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Impact of Mushroom Nutrition on Microbiota and Potential for Preventative Health

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Abstract The current short article reviews the role of mushroom biomass, rich in β-glucans, enzymes, germanium and secondary metabolites, the interaction with the human gut microbiota and the prevention or treatment of various metabolic syndrome-linked diseases. The focus is on mushroom β-glucans compared to other soluble and fermentable dietary fibres, their unique effects on nutrition, human microbiota, health and disease, specifying the significantly differing physicochemical properties depending on the source and type of mushroom or dietary supplement. An overview of definitions and types of fibre, a brief examination of the health benefits associated with β-glucans from mushroom biomass, its possible mechanisms of action, and its potential dietary supplement applications are provided. Despite promising evidence of mushroom biomass on health much research still remains to be done.

Keywords: *mushroom biomass, β-glucans, microbiota*

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1. Introduction

Hippocrates in 400 B.C. was quoted as saying "death sits in the bowels" and "bad digestion is the root of all evil", showing that the importance of the intestines in human health has been long recognized [1].

Virtually all multicellular organisms live in close association with surrounding microbes, and humans are no exception. The human body is host specific and is inhabited by a vast number of bacteria, archaea, viruses, and unicellular eukaryotes. The collection of microorganisms that live in peaceful coexistence with their hosts has been referred to as the microbiota, microbioma, microflora, or normal flora [2].

The microbiota is intimately involved in numerous aspects of normal host physiology, from nutritional status to behaviour and stress response. Additionally, they can be a central or a contributing cause of many diseases. The overall balance in the composition of the gut microbial community is important in ensuring homeostasis. The mechanisms through which microbioma exerts its beneficial or detrimental influences remain largely undefined, but include elaboration of signaling molecules and recognition of bacterial epitopes by both intestinal epithelial and mucosal immune cells[3].

An estimation of human total cell number calculated for a variety of organs and cell types corresponds to a total number of 3.72×10^{13} [4]. As of 2014, it was often reported that there are about 10 times as many microbial cells in the human body than there are human cells. Recently, in 2016, another group published a new estimate considering human cells not so vastly outnumbered by bacteria [5].

As many as 5.1 million fungal species exist. Some 14,000 species of fungi can be considered as mushrooms, and at least 2,000 species are identified as edible. Mushrooms have approximately 12,000 genes controlling the production of more than 200,000 compounds [6]. Only 35 species of mushrooms are grown on a commercial scale, 20 are cultivated on an industrial scale and only 12 have the greatest concentration of β-glucans and used as food supplements.

Unlike a synthetic drug, or even a mushroom extract, a fresh mushroom and mushroom biomass contains thousands of elements, each with a potential effect on the immune system.While mushroom biomass is considered a "dietary or food supplement", mushroom extracts are labelled as "pharmaceutical compounds", "pharmanutrients" or "nutraceuticals".

Biomass is the mycelium with primordia (young fruiting body - before the mushroom blooms). It contains all the nutrients and active compounds, including enzymes, germanium, secondary metabolites and β-glucans. In the view of the FDA, the isolation, concentration and purification targeting a single active ingredient from mushroom extracts designate them as "pharmaceuticals". Using the mushrooms and mycelium in their natural forms as biomass make them "functional foods" or "dietary supplements". Both, extracts or biomass forms, can be considered as acting as "prebiotics" [7].

It is still unclear which mushroom component(s) is(are) responsible for the beneficial impact on human health such as polysaccharides (eg. immunomodulating α- and β-glucans), mettaloids (eg. germanium), glycoproteins (eg. immunomodulating PSK), triterpenes sterols (eg. sistosterols, stigmasterols, campesterols), lipids (eg. cholesterol), modulating proteins (eg. antioxidant enzymes) cyathane derivatives (eg. erinacines and hericenones, nerve growth stimulant factors), secondary metabolites (eg. triterpenes) or all of them in a synergistic manner.

2. Human Gut Microbiota

Our intestines digest the food we eat to provide the body with essential nutrients as well as removing waste material and toxins. Maintaining a healthy digestive system depends on keeping a balance among the billions of bacteria that live there. Scientific evidence is accumulating upsetting the intestinal flora, which may lead to health problems such as indigestion, lowered immunity and susceptibility to diarrhoea. Stress, a poor diet, taking antibiotics, or just tiredness may all upset the natural balance in the gut [8].

It has long been thought that the mesenterium and lower intestines, and in particular the colon, contributed little towards human nutrition. More recent studies, however, have indicated that the mesenterium [9] and the colonic microflora appear to play a vital role in health and it appears that the role of the large bowel in health may have been underestimated [10]. This raises a whole range of interesting philosophical and anatomical questions. The latest thinking presents this vast army of microbes as a vital component in furnishing and maintaining human health even through bacteriotherapy or fecal transplantation [11].

Such is the microbiome's importance that it is now viewed by scientists as a "separate organ" with its own dynamic metabolic activity, by some designated as "The Gut Microflora Party" [12,13,14].

Human gut microbial communities are known to play a major role in health and disease, but an exhaustive analysis of their composition and diversity has only recently become feasible with the advent of new DNA sequencing technologies [15]. Changing the makeup of the microbiome -- whether through changing the diet or consuming prebiotics or probiotics on one hand, or taking antibiotics on the other – may result in great implications for health and well-being.

2.1. Host-Microbe Interactions

The microbiome revolution in medicine is beginning to uncover the underappreciated role our healthy gut bacteria play in nutrition and health as humans live in association with immense populations of bacteria, viruses, fungi and archaea [16].

Researchers have spent more than 100 years studying pathogens with the aim of their eventual elimination, with little regard for what happens in their absence. It is now clear that many 'pathogens' more commonly exist as commensal organisms. Presently, we need to expand our understanding of commensalism and of how these pathogenic organisms may actually be vital to our well-being [17,18].

Our digestive systems are actually directly linked to our brains; in fact, our guts are actually considered our "second brain," with the two sending messages to each other constantly in an attempt to keep things in harmony, influencing mood and well-being, stress and anxiety [19]. The cells in our gut outnumber the cells in the rest of our body by more than 10-1, and the gut digestive system has its own nerve system called the enteric nervous system while there are more neurons in our enteric nervous system than in our brain [20].

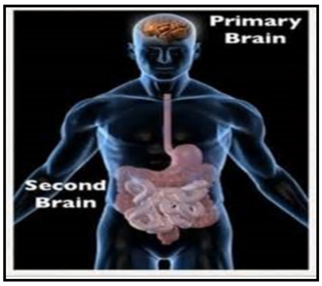


Figure 1. "The second brain"

Most people do not realize that 80 % of human immune system lies in the gastrointestinal tract and 20 % in the back of our mouth (e.g. amygdala) enhancing the fact that an optimal health starts in your gastrointestinal tract [21]. The connection between the gut, brain, and immune function explains why the intestinal health, food and dietary supplements ingested, can affect mental and neurological health [22].

The gastro-intestinal tract contains a delicate microcosm of bacteria (both good and bad) and immune system cells, which take care of both digestion and disease prevention. If things go out of balance, so will your health and mental state. Genes, age, diet, lifestyle and geography all affect which bacteria live in a person's body. How many bacterial species colonize the entire body remains anyone's guess. So does the question of which bacterial species everyone shares [23].

Anaerobes make up the majority of bacteria found in the bacterial flora in the human colon; the most predominant bacteria found are *Bacteroides*. The colon contains over 400 species of organisms and has more than 10^{11} organisms per gram of wet weight [24]. These anerobes enhance health of the human host by helping catabolize complex molecules such as fucosylated glycans [25].

2.2. Nutrition and Inflammation

Inflammation is the body's response to injury or disease. It is a protective mechanism and a necessary part of healing. Evidence supporting the impact of specific foods on inflammation in the body is limited. Some foods have the capacity to suppress inflammation, but it is unclear how often and how much is needed for this benefit. Though there is promising research for the impact of some foods there is no anti-inflammatory miracle food and although diet is important, it is not the only factor [26].

Some mushrooms act directly on inflammation. *Cordyceps synensis*, a mushroom that contains cordycepin, stimulates the production of interleukin 10, an anti-inflammatory cytokine [27]. Reishi mushroom (*Ganoderma lucidum*) contains triterpenes, which *in vitro* have been shown to suppress several markers of inflammation: tumour necrosis factor alpha, interleukin-6, nitric oxide, prostaglandin, NF-kappaB, and COX-2 activity [28]. *Poria cocos* mushroom also contains triterpenes, which have been shown to improve inflammation in mice [29].

Other mushrooms exert an anti-inflammatory effect less directly, by quenching damaging free radicals and counteracting oxidation. Chaga mushroom (*Inonotus obliquus*), for instance, has antioxidant activity, protecting cells against oxidative damage *in vitro* [30]. Oyster mushroom (*Pleurotus ostreatus*) has an antioxidant effect as well [31]. Significant links have been established between gut microbiota and inflammation, sarcopenia and cognitive function partly related to what it is known as the brain-gut axis on a two-way street [32,33]. The bacteria species in our colon today are more or less the same as we had when we were six months old. About 80% of a person's gut microflora are transmitted from the mother during birth, being a very stable system.

The positive health effects of mushroom consumption during inflammation has been demonstrated. However, there were modest effects of *in vivo* consumption of edible mushrooms on induced inflammatory responses. The result is not surprising since it would certainly be harmful to strongly induce or suppress immune function following ingestion of a commonly consumed food [34].

Bacteroides (B. succinogenes and *B. ruminicola), Prevotella* and *Ruminococcus flavefaciens* seem largely distributed in the higher animals. Studies also indicate that long-term diet is strongly associated with the gut microbiome composition. Those who eat plenty of protein and animal fats, typical of Western diet, have predominantly *Bacteroides* bacteria, while for those who consume more carbohydrates, especially fibre, the *Prevotella* species predominate [35]. Bacteria ferment β-glucans in the intestinal tract, producing short-chain volatile fatty acids (acetic, propionic and butyric) [36]. These may stimulate insulin release from the pancreas and alter glycogen breakdown by the liver and therefore play a role in glucose metabolism and protect against insulin resistance [37,38].

The microbiota represent an enormous antigenic burden that must largely be compartmentalized to prevent immune system activation. In the healthy state, intestinal lamina propria cells of both innate and adaptive immune systems cooperate to maintain physiological homeostasis [39].

Imbalances in the composition of the bacterial microbiota, known as dysbiosis, are postulated to be a major factor in human disorders such as inflammatory bowel disease. Molecules of the bacterial microbiota can mediate the critical balance between health and disease [40,41].

Probiotics, prebiotics and synbiotics (a mixture of pro- and prebiotics) are the most frequent components used for the elaboration of functional foods [42]. Probiotics are viable microorganisms able to reach the intestine in an active state and thereby exert positive health effects [43]. A prebiotic is a selectively fermented ingredient that promotes specific changes in the composition and/or activity of the gastrointestinal microbiota which, in turn, confers benefits on host well-being and health [44,45]. Non-digestible oligosaccharides (NDO) fulfill all the criteria for classification as prebiotics. Specifically, the bifidogenic NDO inulin, its hydrolysis product oligofructose, galactooligosaccharides and lactulose are the prototype of prebiotic saccharides [46,47,48].

2.3. β-glucans and the Immune System

Although there is established evidence on the role of dietary fibers in pathologies such as obesity and metabolic syndrome there is a lack of international agreement regarding the definition and classification of fibre [49]. Cereal derived β-glucans have also been ascribed to have immune-stimulating properties [50].

β-glucans are a group of β-D-glucose polysaccharides that are found in the cell walls of bacteria, fungi (mushrooms), yeasts (e.g. *Saccharomyces cerevisae*), algae, lichens and plants (e.g. oats and barley). β-glucans are used in various nutraceutical and cosmetic products, as texturing agents, and as soluble fibre supplements while they have been classified in food as GRAS (Generally Recognised As Safe) by EFSA [51,52,53].

Mushrooms contain dietary fibres including β-glucans, chitin and heteropolysaccharides (e.g. pectinous substances, hemicellulose, polyuronides), making up as much as 10-50% in the dry matter. Much of the active polysaccharides, water soluble or insoluble, isolated from mushrooms, can be classified as dietary fibres (i.e. β-glucan, xyloglucan, heteroglucan, chitinous substance) and their protein complexes [54].

The polysaccharide peptides can be found in the mycelium, while the fruiting body mainly contains polysaccharides. Where there is polysaccharide there is polypeptide and the polysaccharide and peptide of PSP are closely bound and not separated. β-glucans are healthy fibers that humans cannot digest, but that can be digested by some species of gut bacteria [55]. The fungal/mushroom cell wall is a complex structure composed of chitin, glucans, other polymers, and have chitinase or glucanase activity [56]. Not all β-glucans are able to modulate imune functions. Mushroom β-glucans, which consist of a (1,3)-β-linked with small numbers of (1,6)-β-linked side chains, are essentially known for their immuno-modulating effects [57,58].

Based on their effects on the immune system, mushroom β-glucans have been proposed to act as "biological response modifiers" (BRM), enhancing the body's own use of macrophages and T-lymphocytes, rather than directly attacking any tumours [59]. Enterocytes facilitate the transportation of β-(1,3)-glucans and similar compounds across the intestinal cell wall into the lymph, where they begin to interact with macrophages to activate immune function [60].

M cells are specialized cells found in the follicle-associated intestinal epithelium within the Peyer's patches, transporting antigens from the lumen to cells of the immune system [61]. M-cells act as gateways to the mucosal immune system and physically transport the insoluble whole glucan particles into the gut-associated lymphoid tissue [62].

3. Mode of Action of Mushroom Biomass and Extracts on Health

The science is complex, but the bottom line is simple: changing people's bacteria is a more effective strategy for treatment and prevention of disease than changing their diet. The gastrointestinal mucosa forms a barrier between the body and a lumenal environment which not only contains nutrients, but is laden with potentially hostile microorganisms and toxins. The challenge is to allow efficient transport of nutrients across the epithelium while rigorously excluding passage of harmful molecules and organisms into the body [63].

The mechanisms by which mushrooms may influence health benefits remain an active area of investigation. Undeniably, the chemical composition and total intake of mushrooms determine their potential attributes. Mushrooms were reported to enhance the activity of NK (natural killer) cells which are important components of the innate immune system, which is responsible for antiviral and antitumor defense [64,65,66]. The polysaccharides in mushrooms may mimic bacterial polysaccharides and bind to Toll-like receptors expressed in the host. They may function through immune regulation or by altering inflammatory response [67].

Mushrooms may affect gastrointestinal tract healing through their action as prebiotics [68]. Mushrooms also have the potential to improve the development of adaptive immunity after initial exposure to an antigen or pathogen [69,70].

An extract derived from the mushroom Coriolus versicolor, containing polysaccharide K (PSK) and polysaccharide-peptide (PSP), with potential immunomodulating and antineoplastic activities, has been shown to stimulate the production of lymphocytes and cytokines, such as interferons and interleukins, and may exhibit antioxidant activities. However, the precise mechanism of action of these agents is unknown [71].

Because human body cannot digest fibre, it does not provide calories for energy or nutrients for cells. Despite this fact, fibre provides health benefits and is an important part of a healthy diet [72]. Soluble fibre also binds to bile acids in the intestines and promotes their excretion. Eating soluble fibre can help regulate blood cholesterol levels and lower the risk for heart disease. However, it is not clear whether β-glucans can lower triglyceride levels [73,74]. Because insoluble fibre, such as lignin, cellulose and hemicellulose, remains undigested, it increases stool bulk.

While plant cell walls are major sources of dietary fibre, mushroom cell walls can also be considered as food fibre. Mushroom cell walls contain a mixture of fibrillar and matrix components which include chitin (a straight-chain 1,4-β-linked polymer of N-acetyl-glucosamine) and the polysaccharides such as 1,3-β-D-glucans and mannans, respectively. These mushroom cell wall components are non-digestible carbohydrates that are resistant to human enzymes and can be considered as source of dietary fibre [75]. Nevertheless, enterocytes facilitate the transportation of β-(1,3)-glucans and similar compounds across the intestinal cell wall into the lymph, where they begin to interact with macrophages to activate immune function [60].

Chemically, dietary fibre consists of non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, chitins, pectins, β-glucans, and oligosaccharides [76]. M cells within the Peyer's patches physically transport the insoluble whole glucan particles into the gut-associated lymphoid tissue [62].

Commercial quantities of β-glucans are typically obtained from oats (3 - 11%) and barley (2 - 8%) [77]. In developing countries maize is the main staplefood and levels of fibre (2.5%) and β-glucan are also low 3.3% (w/w) [78]. Once the human body completes the process of digestion, bacteria in the colon can degrade fibre molecules. The enzymes produced by gut bacteria degrade the fibre through the process of fermentation. Some 29 to 82 % of cellulose, 56 to 87 % of hemicelluloses and 90 % of pectin degrades as it passes through the colon [79].

3.1. Mushroom Biomass

Both mycelium and primordia (young fruit body) cultivated into a biomass is grown on a sterilized (autoclaved) substrate. This cultivation process ensures the final powder is free from contamination by other fungi and that pesticides and heavy metals are absent. The reason biomass is more resistant to gastric fluid attack is because the nature of the biomass mushroom cells itself, protect the active compounds. The extracted form is more exposed to the action of the proteolytic enzymes since there are no physio-chemical barriers to prevent such exposure, compared to biomass equivalent [80]..

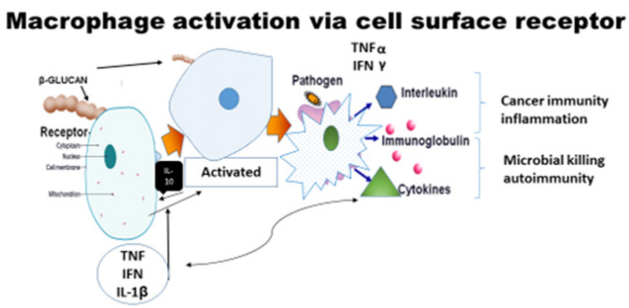
In extracts, proteins, (i.e. enzymes), are denatured by the hot water extraction process which is conducted at temperatures above 62°C and by substances used in the process, such as alcohol and sodium hydroxide [81]. In a study [82] comparing levels of different proteolytic enzymes, β-glucans and secondary metabolites, between biomass and extract forms of *Ganoderma lucidum* mushroom, it was clearly shown the absence of important immune-enhancing enzymes (e.g.protease, glucoamylase, peroxidase) in the extracted form. Nevertheless, mushroom extracts have begun to be sold as dietary supplements with a world-wide market value of over 6 billion US dollars per year [83], a business much higher than for biomass.

3.2. Mushroom Biomass versus Mushroom Extract

Mushrooms are good sources of several bioactive compounds which are able to augment or complement a desired immune response. Such bioactive compounds are polysaccharopeptides, polysaccharide-proteins, functional proteins (ubiquinone-9, nebrodeolysin, ubiquitin-like peptide and glycoprotein), β-glucans, proteoglycans and many others. Most of these bioactive compounds follow the immunomodulatory pathway mechanism of polysaccharide (β-glucans) from mushrooms by stimulating activities for both innate and adaptive immune systems. They proliferate and activate innate immune system components such as natural killer (NK) cells, neutrophils, and macrophages, and stimulate cytokines expression and secretion. These cytokines in turn activate adaptive immunity through the promotion of B cells for antibodies production and stimulation of T-cell differentiation to T helper (Th1 and Th2) cells, which mediate cell and humoral immunities, respectively [84,85].

Besides the antioxidant effect that polysaccharides may have, there are phenolic compounds that occur naturally in the mushrooms. Total phenolic and flavonoid contents occurring in different types of edible mushrooms have been evaluated [86]. Some species of mushrooms synthesize enzymes that may play important functions in the organism. The biomass form of mushrooms contain, not only protein-bound polysaccharides (PSK; PSP), typical on extracts, but also active enzymes responsible for preventing oxidative stress (e.g. laccase, superoxide dismutase), inhibiting cell growth (e.g. proteases, glucoamylases) and promoting detoxification (e.g. peroxidases, cytochrome P450, glucose-2-oxidase) and there are innumerous situations and conditions related with oxidative stress: allergies, arthritis, asthma, atherosclerosis, inflammation (acute, chronic), cancer, cataracts, diabetes, multiple sclerosis, hemorrhage, infections, bruising, intestinal worms, chemotherapy, radiation, stress (physical, mental), tobacco smoke and ulcers [87].

The immune system is made up of white blood cells. There are several different types of white blood cells and each type has a specific job to perform. The immune response that occurs when cells become infected or damaged is the result of interactions between different types of immune cells. When an immune cell recognizes an object or cell as 'foreign' or infected, a cascade of events occurs that ultimately leads to the destruction and removal of the targeted object [88]. This cascade is made of possible messenger molecules produced by the immune cells. These messenger molecules are called cytokines.



TNF-Tumour Necrosis Factor, IL-1 – Interleukin -1, IFN- Interferon

Figure 2. Macrophage activation

Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells [89]. Cytokines include chemokines, interferons, interleukins, lymphokines, tumour necrosis factor but generally not hormones or growth factors. Cytokines (e.g. Interleukin-2 (IL-2) and Alpha Interferon (IFN)) are secreted proteins that allow communication between and within cell types. Different cytokines elicit different responses. Cytokines may exert their effects on other cells as well as the cell that released them. Cytokines can activate, modulate and inhibit immune responses [90].

Published in the journal Nature, a Japanese study shows that butyrate, a by-product of the digestion of dietary fiber (together with actetate and propionate) by gut microbes, acts as an epigenetic switch that boosts the immune system by inducing the production of regulatory T cells in the gut. Previous studies have shown that patients suffering from inflammatory bowel disease lack butyrate-producing bacteria and have lower levels of butyrate in their gut. However, butyrate's anti-inflammatory properties were attributed to its role as main energy source for the cells lining the colon [91].

Hundreds of scientific studies have been produced by prestigious institutions attesting to the immuno-activating and protective effects of β-1,3 /1,6-glucans. The classic model of how β-glucans work is that they are recognized by the immune system as an invasive organism, activating the body's immune defenses as if to fight an infection. [92]. The mushroom mycelium's ability to defend itself from infectious diseases by staving off invasive organisms can act as shields of protection not only through direct anti-microbial activity but also from the production of host-mediated immune cells.

4. Concluding Remarks

There is plenty of evidence that most human major diseases have a physiological or lifestyle basis, but it is probable in some of those that the gut microbiota is a modulating factor that contributes to the overall risk.

One of the things chemotherapy does is suppress the immune system, so the question is whether patients taking mushroom can maintain healthier immune function. Scientists have been studying the effect of β-1,3/1,6-glucans on the immune system for many years, however we have only recently begun to develop a true understanding of the effects of these polysaccharides. β-1,3/1,6-glucans are known as a biological response modifiers as they are able to bond with the surface of certain innate immune cells and improve the immune system's ability to fight off viral, bacterial, fungal and parasitic infections.

Mushroom biomass supplements are a particularly good choice for people with an already weakened immune system. They do not perform miracles neither have a specific role for each illness, rather, it supports the immune system, assisting in preventing or mitigating the effects of a range of several ailments by providing overall immune support via supplementation with β-glucans, enzymes and secondary metabolites.

Mushroom biomass is orally effective, non-toxic with enhanced effectiveness when delivered in small particle sizes to help promoting better ingestion and absorption into the immune system at intestinal level. In this form the product is absorbed to a high percentage into the immune system, not just passed through and expelled by the body as large particle globular glucans. These β-1,3/1,6-glucans are the nutritional fuel for the immune system thus enabling a normalized immune response, fighting back against health invaders and maintaining good health.

β-glucans are the new frontier on mushroom nutrition and medicine, however, much research still remains to be done. Promising evidence, still in its infancy, suggests a positive role for mushrooms and their bioactive components, particularly ergothioneine, vitamin D, β -glucan, and selenium, on immune function, gut function and microbioma balance.

Probing studies in humans are still needed to understand the implications of the observed effects on immune function, gut microbiota, cognition, periodontitis, cancer mechanisms, body composition, and body weight. Studies are also needed to define how much, how often, and perhaps in what pattern specific mushroom species may be consumed to bring about substantial biologic and health responses, as well as to understand the specificity of mushroom impacts on health.

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