga is another fungus that contains measurable secondary metabolites. These are the triterpene compounds inotodiol, trametolenic acid and betulinic acid. They are found in chaga due to the fact that chaga grows on birch trees which produce the precursor compounds. Without the trees, these triterpenes are rarely found in chaga. Mycelium cultured on simple grains like rice or oats do not contain these compounds in detectable quantities.

Without the natural precursors, basidiomycete mycelium in sterile culture produces few of the important secondary metabolites.

Spores

Spores present a special case and only in the last 20 years have they been investigated with some resolve. All mushrooms produce spores, unless sterile, so spores will always be present in a mature fruiting body. But it is well known that spores are indigestible and will pass right through the human alimentary system. Recently, Chinese entrepreneurs invented a novel reishi product that is termed a "cracked" spore. In Asia, cracked spore products have become a big business. The medicinal value of this product has yet to be determined, despite an increasing body of primarily Chinese research that indicates activity. The major active compounds identified from spores are listed as triterpenoids, polysaccharides, ergosterol and long-chain fatty acids. Further analysis and study is needed to confirm the true value of spores treated in this manner [28,29,30]. Investigation of spores is beyond the scope of this analytical program.

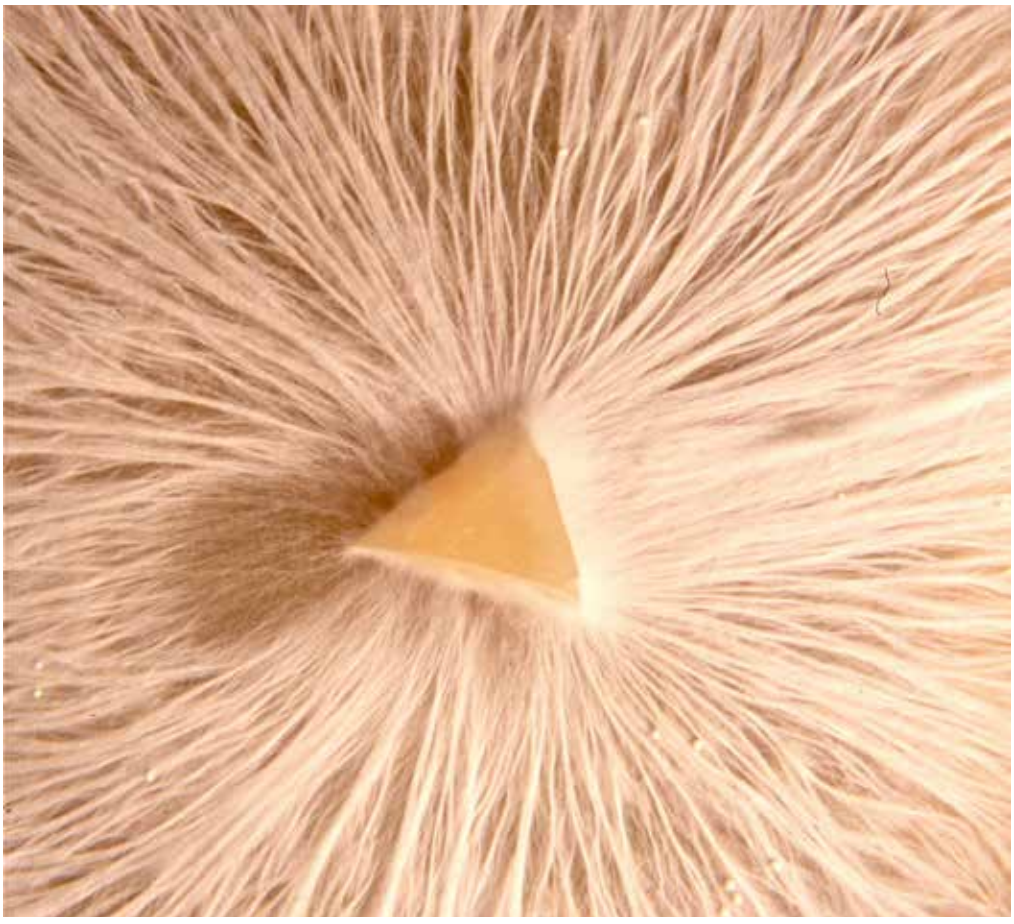
Other Compounds

There are other common as well as unique compounds found in basidiomycetes but very few of these occur in sufficient quantity to allow for practical analysis. This situation may change in the future, especially if any of these compounds are shown to be highly active. But generally speaking, none of these compounds can be demonstrated to have activity or benefit given the amounts that would be normally consumed in a nutritional supplement. Commonly mentioned compounds would be enzymes in general and specifically the common ones such as glucanase, cellulase and ligninase (LMES), all of which are produced by mycelium. Cyathane diterpenes are another category that at present are not readily analyzed.

Whole Basidiomycete and Synergistic Concepts

Some authors have commented on the fact that often it is the full complement of active ingredients that make a specific herbal or basidiomycete product effective. After all, there may be certain benefits that result from the presence of multiple compounds, what could be termed a synergy. I wholeheartedly agree. However, some would use this idea to question the analysis of active compounds as if analysis somehow means leaving other compounds behind. This is simply a misleading perception. Analysis of active marker compounds is one of the best methods of assessing the quality of a herbal or mushroom product.

Others speculate that the combination and therefore interaction of two or more basidiomycete species creates a unique activity that would otherwise not be present in a single basidiomycete. At present this is a synergy theory that has yet to be proven by scientists. Rather than being based on a conceptual foundation like TCM, which puts many different herbs into one formulation, some companies combine 4–16 basidiomycetes into a single product and claim a synergy. This shotgun approach of essentially similar basidiomycete species could actually be a dilution of potency rather than reinforcement of activities. Unless supported by scientific research or traditional formulas, it would be prudent to put only a few of the more well-researched mushrooms together in a complementary blend. For example, *Pleurotus* and shiitake for cholesterol control or 4–5 of the top species for immunopotentiation. (See figure 10.)



Basidiomycete mycelium growing in sterile culture.

The Hundreds of Compounds Theme

In some quarters claims are made as to the hundreds of compounds in a specific commercial basidiomycete product; it may be triterpenes or polysaccharides or even enzymes. It is true that hundreds of triterpenoids have been characterized from reishi mushrooms. It is also true that hundreds of different polysaccharide fractions have been characterized from various mushrooms and mycelia [50]. But these claims have very little meaning. Scientists excel at discovering new compounds and accomplish this by extracting a basic raw material and isolating numerous fractions. Each fraction may be slightly different and also occur in varying concentrations. Fractionation is the process of drug discovery, looking for a fraction with strong activity that can ultimately be purified and manufactured as a drug [44,48].

In the real world of nutritional supplements, this "hundreds" concept is misleading since the vast majority of these fractions occur in very minute amounts, amounts far too minimal to be consequential. A prime example are the triterpenoids characterized in reishi mycelium. HPLC analysis of reishi mycelium consistently shows few if any peaks. The amounts are minuscule and of no consequence yet that doesn't stop some companies from making sweeping triterpenoid claims as if one might gain triterpenoid benefits from their mycelium product. Similar "hundreds" claims are often made about polysaccharides.

It is not the minute amount of some scientifically interesting polysaccharide that will provide one with benefits. It is the total amount of (1,3)(1,6)-beta-glucans that includes all of these fractions that is truly meaningful.

The trap that many basidiomycete producers fall into is the use of scientific research that has no direct application to the products being sold, because the amounts present in the product are far too small, and well below amounts reported in successful clinical trials. The real goal should be an efficacious product, one with scientifically verified active constituents, that can provide greater activity and longer-lasting benefits than a placebo.

Materials and Methods

Now that the major active compounds in basidiomycetes have been identified, verified testing methods can be utilized to assess medicinal basidiomycetes. As this program progresses, more of the identified compounds will be integrated into this screening process. The ultimate goal is to have a comprehensive screen that will give a qualitative and quantitative measurement of a given basidiomycete product. It is my belief a fingerprint for product evaluation should be based upon a dried fruiting body, since that is the stage of a basidiomycete that has been historically utilized as a medicinal herb as well as the stage that most research is based upon.

Additionally, it must be noted that there has been significant research on pure mycelium cultured in flasks or small fermentation vessels and this has led to the discovery of interesting and novel polysaccharide fractions. However, with the exception of Cordyceps species, there are few pure mycelium products that have been commercialized and therefore available for testing.

Beta-D-glucans

Most beta-glucan testing protocols have been designed specifically for grains. Because fungal and cereal endosperm beta-glucans have very different structures, the assay procedures developed and validated for cereal beta-glucans are not applicable to mushroom beta-glucans. [53] Total polysaccharide tests also are not relevant as they will measure all polysaccharides, not just beta-glucan. One such example are the alpha-glucans (starch-type polysaccharides). Starch is a very common polysaccharide that is present in large amounts in staple foods like potatoes, most grains, corn, and rice. The presence of these starchy materials in a mushroom product can elevate a polysaccharide test and give a false positive for the medicinal beta-D-glucans. For this reason it is important to utilize a test method that is specific to mushroom beta-glucans and will not pick up these alpha-glucans.

A new testing method designed for beta-D-glucans in mushrooms and yeast was developed in 2004 by a company called Megazyme International Ireland. The Megazyme test method has been utilized by the USDA and current scientific researchers for the testing of mushroom beta-glucans. This test detects and quantifies soluble and insoluble (1→3)(1→6) beta-D-glucans. Using the Megazyme test we can test all basidiomycete products, whether they are mushroom powders, extract powders, or even mycelium powders [22,31,32,33,34].

But what about the presence of beta-glucans in the grain that is part of mycelium products? Might these beta-glucans show up in our testing? An easy way to determine this is to run samples of cooked grains as a check. By testing the common grains that are used as the media to grow mycelium, it is clearly possible to determine if these grains contain beta-glucans that can be picked up by the Megazyme test [35].

Beta-glucan testing was carried out in 3 batches. The first 2 sets of samples of (45 and 17) were tested by SGS Laboratories, Vancouver, B.C. The final set of 32 samples was tested in the laboratory of Megazyme International Ireland. I wish to thank CEO Barry McCleary for participating in this project. The results derived from Megazyme International Ireland confirmed and validated the results from SGS Vancouver.

Triterpenoids

Even though mushrooms such as reishi and chaga have some classes of triterpenoids in common, one must still procure a pure standard in order to perform an accurate test that measures the quantity of these compounds. Today ganoderic acid standards for reishi are commercially available. This is a good start and it bodes well for chaga and *Antrodia* testing. A verified HPLC analytical method is not available, but there are sufficient methods published in the research to allow in-house testing or method development [36,37,38,39]. Nammex has been utilizing a method developed from this research and has 15 years of experience. We have also developed a method to test chaga triterpenoids [40,41]. All triterpenoid testing was carried out in laboratories at the University of British Columbia.

Ergosterol

USDA, large grain producers, and the agaricus industry have been testing ergosterol for years and the research has been published. These are tests that are readily available. Testing utilized in this screening followed methods used by Phillips et al. for USDA ergosterol content in mushroom research [42].

Starch

The presence of starch in nutritional supplements, especially as a carrier for extract products, has been an ongoing industry issue. Basidiomycete mycelium products present a similar issue since many are grown on grains. Testing for starch will demonstrate the level of grain residue that may be present in the grain-based mycelium, since the grain is not separated from the mycelium in the final product. In fact, testing for starch will allow the determination of how much of the mycelium product is actually mycelium and how much is grain. It will also identify starch carriers used in mushroom extracts.

Mushrooms contain only minor amounts of starch, on average about 3% of the dry weight. Megazyme makes a starch analysis test kit that is perfect for this application [43]. Starch testing was carried out by SGS Laboratories, Vancouver, B.C. and Megazyme International Ireland.

Sample Preparation

Sampling of medicinal basidiomycetes was carried out in the following manner. Samples were selected from three major groupings:

1. Dried mushrooms – 30 samples from 12 species.
2. Commercial mycelium products produced on a grain substrate – 35 samples from 13 companies.
3. Commercial mushroom extracts prepared by hot water extraction – 30 samples from 12 species.

Commercial mycelium products were purchased from online resellers or directly from the manufacturer's website. These were encapsulated products and the powder was removed from the capsules.

Dried mushrooms were selected from retail packaged product from China or product samples from China. These dried mushrooms were identified by the author. A second set of mushrooms were purchased fresh in Vancouver, B.C. markets by mycologist Paul Kroeger, who then dried them.

All extracts were manufactured in China for Nammex. Balanced extracts are concentrated in a ratio of 1:1 using hot water with the mushroom fiber included in the final extract. More concentrated extracts have the mushroom fiber excluded. The identity of the extracts has been confirmed by HP-TLC.

Shiitake mushrooms hand-placed on a screen for sun drying.

Results

Beta-D-Glucan

There were a total of 95 samples tested for beta-glucans and starch, evenly split between crude mycelium cultured on grain and commercially cultivated mushrooms and mushroom extracts.

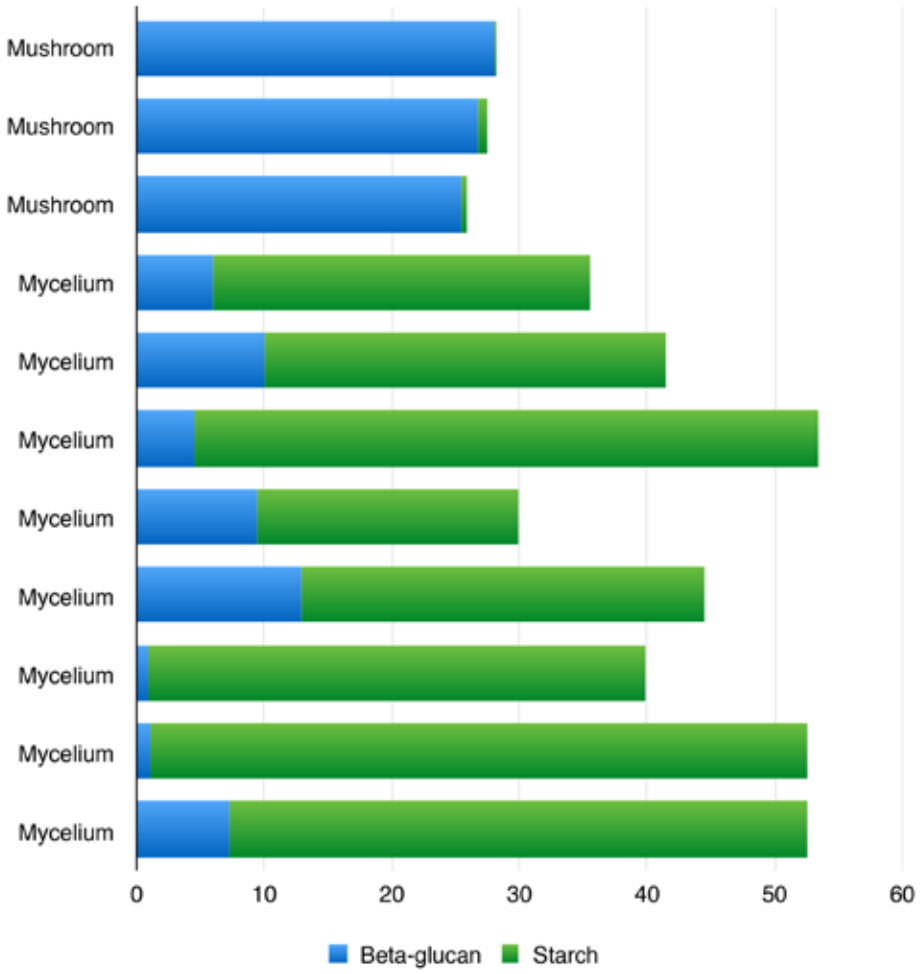


Figure 5. Three different reishi mushrooms and 8 different grain-based reishi mycelium products. All data expressed as percentage of dry weight.

Mushrooms and mushroom extracts contained consistently high levels of beta-glucans, on average 30-40%. The dried mushrooms were either very close to the same level of beta-glucans as the mushroom 1:1 extracts, or in some cases the extract levels were elevated. Some concentrated mushroom extracts showed low levels of beta-glucans. These extracts were exposed as containing high levels of a starch-based excipient, identified as dextrin.

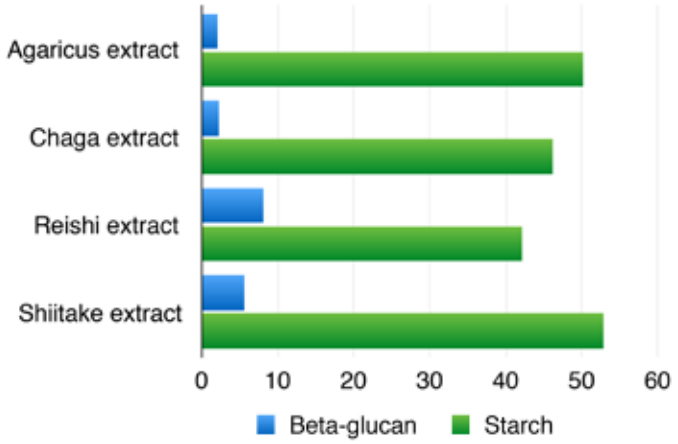


Figure 6. Highly concentrated extracts blended with a starch carrier. The beta-glucan content has declined as a result. All data expressed as percentage of dry weight.

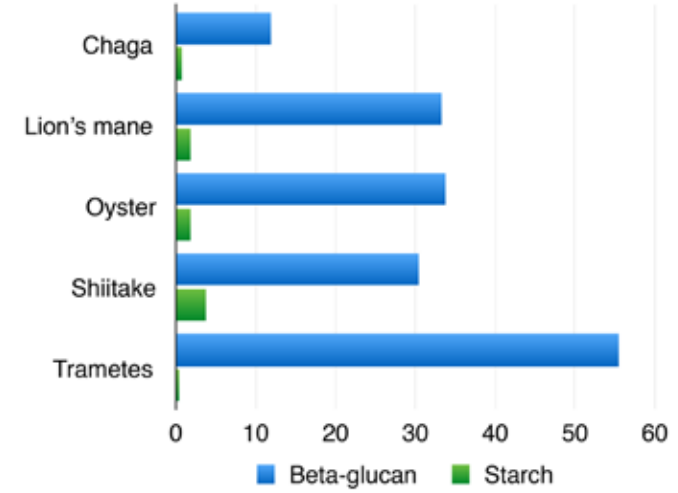


Figure 7. Low concentration mushroom extracts showing a profile consistent with the raw materials. High beta-glucan and low starch. All data expressed as percentage of dry weight.

Mycelium on grain had consistently low levels of beta-glucans. Grains alone had 1–2% of beta-glucans. The lowest of the mycelium on grain products had 0% beta-glucan whereas the highest had 15%. On average the levels were approximately 5-7%.

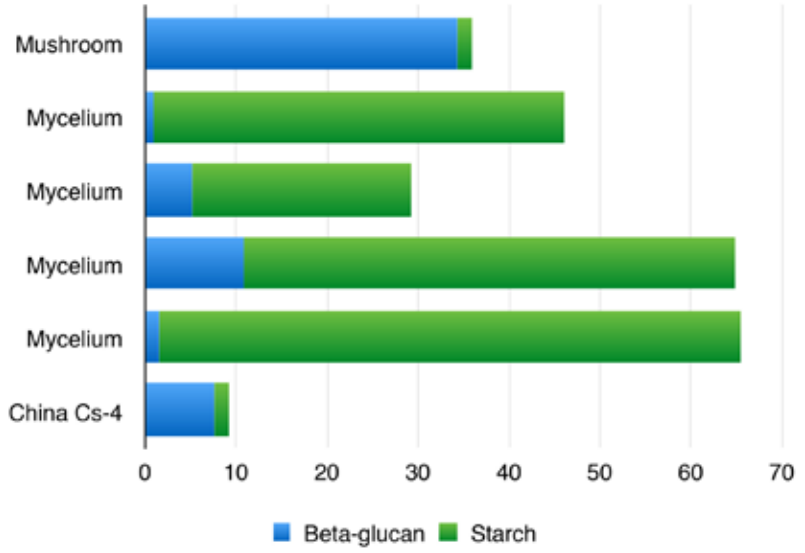


Figure 8. Cordyceps militaris fruiting body, Chinese Cs-4 pure mycelium, and four grain-based Cordyceps mycelium products. All data expressed as percentage of dry weight.

Starch Testing

Mushrooms and mushroom 1:1 extracts contained minimal amounts of starch. This confirms historical nutritional analyses of mushrooms. The highest mushroom tested was shiitake with 3.06% starch, the lowest was reishi with 0.24%. All of the mushroom extracts showed similar low levels of starch with the exception of those that were confirmed to contain dextrin excipients.

Figure 9. Mushroom beta-glucan and starch fingerprint data. Mushrooms with an asterisk indicate the same sample was analyzed by SGS Vancouver and Megazyme International Ireland.

Description	Beta-glucan	Starch
Agaricus bisporus	8.4	1
Agaricus blazei	11.2	2.8
Chaga* - I. obliquus	6.7	0.1
Chaga* - I. obliquus	6.79	1.05
Cordyceps militaris*	34.36	1.65
Cordyceps militaris*	33.7	1.8
Enokitake - F. velutipes	22.6	0.5
Lion's mane - H. erinaceus	37	2.6
Maitake - G. frondosa	33	1.1
Maitake - G. frondosa	32.4	1.6
Maitake - G. frondosa	40.58	2.02
Oyster - P. ostreatus	35	0.9
Oyster - P. eryngii	35.6	0.3
Reishi* - G. lucidum	31.88	0.24
Reishi* - G. lucidum	28.1	0.1
Reishi - G. lucidum	26.8	0.6
Shiitake* - L. edodes	35.39	3.46
Shiitake* - L. edodes	36.4	2.9
Shiitake - L. edodes	28.2	0.7
Shiitake - L. edodes	25.60	3.06
Trametes - T. versicolor	49.3	0.1

All grains contain high levels of starch. Rice contains 74% starch, sorghum has 64%, and oats have 58%. Mycelium grown on these grains also contains high levels of starch. Reishi mycelium on rice contained 49% starch, an actual 10:1 ratio of starch to beta-glucan. Maitake mycelium on sorghum had 40% starch, another 10:1 ratio of starch to beta-glucan. On average, mycelium products on grain contained 35–40% starch. Mycelium grown on the higher starch-containing rice showed consistently higher levels of starch.

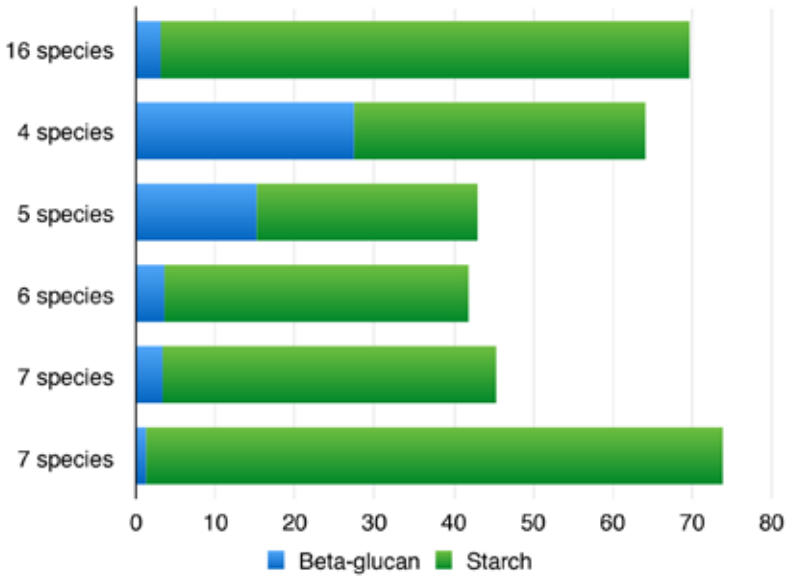
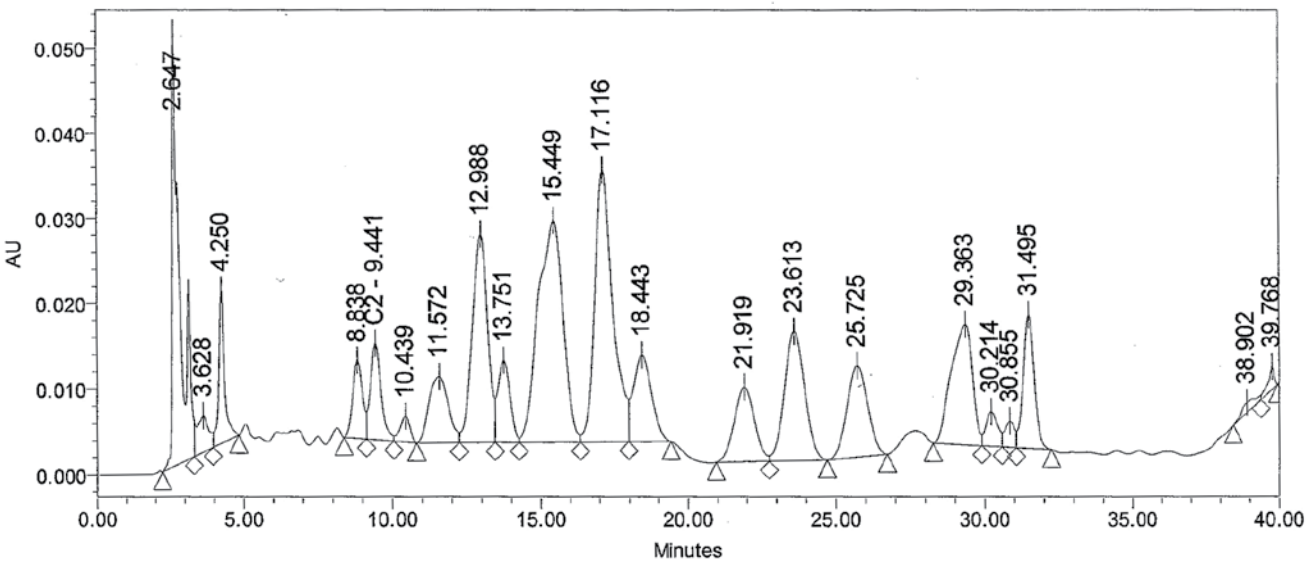


Figure 10. Multi-species grain-based mycelium blends manufactured by various suppliers. All data expressed as percentage of dry weight.

Triterpenoid Testing Results

Reishi mushrooms and mushroom extracts contain measurable amounts of triterpenoids. Reishi mycelium from three different commercial samples showed no significant peaks on the chromatograms. This is consistent with our historical record of triterpenoid testing as far back as 1994. This confirms the basic tenet that without precursor compounds, which primarily occur in natural substrates, important secondary metabolites are not produced in meaningful quantities.

Figure 11. HPLC chromatogram of reishi triterpenoids. Ganoderic acid C2 is identified as the analysis reference standard.



The following chart shows the amount of the major reishi triterpenoids: ganoderic acids A, B, C2, D; the sum total of A-D; and a total for all triterpenoids detected. Also listed is the number of peaks that registered on the chromatogram. (See figure 12.)

Figure 12. Reishi triterpenoid fingerprint. Data expressed as percent of dry weight. n.d. signifies none detected. Naming system per Khoda, H.

"A" samples are soaked for 24 hours, B samples soaked for 48 hours. All mycelium samples are grain based commercial products. Reishi extracts are produced from mushrooms. Extraction ratio expressed numerically. Reishi alcohol extract a and b were run one month apart.

Sample	Peaks	A %	B %	C2 %	D %	A - D %	Total %
Reishi mushroom	13	0.094	0.032	0.012	n.d.	0.138	0.409
Reishi mushroom	20	0.14	0.02	0.02	0.04	0.22	0.51
Reishi mycelium F1a	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium F1b	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium A1a	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium A1b	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium extract A2a	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium extract A2b	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium A3a	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi mycelium A3b	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reishi 1:1 water extract N1a	17	0.027	0.042	0.05	n.d.	0.119	0.372
Reishi 1:1 water extract N1b	17	0.042	0.032	0.049	n.d.	0.122	0.297
Reishi 12:1 water extract N2a	26	0.569	0.354	0.204	0.247	1.375	3.282
Reishi 12:1 water extract N2b	25	0.702	0.384	0.202	0.274	1.563	3.939
Reishi 16:1 alcohol extract N3a	28	1.804	0.41	0.403	0.928	3.545	12.324
Reishi 16:1 alcohol extract N3b	30	1.882	0.411	0.414	1.003	3.71	12.952
Antrodia mushroom extract	25	0.573	0.251	0.241	0.185	1.251	3.326

Ergosterol Testing Results

Ergosterol has been analyzed in the first 45 samples. The results are very important and provide important clues as to the value of specific basidiomycete parts. In fact, a key to current basidiomycete products directly correlates with ergosterol content.

To learn about these important ergosterol tests, contact purescience@nammex.com or call Jeff at 604.886.7799.

Discussion and Conclusion

By using a battery of tests that are specific to basidiomycetes, it is possible to create a fingerprint that can demonstrate with certainty the medicinal potential of a given basidiomycete product. These tests include a Megazyme beta-glucan and starch analysis and an ergosterol analysis as primary methods with the additional use of a HPLC triterpenoid analysis for certain species.

These analyses demonstrate the significant differences between commercial mycelium products produced on a grain substrate and a mushroom or mushroom extract. The tests also reveal the presence of common excipients.

Medicinally, basidiomycete mycelium grown on grain contains low quantities of beta-glucans, high amounts of starch and few secondary metabolites such as triterpenoids. Ergosterol levels demonstrate one of the reasons for this phenomena.

In submerged culture, mycelium is known to produce extracellular beta-glucans, but the production of these compounds in a solid-state grain medium did not prove to be significant. Submerged culture is utilized for the production of the concentrated extracts PSK and PSP, but one cannot compare the activity of these highly refined products to crude mycelium grown on grain especially when the grain is not separated from the final product [33] [46]. Nor are these concentrated mycelium extracts comparable to a crude mycelium biomass grown in a liquid medium utilizing a conventional fermentation process.

It has been noted by many authors that mycelium propagated in fermentation tanks provides a standardized method capable of the production of many of the medicinal compounds identified in this paper [3]. This is certainly true for beta-glucans whereas production of other compounds like triterpenoids will suffer without the presence of precursors. Fermentation of basidiomycete mycelium has yet to be proven economical in North America or there would be companies engaged in this business. There is only the production of mycelium on grain, what is known in the mushroom-growing industry as "grain spawn," using a well-developed and inexpensive process that hasn't changed significantly in 50 years.

Unless grain-bound mycelium can overcome the high amounts of residual starch, which is diluting whatever medicinal compounds are present, it may be difficult to consider these products as genuinely representative of this category.

These tests have established that mushrooms are truly the most important medicinal part of the basidiomycete.

Mushrooms are consistently high in beta-glucans and low in starch. The chitins and other cell-wall compounds add to their activity, especially as prebiotic fiber. Mushrooms also produce secondary metabolites in measurable quantities, such as the ganoderic acids in reishi mushroom and the triterpenoids in chaga and *Antrodia*.

Mushrooms are produced on natural substrates and generally in natural conditions of temperature, light and humidity. The natural substrates are generally similar if not the same as what these mushrooms would grow on in the wild. The cultivation of mushrooms on nutritious natural substrates produces a natural product that is perfectly in tune with end users who desire whole herb, organic, and naturally grown supplements.

Mushrooms are the true "full spectrum" stage of the basidiomycete organism.

This analytical program provides a means for manufacturers to upgrade their product offerings and be confident that the labeling and contents of the product meets the high standards that they have set for their brand.

Endnotes

a. Ophiocordyceps has now replaced Cordyceps as the genus for the species sinensis.

b. Cordyceps mycelium products have always been suspect as to their true taxonomic identity since no one has actually produced an O. sinensis fruiting body, a prerequisite for absolute identification barring genetic ID.

c. Trametes is the genus for the commonly used but outdated basidiomycete name Coriolus versicolor.

d. Ergosterol is a primary life-sustaining compound that performs an important function for basidiomycetes. Most of the medicinally important triterpenoids are secondary metabolites.

e. The various ways in which (1→3)beta-D-glucan and (1→3)(1→6)beta-D-glucan are written in the research literature can become confusing. In this paper I will refer to these specific polysaccharides as beta-glucans or beta-D-glucans unless otherwise noted.

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