Fungi as functional foods: Fungal bioactive compounds with beneficial effects on human health

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Abstract

Food insecurity, malnutrition and unsustainable food production are major challenges faced by humanity today. Edible fungi could play an important role in restructuring current agrifood systems due to their balanced nutritional composition and their cost-effective and eco-friendly production. Many fungal species have the potential to be developed into functional food because they are a source of various bioactive compounds which provide health benefits beyond their macro-nutritional value. This review is aimed at giving a broad overview of fungal bioactive compounds beneficial for human health and investigating the potential of fungi as functional foods. In all the major groups of fungal primary and secondary metabolites that were investigated, a wide variety of bioactivities were found. Frequently occurring bioactivities present in almost all biochemical groups include antioxidant, anti-microbial, anti-cancer and immunomodulating activities, as well as benefits for gut-, brain- and cardiovascular health. Although many compounds that are present in fungi have been shown to possess these bioactivities, their actual effects through dietary intake remain insufficiently investigated. As the presence of specific bioactive compounds differs between fungal species, an assessment of an individual species is recommended. Given the rising demand for healthier and sustainable foods, fungi offer great potential to play an important role in future agrifood systems.

Layman's Summary

De wereld kampt met verschillende uitdagingen op het gebied van voedsel en gezondheid zoals voedselonzekerheid, ondervoeding en niet-duurzame voedselproductie. De huidige voorspelling is dat de omvang van deze problemen zal toenemen vanwege een groeiende wereldbevolking en toenemende conflicten en klimaatextremen. De belangrijkste aanbeveling van het laatste rapport van de voedsel- en landbouworganisatie van de Verenigde Naties is om de kosteneffectiviteit en de beschikbaarheid van voedzaam voedsel te vergroten. Om dit te bewerkstelligen is er een herstructurering van onze huidige agri-voedselsysteem nodig.

Schimmels kunnen bij deze herstructurering een belangrijke rol spelen. Schimmels zijn meercellige micro-organismen en vormen een eigen rijk net als het rijk van de dieren. De meeste schimmels zijn bekend vanwege hun vruchtlichamen die wij kennen als paddenstoelen. Maar naast deze paddenstoelvormende schimmels is er een enorme diversiteit binnen het schimmelrijk, al is er maar weinig bekend over het meeste aantal soorten.

Momenteel worden schimmels al gebruikt in de productie van voedsel en drank. Denk bijvoorbeeld aan de champignon, of aan bakkersgist als ingrediënt van brood. Een voordeel van schimmels is dat ze op een duurzame manier kunnen worden gekweekt. Schimmels kunnen namelijk groeien op dood materiaal zoals afvalproducten uit de landbouw. Daarbij breken ze de grote koolstofmoleculen in het organische materiaal af, en zetten ze dit om naar macronutriënten zoals eiwitten, koolhydraten en gezonde vetten. Daarnaast kunnen schimmels ook worden beschouwd als functioneel voedsel. Functioneel voedsel bevat functionele ingrediënten, ook wel bioactieve stoffen genoemd. Dit zijn stoffen waar na wetenschappelijk onderzoek is aangetoond dat het een gunstig effect heeft op één of meer doelfuncties van het menselijk lichaam.

Dit review artikel geeft een overzicht van de huidige wetenschappelijke literatuur over bioactieve stoffen in schimmels. In alle onderzochte moleculaire categorieën zijn bioactieve stoffen gevonden. In meerdere schimmelsoorten zijn eiwitten, polyssachariden en vetzuren gevonden met een scala aan bioactiviteiten. Zo bevatten schimmels veel antioxidanten, en ontstekingsremmende en antimicrobiële stoffen. Daarnaast zijn er veel stoffen gevonden die potentieel gebruikt kunnen worden om kanker en cardiovasculaire ziekten te voorkomen of te genezen. Verder bevatten schimmels een variëteit aan stoffen die het immuunsysteem of ons darmstelsel beter laten functioneren. Ten slotte zijn ook een aantal stoffen gevonden die het menselijk brein beschermen tegen ziekten zoals Alzheimer en Parkinsons.

Hoewel er is aangetoond dat schimmels vele bioactieve stoffen bevatten, blijven hun werkelijke effecten via orale inname onvoldoende onderzocht. Dit heeft een aantal oorzaken. Er zitten enorme verschillen tussen schimmelsoorten, en dus ook tussen de bioactieve stoffen die ze bevatten. De wetenschap is echter vooral gefocust op enkele soorten waardoor er over veel groepen schimmels weinig informatie bekend is. Daarnaast ontbreekt er veel kennis over het gedrag van deze stoffen in ons darmstelsel. Sommige stoffen kunnen namelijk gedeeltelijk afgebroken worden en verliezen daarmee hun bioactieve functies. Dit wordt ook wel de biologische beschikbaarheid genoemd.

Uit deze review valt te concluderen dat schimmels een enorme potentie hebben als functioneel voedsel om ziektes te voorkomen en te genezen. Er is echter specifiek onderzoek nodig naar individuele schimmelsoorten en de biologische beschikbaarheid van de stoffen die ze produceren voordat er daadwerkelijk gezondheidsclaims gemaakt mogen worden. Schimmels zouden een belangrijke rol kunnen spelen in het toekomstige agri-voedselsysteem aangezien de vraag naar duurzaam en gezond voedsel sterk toeneemt.

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Introduction

Humankind faces many challenges with respect to food & health. These challenges include food insecurity, malnutrition and unsustainable food production. Food insecurity is a growing problem since access to safe, nutritious and affordable food in many low- and middle-income countries have been declining over the last few years (Food and Agriculture Organization of the United Nations, 2022). Moreover, there has been a growth in global populations that are affected by different forms of malnutrition, including both the prevalence of undernourishment and obesity. The magnitude of these problems is predicted to increase, as the world's population continues to grow. Other contributing factors are the worsening of the main drivers behind food insecurity such as conflicts, climate extremes and economic shocks, that are likely to continue in the near future (Food and Agriculture Organization of the United Nations, 2022). The key recommendation of the latest report on the state of food security and nutrition in the world is to increase the cost-effectiveness of nutritious foods and the availability of healthy diets by rethinking current agrifood systems (Food and Agriculture Organization of the United Nations, 2022).

In line with rethinking agrifood systems, there has been increasing interest in the nutritional value of edible fungi. Several factors can explain this growth of interest. Firstly, edible fungi are a rich source of nutrients as they contain a balanced nutritional composition (Mohd Rashidi and Yang, 2016) and can be considered an alternative high-quality protein source with both essential and nonessential amino acids (Bach *et al.*, 2017; González *et al.*, 2020). In addition, edible fungi are a source of carbohydrates, dietary fibre, unsaturated fatty acids, vitamins and minerals (Bach *et al.*, 2017; Ho, Asyikeen Zulkifli and Tan, 2020).
Second, there is a growing interest in the production of healthy and nutrient-rich food of non-animal origin. Animal-based food products usually have high production costs, are resource intensive and are environmentally damaging (González *et al.*, 2020). In contrast, fungi can be grown on agricultural waste products in low-burden fermentation processes, which makes them a sustainable and eco-friendly food source.
Thirdly, the interest in edible fungi can be explained by the converging trends of eastern herbal medicines, organic food product preferences, gut-healthy products and a positive outlook toward sports nutrition (Niego *et al.*, 2021).

Fungi can be considered functional foods as they provide additional health benefits beyond their macro-nutritional value. Edible fungi contain many bioactive compounds with bioactivities such as anti-cancer, antidiabetic, antihypertensive, antimicrobial, anti-viral, anti-inflammatory, antioxidant, immunomodulatory, neurotrophic and neuroprotective properties (Das *et al.*, 2021; Niego *et al.*, 2021; Landi *et al.*, 2022). Anti-cancer bioactivity refers to the ability of a substance to prevent or treat cancer, by exerting a therapeutic effect on cancer cells or the surrounding tissue. This usually involves disrupting one or more processes that are crucial for the survival and growth of cancer cells. The mechanisms by which compounds exert their anti-cancer bioactivities vary significantly. This is true for most of the bioactivities that will be discussed. This review will therefore not go into detail about each specific cellular mechanism behind the observed bioactivity.

In addition to functional foods, many recent scientific articles have been published about nutraceutical compounds present in fungi. Nutraceuticals are a group of products that are more than food but less than pharmaceuticals, as they can be considered a supplement to effective pharmacological treatment proven by clinical testing (Niego *et al.*, 2021). While both nutraceuticals and bioactive compounds have health benefits, nutraceuticals are marketed as supplements of functional foods, while bioactive compounds are found in the functional foods themselves.

The Protein Brewery (TPB) develops food ingredients (Fermotein®) using plant-based substrates and the fermentation capabilities of a Mucorales fungus. The current scientific landscape surrounding the topic of bioactive compounds in edible fungi is almost exclusively focused on Dikarya, the subkingdom of fungi that includes the phyla of the Ascomycota and Basidiomycota, also known as "higher fungi". Less is known about the closely related sister phylum of Mucoromycota, in which the order Mucorales can be found (James *et al.*, 2020). Mucoromycota, were formerly, and according to the conventional four-phylum classification, understood as part of the heterogeneous group of saprotrophic fungi called Zygomycetes (Naranjo-ortiz and Gabald, 2019). Mucoromycota are characterized by having wide filamentous anastomous hyphae lacking septa. The versatile metabolic activities of Mucoromycete fungi can be utilized to produce and accumulate a wide range of valuable metabolites. Mucoromycota are able to co-produce several of these metabolites in single fermentation processes (Dzurendova *et al.*, 2022). Considering their rapid growth and metabolic versatility, Mucoromycota fungi can be considered powerful cell factories for biorefinery application in food production (Pawłowska *et al.*, 2019; Dzurendova *et al.*, 2022).

For fungal-based biorefineries to aid in the transition to sustainable and cost-effective agrifood systems, more knowledge is needed regarding fungal bioactive & nutraceutical compounds. This review is aimed at giving a broad overview of fungal bioactive compounds beneficial for human health and investigating the potential of fungi as functional foods. Since this review is done on behalf of TPB, this review will give special focus to Mucorales species when there is data available. Moreover, this review will summarize the origin of these compounds in terms of species and cellular localisation. The types of bioactive compounds will be categorized into primary metabolites and secondary metabolites. Primary metabolites include proteins, polysaccharides, fats, polyphosphates, organic acids, nucleotides and minerals, and secondary metabolites include polyketides, terpenoids, shikimic acid-derived compounds and non-ribosomal proteins.

Proteins

Along with fats and carbohydrates, proteins are part of the three main macronutrient groups in the human diet. During human digestion proteins are broken down into smaller peptides or single amino acids. Protein has important functions in the body such as providing structure to cells, tissues and organs, as well as various hormonal- and enzymatic activities. Compared to protein production of animal and plant origins, fungal protein is usually faster, cheaper, less space intensive and more sustainable since fungi can be grown on many agricultural waste products (González *et al.*, 2020). Most edible fungi can be considered a high-quality protein source since they have a complete essential amino acid profile (Bach *et al.*, 2017). In a nutritional context, an essential compound indicates that the compound cannot be synthesized in the human body and thus has to be provided by nutritional sources. Compared to plants, the protein profile of fungi is closer to that of meat as animals and fungi have a higher genetic similarity.

Another important factor concerning functional protein is protein digestibility, which refers to the amount of protein available for absorption after the digestion process. Mycoprotein, when consumed in sufficient quantities (< 40 grams), was shown to mount a muscle protein synthetic response, which is evidence of both high quality and bioavailability (Dunlop *et al.*, 2017). It is however important to note that both quality and bioavailability depend on the fungal species, type of substrate and processing conditions (González *et al.*, 2020). This can be partly explained by the fact that some substances present in fungi can act as anti-nutritional factors, interfering in protein digestion. These compounds include dietary fibre, phenolic compounds, phytates, tannins, trypsin inhibitors and hemagglutinins (a type of lectin). Food processing, such as heat treatment or drying can be used to partly inactivate anti-nutritional factors (González *et al.*, 2020).

Bioactive Peptides

Bioactive peptides (BAPs) are amino acid sequences of 2-20 residues long, that have biological health benefits beyond their nutritional value (Zhou *et al.*, 2020). BAPs remain inactive when part of the intact parent protein. When released through hydrolysis during digestion, BAPs, in contrast to their parent proteins, can be absorbed completely by the intestine and enter the circulatory system where they directly produce local effects. BAPs have a high potency, high tissue affinity, are non-toxic and high in stability, which makes them ideal bioactive compounds for functional foods. BAPs lack the same specificity as their parent proteins. This means that BAPs are able to interact with multiple cellular components. Their bioactivity is thus more flexible and sometimes multifunctional. For instance, some fungal BAPs possess both anti-cancer and immunomodulating properties (Zhou *et al.*, 2020).

Hypertension, also known as high blood pressure, leads to cardiovascular diseases, strokes and organ dysfunctions. Blood pressure is regulated in complex biochemical pathways, wherein the renin-angiotensin system (RAS) plays a central role. Within this system, angiotensin converting enzyme (ACE) is a key regulator and is the main target in antihypertensive therapy. Fruiting bodies from a variety of mushrooms, (see Zhou *et al.*, 2020; Landi *et al.*, 2022), are potential sources of ACE inhibitory BAPs which are suggested to act as effective hypotensors in vivo (Ching *et al.*, 2014). Mushroom-derived antihypertensive peptides may have synergistic effects with antihypertensive peptides from other sources and are promising alternatives to synthetic ACE inhibitors due to their high bioavailability and lack of side effects (Zhou *et al.*, 2020). An overview of ACE inhibitory BAPs and their fungal origin can be found in **Tables 1 & 2**.

Table 1. ACE Inhibitory peptides derived from fungi. From (Zhou *et al.*, 2020) *where the references can be found.*

Mushroom species	Amino acid sequence	IC_{50}	Mode of inhibition
Grifola frondosa	VIQKYP	97 μg/mL	Competitive
Tricholoma giganteum	GEP	40 μg/mL	Competitive
Pholiota adiposa	GEGGP	44 μg/mL	Competitive
Pleurotus cornucopiae	RLPSEFDLSAFLRA	0.46 mg/mL	Non-competitive
	RLSGQTIEVTSEYLFRH	1.14 mg/mL	Non-competitive
Hypsizygus marmoreus	LSMGSASLSP	190 μg/mL	Non-competitive
Pleurotus cystidiosus	AHEPVK	62.8 μM	Competitive
	GPSMR	277.5 μM	ND
Agaricus bisporus	AHEPVK	63 μM	Competitive
	RIGLF	116 μM	Competitive
	PSSNK	129 μM	Non-competitive
Tricholoma matsutake	WALKGYK	0.40 μM	Non-competitive
	LLVTLKK	0.95 μM	ND
	IISKIK	1.19 μM	ND
	ILSKLK	4.02 μM	ND
	LIDKVVK	0.62 μM	ND
Ganoderma Lucidum	QLVP	127.9 μM	mixed-type
	QLDL	151.5 μM	Non-competitive
	QDVL	155.8 μM	Competitive

ND, not detected.

Table 2. The N-terminal amino acid sequence of mushroom-derived BAPs. From (Landi *et al.*, 2022) *where the references can be found.*

Peptide Name	Peptide Mr (Da)	N-Terminal Sequence	Peptide Source	Taxonomy NCBI: Txid
ACE inhibitory peptide 1	605.30	$_1$ RIGLF $_5$ [a]	*Agaricus bisporus*	5341
ACE inhibitory peptide 2	679.53	$_1$ AHEPVK $_6$ [a]	*Agaricus bisporus*	5341
ACE inhibitory peptide 3	532.30	$_1$ PSSNK $_5$ [a]	*Agaricus bisporus*	5341
Ubiquitin-like peptide	9500	$_1$ MQIFVK $_6$	*Cyclocybe aegerita*	5400
Agrocybin	9000	$_1$ ANDPQCLYGN VAAKF $_{15}$	*Cyclocybe aegerita*	5400
Cordymin	10,906	$_1$ AMAPPYGYRT PDAAQ $_{15}$	*Cordyceps militaris*	73501
GLP fraction	<10,000	n.r.	*Ganoderma lucidum*	5315
ACE inhibitory peptide	747.42 [b]	$_1$ VIEKYP $_6$ [a]	*Grifola frondosa*	5627
CULP	8500	$_1$ MQIFVKTLTG KTITLEVEES DDIDNVKAKI QDKEG $_{35}$	*Handkea utriformis*	258083
ACE inhibitory peptide	567.30 [b]	$_1$ LSMGSASLSP $_{10}$ [a]	*Hypsizygus marmoreus*	39966
PSULP	9500	$_1$ MQIFVKTLTG KTITL $_{15}$	*Lentinus sajor-caju*	50053
ACE inhibitory peptide	301.00	$_1$ GQP $_3$ [a]	*Macrocybe gigantea*	1491104
ACE inhibitory peptide	414.00	$_1$ GQGGP $_5$ [a]	*Pholiota adiposa*	64639
ACE inhibitory peptide 1	1622.85	$_1$ RLPSEFDLSA FLRA $_{14}$ [a]	*Pleurotus cornucopiae*	5321
ACE inhibitory peptide 2	2037.26	$_1$ RLSGQTIEVT SEYLFRH $_{17}$ [a]	*Pleurotus cornucopiae*	5321
Eryngin	10,000	$_1$ ATRVVYCNRR SGSVVGGDDT VYYEG $_{25}$	*Pleurotus eryngii*	5323
POP	9000	$_1$ GPCYLVAFYE SSGRR $_{15}$	*Pleurotus ostreatus*	5322
Pleurostrin	7000	$_1$ VRPYLVAF $_8$	*Pleurotus ostreatus*	5322
Plectasin	4398.80	$_1$ GFGCNGPWDE DDMQCHNHCK SIKGYKGGYC AKGGFVCKCY $_{40}$ [a]	*Pseudoplectania nigrella*	96584
SU2 peptide	4500	$_1$ KREHGQHCEF $_{10}$	*Russula paludosa*	176813

When present in high amounts, free radicals and reactive oxidant species (ROS) can lead to a multitude of diseases including cancer. Antioxidants can remove free radicals by providing protons or electrons or by enhancing antioxidant enzymatic activity. Fungi can be considered a rich source of antioxidant BAPs with high radical scavenging activities in vitro (Zhou *et al.*, 2020; Landi *et al.*, 2022). Most of the research is focused on the fruiting bodies and their extracts. While mycelium extracts have been shown to possess many anti-oxidant compounds (Sánchez, 2017), no research so far has described mycelium-derived BAPs with anti-oxidant activity. Additionally, fungi contain glutathione (GSH), a tripeptide known as a key regulator in the antioxidant biochemical system and ergothioneine (ERGO), an amino acid with important antioxidant properties (Kalaras *et al.*, 2017). While some antioxidant MBAPs were shown to be effective in vivo, the biostability, bioavailability and antioxidant efficacy of these compounds has yet to be verified. An overview of antioxidant BAPs and their fungal origins can be found in **Table 3**.

Table 3. Antioxidant peptides derived from fungi. From (Zhou *et al.*, 2020) *where the references can be found.*

Mushroom species	Molecular characteristics	Evaluating methods and values
Ganoderma lucidum	Peptide mixtures	Superoxide anion radical scavenging activity, ~45% at 1 mg/mL Hydroxyl radical scavenging activity, IC_{50} = 25 μg/mL Ferric reducing power Soybean lipoxygenase activity inhibition, IC_{50} = 27.1 μg/mL
Ganoderma lucidum	Peptide mixtures	Hepatoprotective effects on D-galactosamine induced liver injury in mice, at a dosage of 180 mg/kg BW ($p < 0.01$)
Ganoderma lucidum	Peptide mixtures	Hepatoprotective effects on CCl_4 induced liver injury in mice, at a dosage of 260 mg/kg BW ($p < 0.05$)
Ganoderma lucidum	3.35 kDa Rich in Phe, Asp, Pro, His and Ile	DPPH radical scavenging activity, 74.21% Superoxide anion radical scavenging activity, 72.16% Hydroxyl radical scavenging activity, 72.87% Lipid peroxidation inhibition (at a dosage of 0.2 mg)
Cordyceps sinensis	10,906 D N-terminal: AMAPPYGYRTPDAAQ	Prevention effect on focal cerebral ischemic/reperfusion injury in rat, at a dosage of 2 mg/kg/day ($p < 0.05$)
Grifola frondosa	<3 kDa fraction (2385 and 1138 Da)	DPPH radical scavenging activity, 89.6% at 2.5 mg/mL Ferric reducing power, 2.71 at 2.5 mg/mL Ferrous ion chelating activity Lipid peroxidation inhibition
Tricholoma matsutake	WALKGYK	DPPH radical scavenging activity, 50% at 10 mg/mL
Agaricus bisporus	1–3 kDa Rich in hydrophobic and negatively charged AAs	DPPH radical scavenging activity, IC_{50} = 0.13 mg/mL Ferrous ion chelating activity Ferric reducing power Lipid peroxidation inhibition
Pleurotus eryngii	Polypeptide	DPPH radical scavenging activity Hydroxy radical scavenging activity, 41.88% at 1 mg/mL Superoxide ion radical scavenging activity Ferric reducing power
Agaricus bisporus	Peptide mixtures	DPPH radical scavenging activity, 73.68% Ferrous ion chelating activity, 11.75% Ferric reducing power, 0.282 Lipid peroxidation inhibition, 79.71% (hydrolysate at 0.25 mg/mL)
Terfezia claveryi	Peptide mixtures	DPPH radical scavenging activity, 51.50% Ferrous ion chelating activity, 21.36% Ferric reducing power, 0.271 Lipid peroxidation inhibition, 85.85% (hydrolysate at 0.25 mg/mL)
Morchella esculenta	Peptide mixtures	DPPH radical scavenging activity, IC_{50} = 6.03 mg/mL ABTS radical scavenging activity, IC_{50} = 0.071 mg/mL H_2O_2 scavenging activity, IC_{50} = 5.28 mg/mL Ferric reducing power, 4.76 μg Vc/mg sample Nitrite and superoxide anion radical scavenging activity Total antioxidant activity
Ganoderma lucidum	Peptide mixtures	DPPH radical scavenging activity, IC_{50} = 22.26 mg/mL ABTS radical scavenging activity, IC_{50} = 7.09 mg/mL Heavy metal ions chelating effect
Cordyceps sinensis	Peptide mixtures	DPPH radical scavenging activity, IC_{50} = 4.79–18.7 mg/mL ABTS radical scavenging activity, IC_{50} = 4.51–14.05 mg/mL Heavy metal ions chelating effect

BW, body weight; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid.

Several anti-microbial BAPs have been identified in fungi. While most studies focus on the fruiting bodies of mushrooms, anti-microbial properties have also been found in the mycelium of *Ganoderma Lucidum* (Mishra *et al.*, 2018). Antimicrobial BAPs can be categorized by their antifungal, antibacterial and antiviral properties (Zhou *et al.*, 2020). Antifungal MBAPs include Cordymin, Pleurostrin, and Eryngin (Landi *et al.*, 2022). Interestingly, Cordymin also showed antiproliferative activity toward breast cancer cells (Wong *et al.*, 2011). Plectasin is a known Antibacterial MBAP that showed anti-infective properties in an in vivo mouse model (Zhou *et al.*, 2020) and has therapeutic potential against several bacterial infections including tuberculosis (Tenland *et al.*, 2018). Several studies also found peptide fractions from *G. Lucidum* and *Terfezia Claveryi* with antibacterial activity against *Escherichia coli* & *Salmonella typhi* and *Bacillus cereus*, respectively (Farzaneh *et al.*, 2018; Mishra *et al.*, 2018). Lastly, antiviral MBAPs with HIV-1 inhibitory activity were found in several fungal species (Sillapachaiyaporn and Chuchawankul, 2020; Landi *et al.*, 2022). An overview of antimicrobial BAPs and their fungal origins can be found in **Table 4**.
So far, there has been only one report of the production of BAPs in the Mucorales order. In vitro ACE-inhibitory BAPs were produced by Mucor Spp during soybean fermentation (Hang and Zhao, 2012).

Table 3. Antimicrobial peptides derived from mushrooms. From (Zhou *et al.*, 2020) *where the references can be found.*

Mushroom species	Bioactivities	Molecular characteristics	Targets and inhibitory values
Pseudoplectania nigrella	Antibacterial	4398.80 Da	*Streptococcus pneumoniae* (in mice), EIC = 10 mg/kg BW
Agaricus bisporus	Antibacterial	Peptide mixtures	*Pseudomonas aeruginosa*, 26.64% at 0.25 mg/mL
Terfezia claveryi	Antibacterial	Peptide mixtures	*Bacillus cereus*, 27.44% at 0.25 mg/mL
Ganoderma lucidum	Antibacterial	Peptide mixtures	*Salmonella typhi*, MIC = 52 μg *Escherichia coli*, MIC = 60 μg
Pleurotus eryngii	Antifungal	10 kDa	*Fusarium oxysporum*, IC_{50} = 1.35 μM *Mycosphaerella arachidicola*, IC_{50} = 3.5 μM
Polyporus alveolaris	Antifungal	28 kDa	*Botrytis cinereal* *F. oxysporum* *M. arachidicola* *Physalospora piricola* EIC = 8 mg/mL for all
Pleurotus ostreatus	Antifungal	7 kDa N-terminal: VRPYLVAF	*F. oxysporum*, 20% *M. arachidicola*, 45% *P. piricola*, 63% (dosage: 15.6 μM)
Agrocybe cylindracea	Antifungal Anti-HIV-1	9 kDa N-terminal: ANDPQCLYGNVAAKF	*F. oxysporum*, IC_{50} = 125 μM *M. arachidicola*, IC_{50} = 60 μM HIV-1 RT, IC_{50} = 60 μM
Cordyceps militaris	Antifungal Anti-HIV-1	10,906 D N-terminal: AMAPPYGYRTPDAAQ	*Mycosphaerella arachidicola*, IC_{50} = 10 μM *Bipolaris maydis*, IC_{50} = 50 μM *Rhizoctonia solani*, IC_{50} = 80 μM *Candida albicans*, IC_{50} = 0.75 mM HIV-1 RT, IC_{50} = 55 μM
Lentinus squarrosulus	Antifungal	17 kDa	*Trichophyton mentagrophytes*, IZD = 25.7 mm *T. rubrum*, IZD = 22.8 mm *Aspergillus niger*, IZD = 12.64 mm *Candida tropicalis*, IZD = 20.54 mm *C. albicans*, IZD = 20.62 mm (dosage: 30 μg/disc)
Russula paludosa	Anti-HIV-1	4.5 kDa N-terminal: KREHGQHCEF	HIV-1 RT, IC_{50} = 11 μM

EIC, effective inhibitory concentration; BW, body weight; MIC, minimal inhibitory concentration; HIV-1, human immunodeficiency virus type 1; RT, reverse transcriptase; IZD, inhibition zone diameter.

Functional Proteins

Many fungal proteins possess bioactivities beneficial for human health. An overview of the fungal proteins discussed below can be found in **Table 5.** Of all fungal proteins, lectins are probably investigated most extensively (Xu *et al.*, 2011). Lectins are carbohydrate-binding proteins and play a role in the recognition between cells, carbohydrates and proteins. Lectins can be found on the cell wall and in the cytoplasm. Lectins have many reported bioactivities including anti-cancer, immunomodulatory, antifungal and HIV-1 reverse transcriptase inhibitory activities (El-maradny *et al.*, 2021; Niego *et al.*, 2021).

Next, many fungal enzymes have been shown to possess anti-cancer potentialities. Ribosome-inactivating proteins (RIPs) are enzymes that can specifically inhibit protein translation and have been shown to possess anti-proliferative and anti-microbial activities. Fungal RIPs with anti-cancer properties are marmorin, flammulin, hypsin, calcaelin, flammin, velin, lyophyllin, pleuturegin and volvarin (Rezvani *et al.*, 2020). Another important group of fungal enzymes with bioactivity are proteases. Proteases are proteolytic enzymes that catalyse the breaking down of proteins. Several types of fungal proteases have been described as potential anti-cancer agents with functions in several cancer stages. Lastly, several fungal laccases and ribonucleases with anti-cancer activities have been found (Xu *et al.*, 2011; Rezvani *et al.*, 2020).

Another group of compounds with anti-proliferative properties are ubiquitin-like proteins (ULPs), which are signalling messengers with roles in proliferation, apoptosis and DNA repair. Several ULPs, including RBUP, PSULP and UbcA1 have been isolated from fungi and have been shown to possess anti-cancer properties in vivo (Rezvani *et al.*, 2020).

Lastly, many fungal immunomodulatory proteins (FIPs) have been discovered during the last decades. This group of bioactive proteins has similar structural characteristics and physicochemical properties. FIPs exert anti-cancer activity either through activation of the immune response or direct cytotoxicity toward tumour cells (Xu *et al.*, 2011; González *et al.*, 2020). The anti-cancer activities of FIPs are summarized in (Li, Zheng and Zhou, 2019).

No bioactivity of proteins derived from Mucorales species have been found. Future research on the presence of bioactive enzymes, RIPs, ULPs and FIPs in Mucorales spp is necessary.

Table 4. Bioactive Fungal proteins. From (Rezvani *et al.*, 2020)*, where the references can be found.*

Table 1 Low-carbohydrate proteins possessing anticancer activity obtained from different mushroom species

	Mushroom species	Type of anticancer protein	Name	Size (kDa)	Assay	Amino acid sequence	IC_{50}
1	*Hypsizigus marmoreus*	Enzyme	Marmorin	9.5	In vitro	AEGTLLGSRATCESGNSMY (N-terminal sequences)	5 μM (MCF-7) 0.15 μM (HepG2)
			Hypsin	20	In vitro	ITFQGDLDARQQVITNADTRRKRDVRAAVR (N-terminal sequences)	–
2	*Calvatia caelata*	Enzyme	Calcaelin	39	In vitro	ANPIYNIDAFRV (N-terminal sequences)	–
3	*Flammulina velutipes*	Enzyme	Flammulin	40	In vitro	APSHFSHPGVLADRAQIDFIXGKVNEGAEPWXSAYN (N-terminal sequences)	0.25 nM
			Flammin	30	In vitro	SPVIPANTFVAFRLYEVGFUPA (N-terminal sequences)	1.4 nM
			Velin	19	In vitro	SGSPLTQAQAEALLKPQGLAYSSGGNT (N-terminal sequences)	2.5 nM
4	*Lyophyllum shimeji*	Enzyme	Lyophyllin	20	In vitro	ITFQGASPARQTVITNAITRARADVRAA (N-terminal sequences)	1 nM
5	*Pleurotus tuber-regium*	Enzyme	Pleuturegin	38	In vitro	ARTQPGNIAPVGDFTLYPNAPRQGHIVA (N-terminal sequences)	0.5 nM
6	*Volvariella volvacea*	Enzyme	Volvarin	30	In vitro/in vivo	nd	0.5 nM
7	*Cordyceps militaris*	Enzyme	CMP	12	In vitro	a)YQXXVTFXDF b)VSXXGDSGVGGN c) NAFNDYTFK	15 μM (MCF-7) 9.3 μM (5637 cells)
8	*Pleurotus eryngii*	Enzyme	Pleureryn	11.5	In vitro	GPQFPEA (N-terminal sequences)	20 nM
9	*Lignosus rhinocerotis*	Enzyme	F5 (code num. GME434 7_g)	a) 31 b) 36	In vitro	(Full sequence can be available in Yap et al. 2015)	3 μg/ml
10	*Coprinus comatus*	Enzyme	CCL	64	In vitro	AIGPVADLKV (N-terminal sequences)	3.46 μM (HepG2) 4.95 μM (MCF-7)

11	*Clitocybe maxima*	Enzyme	nd	62	In vitro	DlGPVTPLAI (N-terminal sequences)	12.3 μM (HepG2) 3.0 μM (MCF-7)
12	*Agrocybe cylindracea*	Enzyme	nd	58	In vitro	SDAQKPFVNL (N-terminal sequences)	5.6 μM (HepG2) 6.5 μM (MCF7)
13	*Pleurotus cornucopiae*	Enzyme	nd	66	In vitro	GELNFHNP (N-terminal sequences)	–
14	*Agrocybe aegerita*	Enzyme	AAD	16	In vitro	145 amino acids (full-length)	> 2 μM
15	*Ganoderma lucidum*	Enzyme	GLR	17.4	In vitro	nd	2.8 μM (HT29) 0.1 μM (HCT116)
16	*Hypsizigus marmoreus*	Enzyme	nd	18	In vitro	TFPDFANPTQNAVITQAFKDAHQLV TLAVSYTTLKGARN (N-terminal sequences)	60 μM
17	*Pleurotus sajor-caju*	Enzyme	nd	12	In vitro	DNGEAGRAAR (N-terminal sequences)	0.22 μM (HepG2) 0.1 μM (L1210)
18	*Hohenbuehelia serotina*	Enzyme	nd	27	In vitro	TVGGSLAEKGN (N-terminal sequences)	25 μM (L1210) 40 μM (MBL2)
19	*Lyophyllum shimeiji*	Enzyme	nd	14.5	In vitro	AATCWKTSTA (N-terminal sequences)	10 μM (HepG2) 6.2 μM (MCF7)
20	*Pleurotus ostreatus*	Enzyme	nd	9	In vitro	GPCYLVAFYESSGRR (N-terminal sequences)	15 nM
						VRPYLVAFYESH (N-terminal sequences)	41 μM
21	*Lactarius flavidulus*	Enzyme	nd	14.6	In vitro	ATFVATIATNTLGTN (N-terminal sequences)	3.19 μM (HepG2) 6.52 μM (L1210)
22	*Russula delica*	Enzyme	nd	14	In vitro	GCGATACKQV (N-terminal sequences)	8.6 μM (HepG2) 7.2 μM (MCF-7)
23	*Pleurotus djamor*	Enzyme	nd	15	In vitro	DTACNCRKQV (N-terminal sequences)	3.9 μM (MCF-7) 3.4 μM (HepG2)
24	*Calvatia cuelata*	Ubiquitin-like peptide	CULP	8	In vitro	a)MQIFVKTLTG b)KTITLEVEESDDIDNVKAKI c)QDKEG (N-terminal sequences)	100 nM
25	*Agrocybe cylindracea*	Ubiquitin-like peptide	nd	9.5	In vitro	MQIFVK (N-terminal sequences)	10 μM (M1) 100 μM (HepG2)
26	*Ramaria botrytis*	Ubiquitin-like protein	RBUP	18.5	In vitro	Similar to *Coprinellus congregates* ubiquitin (gi|136,667)	15.93 μM
27	*Pleurotus sajor-caju*	Ubiquitin-like protein	PSULP	9.5	In vitro	MQIFVKTLTGKTITL (N-terminal sequences)	30 nM
28	*Oudemansiella radicata*	Ubiquitin-like protein	nd	13.5	–	–	–
29	*Agrocybe aegerita*	Ubiquitin-like protein	UbcA1	42	–	–	–
30	*Pleurotus citrinopileatus*	Non-classified	YP3	27.6	In vitro	NRDVAACARFIDDFCDTLTP (N-terminal sequences)	20 mg/L
31	*Pholiota nameko*	Non-classified	PNAP	18.5	In vitro	AGRTFIGYNG (N-terminal sequences)	9.97 μM (MCF-7) 12.11 μM (HeLa cells)
32	*Pleurotus eryngii*	Non-classified	PEP	40	In vitro	Similar to the protein Pleery1 (protein ID: 1310667)	–
					In vitro/in vivo		85 μg/mL (HCT116) 198 μg/mL (MC38)
33	*Pleurotus eryngii*	Non-classified	PEMP	nd	In vitro	nd	Range 0.05–2 mg/mL
34	*Pleurotus nebrodensis*	Non-classified	Nebrodeolysin	27	In vitro	nd	40 μg/mL
35	*Lentinus edodes*	Non-classified	Lentin	27.5	In vitro	CQRAFNNPRDDAIRW	0.2 μM
36	*Grifola frondosa*	Non-classified	GFP	83	In vitro/in vivo	nd	2 μg/mL
37	*Lentinula edodes* $C_{\varphi 1-3}$	Non-classified	LFP $_{\varphi 1-3}$A2	26	In vitro	a) NVAVPLYNR b)SDGANGLLTK c) NKYEDELNKR	Range 5–15 μg/mL
			LP1	116	In vitro	Full sequence can be available in Liu et al.	–
			LP-3	44.5	In vitro	GenBank accession number: KF682440	60 μg/ml
			LP4 RCC1	nd	In vitro	nd	–
			LP11	13.09	In vitro	nd	100 μg/mL
			LP13	46.7	In vitro	Full sequence can be available in Wang et al. (2016)	200 μg/mL
			LP15 RCC1	45	In vitro	Full sequence can be available in Tian et al. (2016)	–
38	*Ganoderma lucidum*	FIP[1]	LZ-8	13	In vitro	nd	3.13 μg/m
39	*Flammulina velutipes*	FIP[1]	FIP-fve	12.7	In vitro/in vivo	nd	–
				12.7	In vitro/in vivo	(Full sequence can be available in Ko et al. 1994)	–
				nd	In vitro/in vivo	Similar to Zhi-8 (LZ-8) and FIP-GTS	–
40	*Volvariella volvacea*	FIP[1]	FIP-vvo	15	In vitro/in vivo	112 amino acid (Full sequence can be available in Hsu et al. 1997)	–
41	*Ganoderma tsugae*	FIP[1]	FIP-gts	13	In vitro	nd	–
42	*Auricularia polytricha*	FIP[1]	APP	13.4	In vitro	nd	9.97 μM
43	*Pleurotus eryngii*	FIP[1]	PEP 1b	21.9	In vitro	nd	–
44	*Pleurotus citrinopileatus*	FIP[1]	PCiP	15	In vitro	QSLLTGTNYNSLGSNL (N-terminal sequences)	Range 5 – 20 μg/mL
45	*Trametes versicolor*	FIP[1]	TVC	15	In vitro	VAQLDTSKTSLTQN(N-terminal sequences)	60 μg/mL

[1] In addition to the low-carbohydrate FIPs mentioned in this table, there are several other recombinant FIPs most of which have previously been reviewed (Li et al. 2019). *Nd N*

Polysaccharides

Polysaccharides (PS) are long-chain polymeric carbohydrates composed of monosaccharide (MS) units bound together by glycosidic linkages. Homopolysaccharides are PSs consisting of a single type of MS. In contrast, hetero-PSs consist of multiple types of MS (Wang, 2017). Fungal PSs are synthesized using intracellular nucleotide sugars. Together, various polysaccharides form a gel-like matrix on the hyphal cell wall surface. This matrix is important for energy storage, cell wall structure, cell-cell interaction and host-pathogen interactions (Wang, 2017). The composition and organisation of PS in the fungal cell wall vary between species.

Polysaccharides have been described as the most potent bioactive compounds derived from mushrooms and are responsible for a variety of physiological activities (Valverde, Hernández-pérez and Paredes-lópez, 2015; Niego *et al.*, 2021). Although the bioactivities of PSs are well established, little is known about the relation between bioactive effects and monosaccharide composition (Wang, 2017). Since bioactivities of many polysaccharide fractions from a multitude of fungi have been reported (Yang, Zhou and Zhang, 2019; Niego *et al.*, 2021; Garcia *et al.*, 2022), this review will focus on the main types of fungal polysaccharides, and summarize their common bioactivities.

Glycans

β-glucans are the main polysaccharides found in fungi and comprise a group of β-D-glucose polysaccharides. Many studies have shown that β-glucans possess bioactivities with a beneficial effect on human health. Since these compounds are not synthesized in the human body, they can induce a wide range of immune responses, thereby indirectly stimulating the body's immune function (Mingyi *et al.*, 2019). The biologically active fungal β-glucans are those comprising β (1-3) linked glucose with side chains of glucose with β (1-6) linkages (Yang, Zhou and Zhang, 2019).
Lentinan, a β-glucan isolated from *Lentina edodes,* has been shown in clinical studies to affect the growth of various cancers (Yang, Zhou and Zhang, 2019). In addition, Lentinan has demonstrated antioxidative, anti-inflammatory and antimicrobial activity (Niego *et al.*, 2021). Schizophyllan, produced by *Schizophyllum commune*, has similar immunomodulatory and anti-cancer properties as lentinan. Both Lentinan and Schizophyllan are used in Japan as adjuvants for cancer therapies (Yang, Zhou and Zhang, 2019; Garcia *et al.*, 2022). There exists a multitude of other β-glucans with bioactivities. These include Pleuran, Calocyban, Polysaccharide Krestin (PSK), Ganoderan, Grifolan, Pachymaran and Tramesan. The biological activities and origins of these compounds can be found in (Yang, Zhou and Zhang, 2019; Niego *et al.*, 2021; Garcia *et al.*, 2022) and **Table 6**.

Another well-known group of fungal PSs are mannans, which are composed of α-glycosidic bond-linked mannoses. Although the anti-cancer effects of mannans are generally weaker than β-glucans, they are still found to be effective in animal models (Yang, Zhou and Zhang, 2019).
While β-glucans are the best-studied glycans in terms of bioactivity, many papers found complete glycan fractions that possess anti-oxidative, anti-cancer and hypoglycemic effects. The variety in different biological activities of different fungal PSs might suggest that the unique PS combinations, rather than the β-glucans alone, contribute to the observed clinical efficacies (Yang, Zhou and Zhang, 2019).

Table 5. Bioactive fungal polysaccharides and their origins. From (Niego *et al.*, 2021) *where the references can be found.*

Mushroom Species	Name of Fraction(s)	Bioactivity	Target Cells/Experimental Subjects
Agaricus bisporus	*Agaricus bisporus* neutral polysaccharides (Abnp1001 and Abnp1002) and *Agaricus bisporus* all polysaccharides (Abap1001, and Abap1002)	Hepato-protective activity	CCl_4-induced hepatic injury in mice
	AlAPS and their three purified fractions (AlAPS-1, AlAPS-2, and AlAPS-3)	Antiaging, antioxidant, and hepatoprotective effects, prevent age-related diseases	Fresh liver and blood samples of male Kunming strain mice
	Mannogalactoglucan polysaccharide	Antitumor activity (lung cancer)	Human hepatocarcinoma cells (HepG2)
	AcAPS and its major purified fractions (AcAPS-1, AcAPS-2 and AcAPS-3)	Antiaging and antioxidant effects	Fresh liver and kidney samples of male Kunming strain mice
	Agaricus bisporus fruiting body polysaccharide (FPS)	Hepato-protective activity	CCl_4-induced liver injury in mice
	Glucogalactomanan polysaccharide TJ3	Immunostimulatory activity	RAW 264.7 cells
Ganoderma lingzhi/G. sichuanense (as *Ganoderma lucidum*)	*Ganoderma lucidum* polysaccharides (GLP)	Immunomodulatory effect	Mice immunized with GLPL/OVA
	GLP	Antitumor activity (colorectal cancer)	Colorectal cancer HT29 (p53R273H) and SW480 (p53R273H&P309S) cells
	GLP	Neuroprotective effects	Rat cerebellar granule cells (CGCs)
	GLP	Anticancer activity (prostate cancer)	Human prostate cancer cells LNCaP
	GLP	Antitumor (brain glioma) and immunomodulatory activities	Glioma-bearing rats
	GLP	Hypoglycemic effect	Type 2 diabetes mellitus (T2DM) rats' blood liver and skeletal muscles
	Degraded *Ganoderma lucidum* polysaccharides (GLP_{UD})	Hypolipidemic and antioxidant activities	Blood, heart, spleen, liver and kidney of male Kunming mice
	GLP	Antidiabetic activity	T2DM rats' blood
Grifola frondosa	*Grifola frondosa* polysaccharides (GFP)	Anticancer activity (breast cancer)	MCF-7 and MDA-MB-231 cells, as well as in nude mice bearing MCF-7 tumor xenografts.
	GFP	Memory enhancement and antiaging activities	20-month-old rats
	GFP-N	Hypoglycemic and prebiotic activities	Diabetic mouse livers
	GFP	Hypoglycemic and hypolipidemic activities	Diabetic mice induced by HFD and streptozotocin (STZ)
Hericium erinaceus	Hydroxyethylated derivative of HEP	Immunomodulatory activities	RAW264.7 macrophages
	Selenium derivatives (sHEPs)	Immunostimulant activity	Dendritic cells
	Hericium erinaceus crude polysaccharide (HECP) and *Hericium erinaceus* refined polysaccharide (HERP)	Gastroprotective activity	Sprague–Dawley rats' stomach
	Novel *Hericium erinaceus* polysaccharide HEP_N	Gastroprotective activity	Human gastric epithelium (GES-1) cells
	Hericium erinaceus fruiting body polysaccharide (HEFP)-2b	Anticancer activity (colon cancer)	Colon cancer cells (HCT-116)
	Enzymatic hydrolysis of *Hericium erinaceus* polysaccharide (EHEP)	Immune-enhancement activity	Female Balb/c mice

Lentinula edodes	Mannogalactoglucan-type polysaccharides (WPLE-N-2 and WPLE-A0.5-2)	Anticancer and immunomodulating activities	Sarcoma 180-bearing mice
	Myeloid-derived suppressor cells	Immunosuppressive effects	Immortalized myeloid immune suppressor cell line (MSC2)
	Lentinula edodes polysaccharide (LEP)1	Antitumor activity	Human cervical carcinoma HeLa cells
	Residue polysaccharide (RPS) and its enzymatic-RPS (ERPS)	Antioxidant and anti-inflammatory activities	LPS-induced sepsis in mice
	LEP	Anticancer (colon cancer)	HT-29 colon cancer cells
	Acidic spent mushroom compost polysaccharides (ASMCP)	Antioxidant, anti-inflammatory and renoprotective effects	LPS-induced KI in mice
	Polysaccharide fractions (F1, F2 and F3)	Immunomodulatory effects	Female BALB/c mice

Mushroom Species	**Name of Fraction(s)**	**Bioactivity**	**Target Cells/Experimental Subjects**
Ophiocordyceps sinensis (as *Cordyceps sinensis*)	*Cordyceps sinensis* polysaccharide (CSP1-2)	Antihypertensive effect	Spontaneously hypertensive rats (SHR)
	CPS-A	Protective effect	L02 cells
	CSP	Prebiotics	Cyclophosphamide (Cy)-induced intestinal mucosal immunosuppression and microbial dysbiosis in mice
	CSP	Anti-obesity	High-fat diet (HFD)-feeding C57BL/6J mice
	Docetaxel-loaded acetic acid conjugated *Cordyceps sinensis* polysaccharide (DTX-AA-CSP)	Drug carrier and anticancer (liver and colon cancers)	Human umbilical vein endothelial cells; human liver HepG2; colon cancer cells SW480
	CSP	Anticancer activity (colon cancer)	Colon cancer cell line HCT116
Pleurotus eryngii	*Pleurotus eryngii* polysaccharides PEP-1 and PEP-2	Antitumor	Human hepatoblastoma HepG-2 cells
	Pleurotus eryngii polysaccharide (PEP)	Hypolipidemic and hypoglycemic activities	KK-A^{y} mice
	water-soluble polysaccharide EPA-1	Immunoregulatory activity	RAW 264.7 cells
	PEP	Hypolipidemic effect	Mice with hyperlipidemia
	PEP	Neuroprotective effect	β-amyloid-induced neurotoxicity in cultured rat pheochromocytoma (PC12) cells
Pleurotus ostreatus	*Pleurotus ostreatus* polysaccharide (POP)	Regulating dyslipidemia effect	STZ-induced diabetic rats
	POP	Anticancer activity	Sarcoma 180 tumor cells
	POP	Regulating dyslipidemia effect	Fat-emulsion-induced hyperlipidemia rats
	POP	Anticancer (lymphoid cancer)	Murine lymphoid cancer cell line
	Selenium polysaccharide fraction (Se-POP-3)	Antitumor activity	Human cancer cell lines HepG2, MCF-7, SKOV3, HeLa, and PC-3
	Phosphorylated *Pleurotus ostreatus* polysaccharide (PPOP)	Hepatoprotective effect	Carbon tetrachloride-induced liver injury in mice
Trametes versicolor	Polysaccharopeptides PSPs-EH80	Antioxidative effect	HaCaT cells
	Trametes versicolor polysaccharide (TVP)	Anti-proliferative and anti-invasive effects	LoVo and HT-29 human colon cancer cells
	Intracellular polysaccharide extract of *Trametes versicolor* (IPTV) and extracellular polysaccharide extracts of *T. versicolor* (EPTV)	Antihyperlipidemic effects	HFD-induced hyperlipidemic mice

Chitin and Chitosan

Chitin and its partially deacetylated analogue called chitosan, are two other major polysaccharides present in the fungal cell wall. Chitin is a long-chain polymer composed of repeating β(1,4)-N-acetylglucosamine (Shamshina, Oldham and Rogers, 2019). Several studies in both animals and humans have demonstrated potential nutritional benefits of chitosan such as reducing blood LDL-cholesterol levels and decreasing lipid digestion, which can be useful in obesity treatment and hypercholesterolemia (Shamshina, Oldham and Rogers, 2019). Chitosan, as part of a nutraceutical formulation together with three other compounds, is effective in lowering cholesterol levels in a 12-week trial (Spigoni *et al.*, 2017). Other bioactivities attributed to chitosan include antimicrobial, antioxidant and anti-cancer and anti-inflammatory activity (Iqbal *et al.*, 2021).

It is however still unclear which specific structural and chemical properties of chitosan are most effective. Taken together with the fact that most of these studies have used crustacean-derived chitosan, it is still too early to ascribe nutritional benefits to fungal chitosan. Most of the bioactivities found in the scientific literature are ascribed to chitosan, and not chitin. Compared to fungi from the subkingdom Dikarya, several mucor species were found to possess a higher chitin/chitosan content (Rousta *et al.*, 2023). The spores and hyphae of Mucorales species contain a chitin/chitosan proportion of 12% and 40%, respectively. These findings point to Mucorales species as potential candidates for functional foods with respect to their chitosan content.

Dietary Fibres

Next to their above-mentioned bioactivities, fungal PSs have also beneficial effects on human health as dietary fibres (DFs) (Shamshina, Oldham and Rogers, 2019). DFs are non-starch polysaccharides that cannot be broken down in the human digestive system. The fungal DF content is mainly comprised of β-glucans, chitin and hemicellulose. (Zhang *et al.*, 2021). Dietary fibre consumption aids in regulating the gut microbiota, prevents constipation, promotes intestinal health and reduces the risks of cardiovascular disease (He *et al.*, 2022). The health benefits of DF from fungal origin specifically, include anti-cancer and immune-enhancing activities, as well as beneficial attenuation of glucose-, lipid- and cholesterol-blood levels (Zhang *et al.*, 2021).

Considering their wide varieties in biological activities, fungal polysaccharides are promising functional compounds with the potential for food and nutraceutical applications (Zhang *et al.*, 2021). To date, there are already eight fungal PS-based drugs approved by the Chinese Food and Drug Administration (Yang, Zhou and Zhang, 2019). However, how the bioactivity mechanisms of PSs relate to their structural- and biochemical compositions remains partly unclear. The ability of PSs to induce immunostimulatory effects is related to the degree of branching, molecular weight, conformation and functional groups present (Ferreira *et al.*, 2015). The structure-function relationships differ per type of PS. This means that the assessment of specific PS fractions and their bioactivities is necessary to make claims about their potential as functional ingredients.

Although the precise structure of cell wall polysaccharides of Mucorales species remains unknown, some PS have been described (Figure 1). These include mucoran, mucoric acid, chitin and chitosan which have been found in *M. circinelloides* and *M. mucedo* (Lecointe, 2019). Currently, no bioactivities are ascribed to the PSs found specifically in Mucorales species.

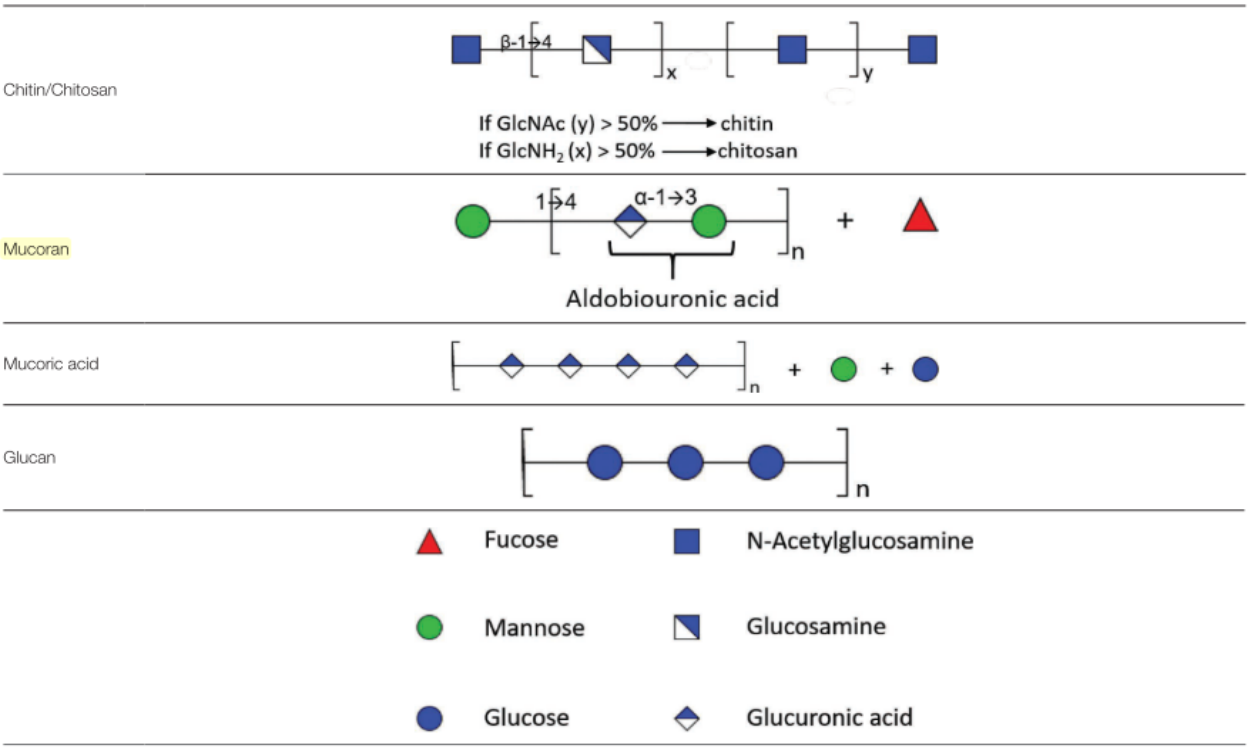

Figure 1 Partial structure of cell wall polysaccharides found in Mucorales. From (Lecointe, 2019).

Fats

Fats are a group of molecules that are insoluble in water, but soluble in non-polar solvents. They include mono-, di- and triglycerides, phospholipids, sterols and waxes. Most fungal-derived fats can be found in the fungal cell wall. Compared to carbohydrates and proteins, the amount of fat in most fungi is relatively low (Niego *et al.*, 2021).

Fatty Acids

The primary component of dietary fats are fatty acids (FA). Nutritionists recommend increased polyunsaturated fatty acids (PUFAs) and reduced intake of saturated fatty acids (SFA). Fungi have been shown to have a PUFA/SFA ratio higher than 0.45 which is recommended for a healthy diet (Ogwok, Muyinda and Bamuwamye, 2016). PUFAs are essential components of the cellular membrane and regulate many cellular processes (Abedi and Sahari, 2014). Fungi are a source of various PUFAs, including linoleic acids (LA), alpha-linolenic acid (ALA), gamma-linolenic acid (GLA), arachidonic acid (AA), and docosahexaenoic acid (DHA) (Kosa *et al.*, 2018; Copetti, 2019; Dimitrijevic *et al.*, 2019). From these PUFAs, ALA and LA are essential fatty acids.

PUFAs have many biological activities with health benefits. They have a positive effect on cardiovascular health by lowering low-density lipoprotein (LDL) levels and reducing blood pressure (Abedi and Sahari, 2014). PUFAs influence neurotransmitter biosynthesis and uptake and have additional positive effects on brain functioning. Lipid extracts from *Cordyceps militaris* were shown to have the potential to treat neurodegenerative diseases by lowering inflammation in microglial cell models (Nallathamby et al, 2020). Furthermore, PUFAs are shown to have beneficial effects on skin diseases, asthma, arthritis and multiple sclerosis. Lastly, PUFAs play a role in the prevention of cancer and autoimmune disease, diabetes and Crohn's disease (Abedi and Sahari, 2014; Akpinar-Bayizit, 2014).

Next to the amount of dietary PUFA intake, the omega-3/omega-6 ratio is another important factor for their nutritional value. In the west, people overconsume omega-6 PUFAs and need more omega-3 PUFAs in their diet (Abedi and Sahari, 2014). The omega-6/omega-3 ratio in several fungi is found to be well above the recommended dietary ratio of 1-4:1 (Ogwok, Muyinda and Bamuwamye, 2016). The high ratio could be explained by a high amount of LA. It is however important to note that because of the low amount of fat in most fungi, the high ratio doesn't make it detrimental to health (Ogwok, Muyinda and Bamuwamye, 2016), especially considering the health benefits mentioned above.

Sterols

Sterols play an essential role in eukaryotic cells where it influences the membrane's fluidity and acts as a secondary messenger. Mycosterols are a class of sterols that are produced by fungi. They can differ in their side chains and functional groups when compared to human sterols. Ergosterol and its derivatives are considered the main sterols present in fungi. Although for some other fungi other sterol products e.g., cholesterol derivatives and brassicasterol are the main sterols present. Ergosterol has many health benefits for humans and is found to possess anti-inflammatory, anti-cancer and antimicrobial activities (Peralta, Bracht and Ferreira, 2017). Furthermore, mycosterols have been suggested as functional compounds to treat hyperlipidaemia disorder (Das and Kumar, 2021). Lastly, ergosterol, when exposed to ultraviolet light, is converted into Vitamin D2 and consequently offers Vitamin D-related health benefits including enhancement of immune, muscle, bone and cognitive functioning (Peralta, Bracht and Ferreira, 2017).

Another known mycosterol is 22-Dihydroergocalciferol, which is commonly known as vitamin D4. Vitamin D4 has been investigated for its therapeutic value in the context of hyperproliferative diseases, multiple sclerosis, rheumatoid arthritis, Crohn's disease and type 1 diabetes (Niego *et al.*, 2021). To date, several sterols have been identified in Mucorales species. These include ergosterol, 22-dihydroergosterol and episterol (Das and Kumar, 2021).

Phospholipids

Phospholipids (PLs) are another major component of eukaryotic membranes. They are a class of lipids containing phosphorous in their chemical structure and are present in several food sources. The main fungal phospholipids include phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylinositol (PI). Less abundant fungal phospholipids include phosphatidylserine (PS), phosphatidic acid (PA) and cardiolipin (CL).
Nutraceutical properties of PLs include gastrointestinal infection protection, cognitive improvement across the lifespan (Schverer *et al.*, 2021), reduced cholesterol absorption and exercise-induced stress reduction (Ali *et al.*, 2017). Phosphatidylcholine has been shown to have the potential to reduce liver damage caused by toxins such as alcohol and was shown to be effective as an adjuvant to treat non-alcoholic fatty liver disease (Maev *et al.*, 2020). These benefits are found with the use of PLs of animal- and/or plant-based origin, however, to date no nutritional health benefits of fungal PLs have been described.

Other fats

Furan fatty acids (FuFAs) are a group of FAs that contain a furan ring. They are known to protect against oxidative stress because of their radical scavenging properties and the ability to protect PUFAs from peroxidation (Hermann-ene *et al.*, 2022). Although present in low amounts compared to animal sources, FUFAs were found in 29 out of the 37 edible fungi tested (Hermann-ene *et al.*, 2022).
Next to the main three classes of fats which were covered already, more classes exist, most notably waxes and sphingolipids. However, to date, these fungal compounds have not been described in terms of their nutritional and nutraceutical values.

Among fungi, Mucoromycota species are considered potential candidates for high-value lipid production as these species are naturally oleaginous (Mohamed *et al.*, 2020; Zhao *et al.*, 2021; Dzurendova *et al.*, 2022). Lipid extracts of Mucor species were shown to be rich in PUFAs, especially the bioactive compound GLA.

Polyphosphates

Polyphosphates (PolyPs) are linear polymers containing tens to hundreds of phosphate residues linked by energy-rich phosphoanhydride bonds (Achbergerová and Nahálka, 2011). PolyPs are localized in different organelles including the cell wall, cell membrane, cytoplasm, and various vacuolar and vesicular structures. In addition to their primary function as energy storage, PolyPs play a role in controlling homeostasis and protection against environmental stress (Werner, Amrhein and Freimoser, 2007).

Polyphosphates are used to improve the sensory properties and shelf-life of many foods as PolyPs possess high buffering capacity, water-retaining ability and antibacterial activity (Kulakovskaya, Vagabov and Kulaev, 2012). Most PolyPs are broken down to single phosphate units in the stomach by intestinal phosphatases. While PolyPs are nontoxic, overconsumption of PolyPs may have undesired effects on human health, such as an increased risk of cardiovascular disease and impaired kidney function, although the current for this evidence remains inconclusive (Kulakovskaya, Vagabov and Kulaev, 2012).
Beneficial health effects and bioactivities of Polyphosphates have also been reported. Probiotic-derived polyphosphates were found to enhance epithelial barrier function and have the potential to alleviate intestinal inflammation (Segawa *et al.*, 2011). Furthermore, PolyPs could be used in therapies for several blood diseases because of the ability to restore defective plasma clotting and treat bleeding episodes in patients with haemophilia (Kulakovskaya, Vagabov and Kulaev, 2012). However, the health benefits of fungal-derived PolyPs have not yet been researched. Several Mucoromycota species have been shown to possess polyphosphate accumulation properties. The highest rate of phosphorus uptake in these species is observed during the exponential growth phase (Dzurendova *et al.*, 2022).

Organic acids

Fungal organic acids are produced by fungi through various metabolic pathways. Organic acids are involved in nutrient acquisition, energy metabolism and communication between cells and other organisms. Fungal organic acids have been shown to have various beneficial effects on human health (Naraian and Kumari, 2018). First of all, many edible fungi contain ascorbic acid, which is commonly known as vitamin C. Ascorbic acid is an essential nutrient required for a multitude of enzymatic reactions in the human body and which functions as an antioxidant (Al-Obaidi, Jambari and Ahmad-Kamil, 2021). Fungi can also be considered a good source of pantothenic acid (vitamin B5) and folic acid (vitamin B9) which both are essential nutrients for humans with various beneficial health effects.

Organic acids, such as lactic acid produced by fungi of both Saccharomyces and Aspergillus species, can improve gut health by promoting the growth of beneficial gut bacteria and reducing gut inflammation, leading to improved digestion and absorption of nutrients (Parvez *et al.*, 2006). Several organic acids (i.e., citric, acetic, gluconic, glucuronic, lactic, tartaric, citric and malic acid) possess anti-oxidant properties. Of these, acetic acid, lactic acid and glucuronic acid also exhibit antimicrobial activities (Naraian and Kumari, 2018; Abaci *et al.*, 2022). Furthermore, acetic acid in kombucha, a drink which is produced in a symbiotic fermentation process by yeast and bacterial species, is likely to play a role in cytotoxicity towards cancer cells and anti-hypercholesterolemic effects (Abaci *et al.*, 2022). The dietary addition of fungal organic acids has been shown to improve gut health and nutrient absorption in poultry (Khan and Iqbal, 2016). However, further research is needed to demonstrate the benefits of fungal organic acids on human health. So far, the presence of tartaric acid, malic acids, citric acid, butane diacid, ethylic acid and oxalic acid has been described in Mucor-type fungi (He *et al.*, 2019).

Nucleotides

Nucleotides (NTs) are composed of a nitrogenous base, a pentose sugar and a phosphate and are the basic units of nucleic acid macromolecules such as DNA and RNA. NTs also act in various cellular processes and pathways. Humans can synthesize NTs themselves. However, over the last centuries, it has become clear that exogenous NTs have several additional biological functions (Ding *et al.*, 2021). Dietary NTs are able to modify intestinal flora, modulate immune function, increase resistance to infection, maintain liver function and improve antioxidant activities (Ding *et al.*, 2021).
So far, only two recent papers have looked into the bioactivities of NTs derived from fungi. The studies used dietary yeast nucleotides to feed tilapia (Xu *et al.*, 2015) and pacific white shrimp (Jin *et al.*, 2018). Both studies found an improved growth performance, enhanced innate immunity and an improvement in intestinal morphology.
Research on the benefits of dietary nucleic acid derivatives is scarce as well. Currently, only one such known fungal compound has been found. Eritadenine, isolated from the fruiting body of *L. edodes* has been shown to possess cholesterol-lowering and ACE-inhibitory activity (Fukushima-sakuno, 2020). While some of these results are promising, more research is needed on dietary fungal NTs and their derivatives before any conclusion can be made. No research with respect to dietary NTs from Mucorales species could be found.

Minerals

In a nutritional context, minerals are chemical elements that are essential nutrients for the survival of organisms. Excluding the four major structural elements; oxygen, hydrogen, carbon and nitrogen. The most abundant minerals found in fungi are potassium [K] phosphorous [P], sodium [Na], calcium [Ca] and magnesium [Mg]. Minor mineral constituents include copper [Cu], zinc [Zn], iron [Fe], manganese [Mn] and selenium [Se] (Dimitrijevic *et al.*, 2019; Niego *et al.*, 2021). The content of minerals is dependent on both the substrate and fungal species (Niego *et al.*, 2021). Since the beneficial health benefits of minerals are already extensively reported, this review will instead focus on the bioactive organic compounds which are more specific for fungi.

Secondary Metabolites

Fungal secondary metabolites (SM) are organic compounds which are not directly involved in the normal growth, development or reproduction of the fungus that produces the metabolite. Instead, these compounds often have functions in environmental interactions. Fungal SMs are categorized by their biosynthetic origin into; polyketides, terpenoids, nonribosomal peptides, and shikimic acid-derived compounds (Devi *et al.*, 2020). However, it must be noted that molecules with mixed biosynthetic origin are common (Skellam, 2022). The subcellular localisation of secondary metabolites is extremely complex. Pathways and enzymes involved in SM synthesis are located differently in different fungi (Skellam, 2022).

Polyketides

Polyketides (PKS) are a diverse class of molecules consisting of a chain of alternating ketone and methylene groups. These compounds possess many bioactivities including antibiotic, anti-microbial and immunosuppressive properties (Staunton and Weissman, 2001). Many of today's pharmaceuticals are derived from polyketides. A well-studied fungal polyketide is lovastatin which is used as a drug to treat high blood cholesterol and reduce the risk of cardiovascular disease. Recent studies have also researched lovastatin as an adjuvant of anti-cancer drugs for its chemotherapeutic activity against several types of cancer (Xie *et al.*, 2021). Two types of fungal pentaketides, formylanserinone B and anserinones B, also possess anti-cancer activities (Lin and Xu, 2020). Although much is known about polyketide biosynthesis in fungi and the pharmaceutical potential of polyketides derived from other organisms (Staunton and Weissman, 2001), research on specific bioactivities of polyketides derived from fungi, including mucor-species, is lacking.

Terpenoids

Terpenoids are a group of compounds containing an aliphatic polyene chain derived from the 5-carbon compound isoprene. Molecules within this group are classified according to their number of isoprene units. Many fungal terpenoids are shown to have various bioactivities including anti-cancer, anti-microbial and anti-inflammatory activities (Amirzakariya and Shakeri, 2022) (Devi *et al.*, 2020). Tetraterpenoids, better known as carotenoids are shown to have various bioactivities including their antioxidant properties linked to the prevention of different types of cancer and immune system enhancement (Mohamed *et al.*, 2020). Moreover, carotenoids lower the risk of diseases such as cancer, cardiovascular diseases and age-related eye disorders (Lin and Xu, 2020). A triterpenoid named Ganoderic acid, produced by *G. lucidum* is used as an adjuvant in therapies aimed to treat hepatitis, fatigue syndrome and prostate cancer. Moreover, Ganoderic acid has been shown to possess antioxidant and anti-cancer activities (Niego *et al.*, 2021). Astaxanthin, produced by *Xanthophyllomyces dendrorhous*, is another fungal carotenoid that has gained attention in the pharmaceutical- and food industries (Fang *et al.*, 2019). It has shown great potential in treating diseases such as Alzheimer's, Parkinson's, stroke, and high cholesterol, as well as preventing cancer. Terpenoids were found to be widely present in eleven Mucor species tested. β-carotene and zeaxanthin were found to be the major carotenoids in these Mucor species (Mohamed *et al.*, 2020). Zeaxanthin has a multitude of health benefits, including neuroprotective, antimicrobial, antiosteoporosis, UV-protective, anti-allergic and ophthalmological activities (Bouyahya *et al.*, 2021). β-carotene is the precursor of vitamin A and is considered a potent antioxidant. Moreover, it has been shown to lower the risk of heart disease and certain types of cancer, and to protect against age-related macular degeneration (Gul *et al.*, 2015).

Shikimic acid-derived compounds

Phenolic compounds

Phenolic compounds contain an aromatic ring with one or more hydroxyl groups. Eight edible fungi have been shown to contain 1-6 mg of phenolics per gram of dried mushroom. They exhibit a variety of bioactivities beneficial for human health. The best-documented bioactivity is their antioxidant activity as free radical scavengers, single oxygen quenchers and metal ion chelators. Fungal phenolic compounds with antioxidant activity include gallic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, cinnamic acid, p-coumaric acid, caffeic and ferulic acids (Heleno *et al.*, 2015). In addition, these compounds often possess other bioactivities such as antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, antithrombotic, anti-cancer and cardioprotective activity (Heleno *et al.*, 2015; Valverde, Hernández-pérez and Paredes-lópez, 2015; Niego *et al.*, 2021). Three strains of Mucor circinelloides were investigated for their antioxidant potential. Phenolic compounds, such as tannins and flavonoids were found to be the major effective antioxidant components in different in vitro assays (Hameed *et al.*, 2017).

There is however still debate about the actual effects in vivo because these compounds are subject to several biochemical reactions in the gastrointestinal tracts and blood plasm. This can change their structures, and influence their bioactivities. For ferulic- and caffeic acid it has been shown that their metabolites still exert a significant antioxidant action in vivo, and even possess greater anti-cancer potential than their parental compounds (Heleno *et al.*, 2015). Since the bioavailability of phenolic compounds is crucial for their bioactivities, more research is needed for many of the above-mentioned compounds, including those of Mucorales, to ascertain their beneficial health benefits in humans.

Alkaloids

The group of organic compounds named alkaloids contain great structural diversity and are characterized by having at least one nitrogen atom. Alkaloids derived from several fungal species have been shown to possess various bioactivities. Cordycepin is a nucleoside fungal alkaloid derived from Cordyceps mushrooms. It possesses anti-diabetic, anti-malarial, anti-arthritic, anti-osteoporosis, antioxidant bioactivities, and beneficial immunomodulatory effects (Ashraf *et al.*, 2020). Ergot alkaloids, of which some are produced by species in the genus Rhizopus from the order Mucorales, have nutraceutical potential and have been used for their vasoconstrictive effects (Guerre, 2015). Another alkaloid compound, Pseurotin A, obtained from aspergillus, has been shown to possess antioxidant, anti-inflammatory and anti-cancer properties (Helal *et al.*, 2019). Lastly, several indole alkaloids derived from Chaetomium species have anti-cancer properties. These compounds include vinblastine, vincristine, chaetoglobosins and ischaetoglobosin D (Zhang *et al.*, 2012; Manganyi, 2020).

Nonribosomal peptides

Non-ribosomal peptides (NRPs) are a class of organic peptides that are synthesized by non-ribosomal peptide synthetases. Filamentous fungi are considered highly productive NRP producers (Vassaux *et al.*, 2019). Bioactivities of NRPs include antimicrobial, anti-cancer and anti-inflammatory as well as immunomodulatory and neuroprotective properties. A well-researched fungal NRP is cyclosporin A, which is used as an immunosuppressant drug for organ transplants (Devi *et al.*, 2020). Fungal NRPs with anti-cancer properties include Gliotoxin (*Aspergillus sp*.) (Nguyen *et al.*, 2014), Trichokonin VI (*Trichoderma pseudokongii*), Lipovelutibols B and D (*T. velutinum*), Culicinin D (*Culicinomyces clavisporus*), and several trichobrachins (*T. longibrachiatum*) (Hou *et al.*, 2022). A great number of bioactive peptaibols, a subgroup of NRPs, are summarized in (Hou *et al.*, 2022).

Gene clusters for secondary metabolic pathways are common in filamentous fungi. The greatest diversity of secondary metabolite clusters (SMCs) among non-Dikarya fungi was found in Mucorales species (Koczyk and Pawłowska, 2021). This includes clusters involved in polyketide-, nonribosomal peptide- and terpenoid synthesis (Figure 2). Considering the wide variety of SMCs present, Mucorales species offer great potential to possess bioactive secondary metabolites (Lebreton *et al.*, 2019).

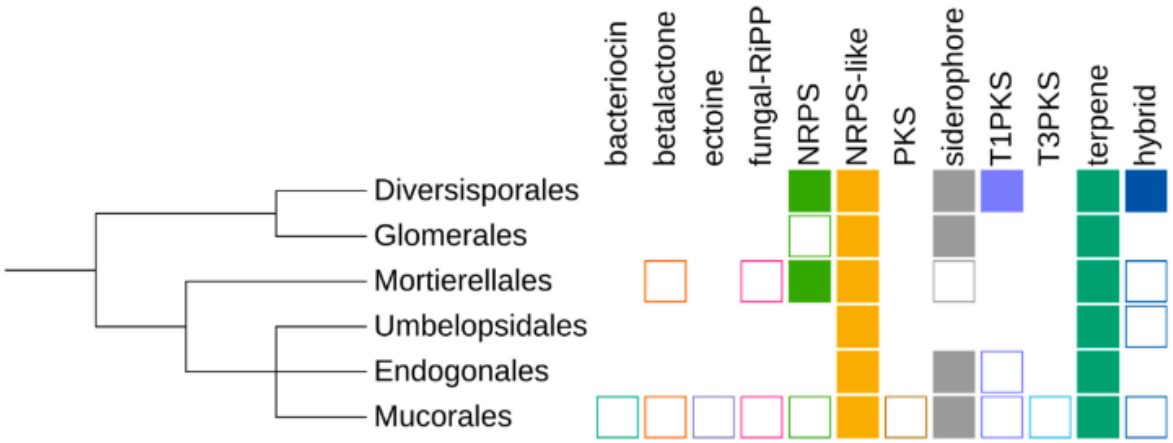

Figure 2 Taxonomic distribution of secondary metabolite clusters (SMCs) types. Filled rectangles, clusters of this type are present in more than 50% of isolates of the taxon; empty shape, in less than 50% of isolates; no shape, no clusters. From (Koczyk and Pawłowska, 2021).

Discussion and Conclusion

The present work aims to give a broad overview of fungal bioactive compounds beneficial for human health. The current scientific landscape surrounding bioactive compounds of fungal origin is extremely scattered in terms of the compounds investigated, as well as the biochemical categories in which these compounds are placed. In this review, major groups of fungal primary and secondary metabolites were investigated. The primary metabolites include proteins, polysaccharides, fats, polyphosphates, organic acids, nucleotides and minerals. The secondary metabolites include polyketides, terpenoids, shikimic acid-derived compounds and non-ribosomal proteins.

This review further emphasises the potential of fungi as functional foods. It can be concluded fungi have many potential health benefits beyond their basic nutritional value, considering the multitude of bioactive compounds found throughout the fungal kingdom (Niego *et al.*, 2021). In all of the biochemical groups investigated in this review, a wide variety of bioactive compounds were found, often with similar potential benefits for human health. Frequently occurring bioactivities present in almost all biochemical groups include antioxidant, anti-microbial, anti-cancer and immunomodulating activities, as well as benefits for gut-, brain- and cardiovascular health. An overview of these compounds together with their bioactivities is given in Figure 3. Since most studies have investigated individual compounds or specific categories of compounds, it will be interesting to research the effects of certain combinations of compounds as it could be possible that this will lead to the discovery of synergistic effects between them. It is important to note that are no strict boundaries between the reported bioactivities. For instance, immunomodulating or antioxidant activity is often associated with anti-cancer or gut-protective properties, and vice-versa (Zhou *et al.*, 2020).

Since The Protein Brewery utilizes a Mucorales fungus for the production of Fermotein®, there was an additional focus on Mucorales species in this review. It can be concluded that Mucorales has great potential in the production of functional foods. Mucorales species offer great potential to possess bioactive secondary metabolites (Koczyk and Pawłowska, 2021). Moreover, they have been shown to possess many high-value lipid products (Dzurendova *et al.*, 2021) as well as BAPs with ACE-inhibitory ability (Hang and Zhao, 2012). However, since polysaccharides are sometimes described as the most potent bioactive compounds from fungi, further research is needed with regards to the PS found such as mucoran and mucoric acid. Mucoromycota have been shown to possess a versatile metabolism that can be used in fermentation processes to produce high-value products in terms of their nutritional value and bioactivities. Further research into co-production strategies is required to improve the efficiency and sustainability of mucoromycota-based fermentations (Dzurendova *et al.*, 2022).

Although many compounds present in fungi have been shown to possess bioactivities, their actual effects through dietary intake remain insufficiently investigated. This can be explained by the following factors. First, the fungal kingdom remains a relatively understudied group of organisms, even though they have the biotechnological potential for many industrial applications (Hyde *et al.*, 2019). This scarcity is also reflected in research on bioactive compounds from fungal origins. Most of the existing literature in this scientific landscape is instead focused on their plant- or animal-derived counterparts. This is particularly true for fungal chitosan, furan fatty acids, polyphosphates, nucleotides, polyketides and nonribosomal proteins. Conversely, this offers many potentials for future research.

Second, even within the fungal kingdom most of the research is focused on only a handful of fungal species from Dikarya, all having the same characteristic of fruiting body formation. Considering the differences in genetic and biochemical composition between fungal taxa, as represented in figure 2, it is hard to make conclusions about other taxa. This is made even more difficult when considering the

complexity and multifactorial nature of structure-function relationships of bioactive compounds. Small changes in biochemical structure can have significant effects on the ability of a compound to elicit its proposed bioactivity (Ferreira *et al.*, 2015).
Thirdly, some of the bioactivities reported in this review were found in *in vitro* studies. Although *in vitro* studies offer valuable information regarding the potentiality of bioactive compounds, *in vivo* studies offer a more accurate picture of the bioactivity and the underlying cellular mechanisms involved. Continuing, even *in vivo* studies do not offer a complete picture considering the complex nature and importance of bioavailability. Bioavailability is crucial to determine the actual bioactivities in the human body as many barriers and biochemical reactions in the gastrointestinal tracts affect the bioactive potential. The bioactivity of dietary fibres, BAPs and some phenolic compounds have been discussed in the light of bioavailability. Their bioavailability is similar, or even enhanced by the biochemical reactions in the gastrointestinal tracts. However, for most of the bioactive fungal compounds discussed, no information regarding their bioavailability is available.
When considering fungi as functional foods, the anti-nutritional factors present in fungi are another major element that is currently overlooked by many of the scientific papers reviewed. Anti-nutritional factors are compounds that reduce nutrient utilization and uptake. For example, dietary fibre, phenolic compounds, tannins and phytates are known to interfere with protein digestion which could decrease the bioavailability of protein and BAPs. Interactions between the biochemical components of fungi during digestion remain understudied. It is known that this can be influenced by processing methods and the type of substrate used in the fermentation process (González *et al.*, 2020). This is however beyond the scope of this review.

To conclude, fungi possess a multitude of bioactive compounds beneficial for human health. These could be developed into functional foods for the prevention and treatment of several diseases. There are many species of edible fungi and types of fungal biochemical compounds that are yet to be explored. Before fungi and their bioactive compound can be fully considered functional foods, further research is needed regarding their mechanism of action and bioavailability. This research should be done for each fungal species individually as different fungi have been shown to possess different bioactive compounds. Given the rising demand for healthier and sustainable foods, fungi offer great potential to play an important role in future agrifood systems.

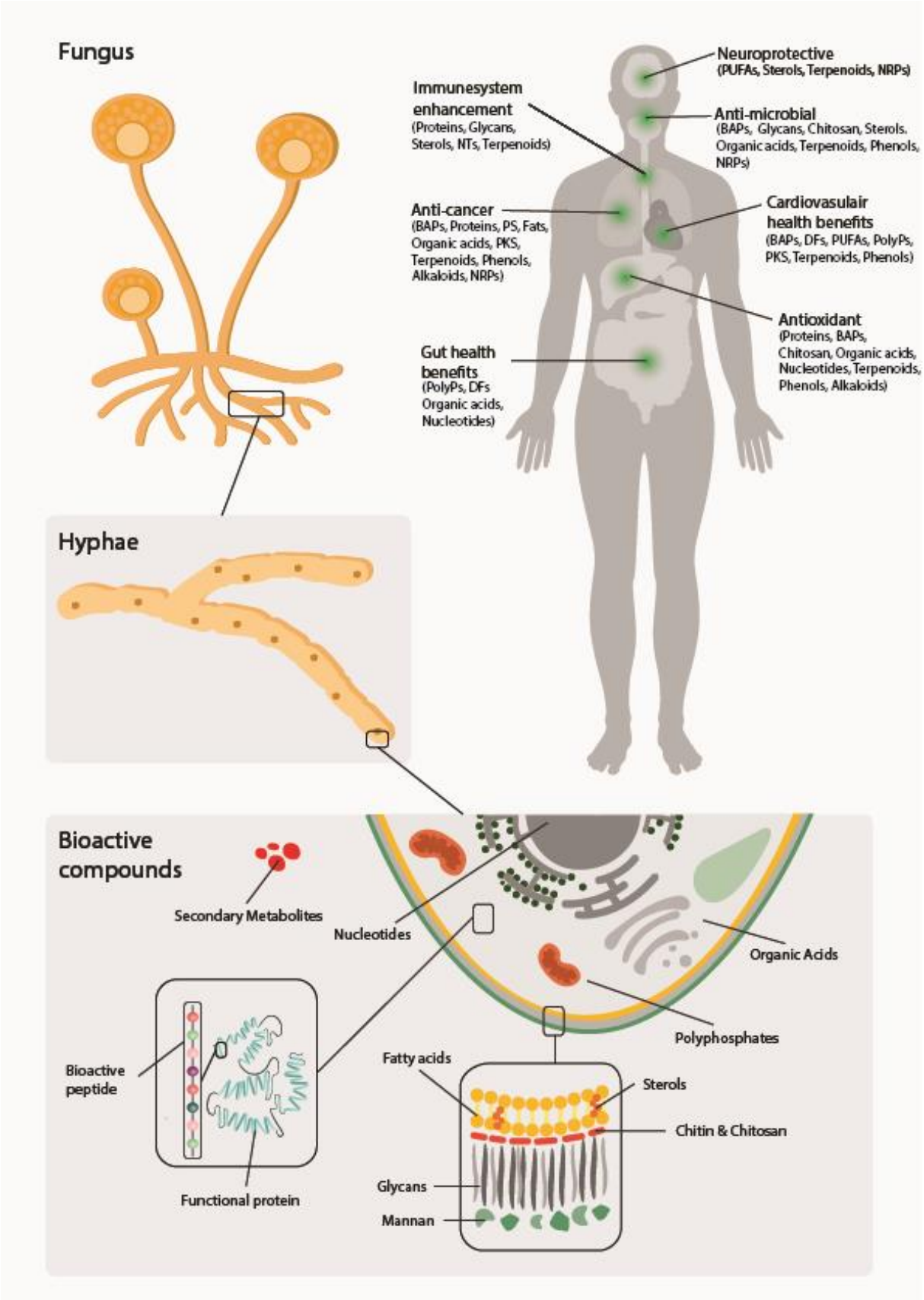

Figure 3. Visual representation of bioactive compounds in the fungal cell. The figure shown is for illustration purposes only and is not an exact representation of a fungal cell from a specific species. The localisations for both the bioactive compounds in the fungal cell as well as their bioactivities in the human body should not be considered as their exact locations.

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