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Review

# Peptides and proteins from fungi

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**Abstract**

The peptides and proteins secreted by fungi are reviewed in this article. They include ribosome inactivating peptides and proteins, antifungal peptides and proteins, lectins, ubiquitin-like peptides and proteins, peptides and proteins with nucleolytic activity, proteases, xylanases, cellulases, sugar oxidoreductases, laccases, invertases, trehalose phosphorylases, and various enzymes with applications in food industry, chemical production and the medical sector.


*Keywords:* Fungal; Enzymes; Fungal peptides; Fungal proteins

## 1. Introduction

Fungi form an important group of microorganisms, some of which have beneficial effects while others are pathogenic. One class of fungi, the mushrooms, are abundant in proteins. In fact mushrooms form favorite dishes in Oriental as well as Western families. A variety of proteins with interesting biological actions is elaborated by fungi and many of these proteins have potentially applicable activities. Fungal proteins that have been purified and characterized are reviewed in the following. They include ribosome inactivating proteins, antifungal proteins, ribonucleases, ubiquitin-like proteins and peptides, lectins, cellulases, xylanases, laccases, invertases and trehalose phosphory lases.

Ribosome inactivating proteins are included in this review on account of their potential usefulness. A large number of ribosome inactivating proteins and peptides has been isolated from flowering plants but only several proteins have so far been isolated from fungi. Most of the plant ribosome inactivating proteins belong to type 1 and possess a molecular mass of 25–32 kDa [10,107,143]. They are endowed with a variety of activities including immunosuppressive/antimitogenic, antitumor/anti-proliferative, and antiviral activities [107]. Type 2 ribosome inactivating proteins are composed of a ribosome inactivating protein chain and a lectin chain [10]. Another type of ribosome inactivating protein is a peptide with a molecular mass below 10 kDa. They have N-terminal sequences that are rich in arginine and glutamate residues [110,168]. Ribosome inactivating proteins have been used as immunotoxins.

Antifungal proteins are defense molecules produced by diverse organisms including plants and animals. They are protective against the destructive effects of pathogenic fungi. Plant antifungal proteins and peptides are structurally diverse and can be further classified into thaumatin-like proteins, chitinases, miraculin-like proteins, ribosome inactivating proteins, and cyclophilin-like proteins. Antifungal proteins and ribosome inactivating proteins alike can inhibit enzymes crucial to the HIV life cycle [106]. In contrast to the voluminous information on plant antifungal proteins, there is only scanty information available about antifungal proteins of fungal origin.

There is a wealth of information pertaining to animal and plant ribonucleases [30]. Elucidation of the structures and functions of ribonucleases has led to the award of several Nobel Prizes [30]. However, data about fungal ribonucleases are confined to a few species despite the existence of a vast number of fungal species.

By comparison, there is a much larger collection of papers on fungal lectins and a number of reviews have appeared on this topic [54,64,181]. The huge number of books and articles on plant and animal lectins attests to the importance of lectins. Mannose- and *N*-acetylglucosamine-specific macrophage lectins mediate binding and phagocytosis of microorganisms. β-Galactoside-specific animal lectins regulate differentation of organ formation. Lectins also play a role in the migration of lymphocytes from the blood stream

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into the lymphoid organs and in metastasis of cancer cells. Antitumor and immunomodulatory activities are inherent in some lectins [181]. This explains why lectins have captured the attention of a large number of investigators.

A considerable amount of literature regarding fungal cellulases, xylanases, and laccases exists. These enzymes play an important role in the saprophytic mode of life in fungi. Together with proteases, these enzymes find important applications in biotechnology and industry.

Proteins covalently conjugated to ubiquitin are targeted for degradation by 26S proteasome. These important regulatory proteins encompasss cell-cycle regulatory proteins, P53 tumor suppressor, the transcriptional regulator NF-KB and its inhibitor, many transcription factors, and the *mos*-protooncogene. The ubiquitin-mediated pathway is of paramount importance to the control of processes such as cell-cycle progression, signal transcriptional regulation, receptor down-regulation, endocytosis, immune response, development, and apoptosis. Defects in ubiquitin-mediated events have been implicated in the development of pathological conditions including malignant transformation [26,60]. The physiological importance of ubiquitin in the life of fungi may be related to the functions mentioned above. As its name implies, ubiquitin occurs in a diversity of organisms. There are a few reports in the literature about the presence of ubiquitin-like peptides in fungi.

Invertase and trehalose phosphorylase are fungal enzymes inducible by specific saccharides. Ribosome inactivating proteins, antifungal proteins, and lectins are clearly defense proteins. Ribonucleases and proteases may also play a protective function and eliminate foreign informational molecules such as ribonucleic acids and proteins. The intent of this review is to summarize the existing knowledge about the aforementioned fungal proteins that play a significant part in the life of various fungi.

## 2. Ribosome inactivating peptides and proteins

### 2.1. Ribosome inactivating proteins from mushrooms

Ribosome inactivating proteins are well known for their ability to inhibit translation in a cell-free rabbit reticulocyte lysate system. They attack ribosomes and catalitically cleave the N-glycosidic bond at A4324 in rat 28S RNA resulting in arrest of protein synthesis [10]. From the fruiting bodies of *Flammulina velutipes* a ribosome inactivating proteins (RIP) designated flammulin and possessing a molecular mass of 40 kDa, and a small ribosome RIP designated velutin and possessing a molecular mass of 13.8 kDa, have been isolated [165,166]. The two RIPs exhibit distinct N-terminal sequences. Flammulin and velutin inhibit cell-free translation in the rabbit reticulocyte lysate system with $IC_{50}$ values of 0.25 and 0.29 nM, respectively. Neither flammulin nor velutin exhibits ribonuclease activity. The inhibitory effect of velutin on HIV-1 reverse transcriptase can be considerably augmented by succinylation. Velutin can also inhibit β-glucosidase and β-glucuronidase, which are implicated in viral infection. Flammin and velin are, respectively, 30 and 19-kDa proteins isolated from *Flammulina velutipes*. They inhibit translation with an $IC_{50}$ of 1.4 and 2.5 nM, respectively [113].

Pleuturegin, the RIP from *Pleurotus tuber-regium*, has a molecular mass of 38 kDa. It inhibits cell-free translation with an $IC_{50}$ of 0.5 nM and is devoid of ribonuclease activity. Pleuturegin is unlike RIPs from other mushrooms in that it is adsorbed on DEAE-cellulose and unadsorbed on SP-Sepharose [167]. Other mushroom RIPs are unadsorbed on DEAE-cellulose and adsorbed on CM- or SP-ion exchangers [88].

Lyophyllin, the RIP from *Lyophyllum shimeiji*, exhibits a molecular mass of 20 kDa. It inhibits cell-free translation with an $IC_{50}$ of 1 nM and HIV-1 reverse transcriptase with an $IC_{50}$ of 8 nM. Lyophyllin does not display any ribonuclease activity. Like angiosperm RIPs, lyophyllin inhibits [$^3$H-methyl]-thymidine incorporation into mouse splenocytes. Lyophyllin manifests antifungal activity toward *Coprinus comatus* and *Physalospora piricola* [88].

Hypsin, an RIP with a molecular mass of 20 kDa and an N-terminal sequence showing remarkable homology to lyophyllin, has been isolated from *Hysizigus marmoreus*, a species closely related to and morphologically similar to *L. shimeiji* [89].

Calcaelin is an RIP from the puffball mushroom *Calvatia caelata* with an N-terminal sequence resembling those of American ginseng and Chinese ginseng RIPs. It demonstrates anti-mitogenic activity toward spleen cells and antiproliferative activity toward tumor cells [108]. Adustin is a 16.5-kDa polypeptide with translation-inhibiting activity isolated from the wild mushroom *Polyporus adusta* [112].

A comparison of the N-terminal sequences of ribosome inactivating proteins from various mushrooms and flowering plants is presented in Table 2.

### 2.2. Ribosome inactivating proteins from other fungi

Gigantin, purified from the culture medium of *Aspergillus giganteus* IFO 5818, is a ribonuclease with a molecular mass of 17 kDa. It exhibits differences in only 9 amino acid residues from α-sarcin, a ribosome inactivating protein from *A. giganteus* MDH 18894 [138,190]. However, gigantin exhibits preference for poly C and poly U while α-sarcin shows preference for poly A and poly I. Gigantin and α-sarcin are immunologically distinct. Gigantin demonstrates a pH optimum around 7.0 and a temperature optimum at 45–55 °C.

Clavin, also known as c-sarcin [62], is a type 1 ribosome inactivating protein from *Aspergillus clavatus*. It has a molecular mass of 17 kDa [29]. Restrictocin and mitogillin are other well-known ribosome inactivating proteins, also referred to as ribotoxins. Structurally related ribotoxins have been demonstrated in other species [94].

Immunotoxins based on clavin [29] and restrictocin [123] have been constructed. Histidine 137 is located at the active site of α-sarcin since replacement by glutamine abolishes the catalytic activity of α-sarcin [86].

Tricholin is a 14 kDa ribosome inactivating protein elaborated by soil-borne *Trichoderma viride*. It acts on reticulocyte ribosomal RNA to produce an α-sarcin RNA fragment. Interestingly, anti-α-sarcin antibodies show a strong cross-reaction with tricholin but anti-tricholin antibodies cross-react only weakly with α-sarcin [93].

## 3. Antifungal peptides and proteins

Antifungal peptides and proteins have great economic implications because they protect crops from the devastating damage brought about by fungal infections.

### 3.1. Antifungal proteins from mushrooms

Proteins with suppressive effects on fungal growth are produced by mushrooms and other fungi. It has been demonstrated that both angiosperm ribosome inactivating proteins and antifungal proteins exert anti-fungal activity. The same occurs in the mushroom *L. shimeiji* [88]. A 14-kDa antifungal protein designated *Lyophyllum* antifungal protein (LAP) has been isolated from fruiting bodies of *L. shimeji*. Its antifungal potency is higher than that of lyophyllin, a ribosome inactivating protein from the same mushroom. Lyophyllin is 30 times more potent than LAP toward the fungus *P. piricola*. LAP suppresses cell-free translation with a low potency ($IC_{50}$ = 70 μM) but inhibits HIV-1 reverse transcriptase with a high potency ($IC_{50}$ = 5 nM). The chromatographic behavior of LAP in general resembles that of lyophyllin. It is eluted from a Mono S column slightly earlier than lyophyllin [88].

Thaumatin-like proteins from the mushrooms *Lentinus edodes* and *Irpex lacteus* and the fungus *Rhizoctonia solani* inhibit the growth of *Saccharomyces cerevisiae*, and are capable of hydrolyzing polymeric carboxymethylated-pachyman in an in-gel β-1,3-glucanase assay [52]. An endo-1,3-β-glucanase from *Agaricus bisporus* was characterized with regard to substrate specificity, optimum temperature and optimum pH. Antifungal activity was not investigated, however [42].

Erygin is an antifungal peptide from the mushroom Pleurotus erygii with a molecular mass of 10 kDa and inhibitory activity toward *Fusarium oxysporum* and *Mycosphaerella arachidicola* [173]. The N-terminal sequences of some mushroom antifungal proteins are present in Table 1.

### 3.2. Antifungal proteins and peptides from other fungi

An antifungal peptide of 51 amino acid residues, secreted by *A. giganteus*, has been purified and characterized [87].

Table 1
N-terminal sequences of some mushroom antifungal proteins

| | |
|---|---|
| Eryngin | ATRVVYCNRRSGSVVGGDDTVYYEG |
| *Lyophyllum* antifungal protein | AGTEIVTCYNAGTKVPRGPSAXGGAIDFFN |

Data are taken from [88] and [173].

It inhibits the growth of various filamentous fungi including the phytopathogenic fungi *Fusarium moniliforme* and *Magnaporthe grisea* and the oomycete pathogen *Phytophthora infestans* [158], but is devoid of any effect on yeasts and bacteria [87]. It forms a loop structure and is similar to phospholipase A2 [104]. Its precursor consists of 94 amino acids and is processed to the mature peptide in 2 steps [190]. Studies by Martinez-Ruiz et al. [95] have disclosed another precursor which has six extra amino acid residues when compared to the antifungal peptide.

The molecular cloning, sequence analysis and expression of the gene encoding *A. giganteus* antifungal protein have been achieved [191]. The transcriptional regulation of *A. giganteus* antifungal protein has been investigated [101]. An alkaline ambient pH, heat shock, presence of excess sodium chloride and ethanol and carbon skeleton tend to up-regulate its expression. Presence of $H_2O_2$ and nitrogen starvation result in a slight reduction in expression. The antifungal protein and α-sarcin are the two major proteins produced by *A. giganteus* in culture [95]. They can be purified simultaneously from the culture medium by employing blue-Sepharose CL-6B [56].

In the mould *T. viride*, a silent antifungal protein-like gene lacking two introns has been demonstrated [56]. However, the antifungal protein cannot be detected in the medium used to culture the mould.

An exo-α-1,3-glucanase with antifungal activity from *T. harzianum* has been reported [2].

## 4. Ubiquitin-like peptides and proteins

An 8-kDa ubiquitin-like peptide has been isolated from the mushroom *Calvatia caelata* [90]. A similar ubiquitin-like protein and a peptide have been purified from *Pleurotus ostreatus* [164] and *P. sajor-caju* cv hsiu tseng [109], respectively. All three of them inhibit cell-free translation, and demonstrate ribonuclease and N-glycosidase activities, albeit with different potencies. A ubiquitin-like peptide with ribonuclease activity against various polyhomoribonucleotides has been purified from the yellow mushroom *Cantharellus cibarius* [185]. Their sequences are distinct from those of ribonucleases discussed in the following. The peptide from *C. caelata* demonstrates antimitogenic activity toward mouse splenocytes and antiproliferative activity toward human breast cancer cells. The ubiquitin-like peptide from *Agrocybe cylindracea* exerts immunostimulating and antiproliferative activities [116]. The ubiquitin-like

peptides and proteins are unadsorbed on DEAE-cellulose and adsorbed on Affi-gel blue gel and Mono S.

Ubiquitin-conjugated proteins including cell cycle regulatory proteins, p53 tumor suppressor, the transcriptional regulator NF-kB and its inhibitor, many transcription factors, and the *mos* protooncogene, are targets for degradation by the 26S proteasome. The ubiquitin-meidated pathway regulates cell-cycle progression, signal transcription regulation, receptor down-regulation, endocytosis, immune response, development, and apoptosis. Defects in ubiquitin-mediated events may be involved in the development of pathological conditions including malignant transformation [20,60]. Whether ubiquitins have similar significance in the life of mushrooms remain to be elucidated, but it is likely in view of the conserved sequence exhibited by ubiquitins.

## 5. Peptides and proteins with nuclease activity

Ribonucleases distinct from ubiquitin-like peotides and proteins have been purified from several mushroom species including *Ganoderma lucidum* [184], *I. lacteus* [189], *L. edodes* [79], *Pleurotus eryngii* [174], *P. ostreatus* [118,199], *P. sajor-caju* [115], *P. tuber-regium* [180], *P. pulmonarius* [198], *Russulus virescens* [183], *Termitomyces globules* [172], and *Volvariella volvacea* [163]. Sequence homology is discernible only among *I. lacteus* and *L. edodes* RNases. The isolated RNases exhibit a range of RNase activities, encompassing 650 U/mg for *P. ostreatus* peptide [199], 407 u/mg for *V. volvacea* RNase [163], 217 u/mg for *R. virescens* RNase, and 39,000 U/mg for *P. tuber-regium* RNase [170]. Polyhomoribonucleotide specificity also differs from one RNase to another e.g. poly G for *P. tuber-regium* RNase [170,180] and *P. ostreatus* RNase [118], poly C for *P. pulmonarius* RNase [183], poly U for *P. sajor-caju* RNase [115], co-specificity toward poly A and poly C for *R. virescens* and *T. globulus* RNases [172,183], and co-specificity toward poly A and poly G for *P. eryngii* RNase [174]. The range of molecular masses runs from 9 kDa for *P. ostreatus* peptide [184], 12 kDa for *P. sajor-caju* RNase [115], 13 kDa for *T. globulus* RNase [172], 14 kDa for *P. pulmonarius* RNase [198], 16 kDa for *P. eryngii* RNase [174], 28 kDa for *R. virescens* RNase [183], and 29 kDa for *P. tuber-regium* RNase [170,180] to 42 kDa for *V. volvacea* RNases [163] and *G. lucidum* RNase [184]. The optimum pH is 8.0 for *P. ostreatus* RNase, 6.5 for *P. tuber-regium* and *P. eryngii* RNases, 7.0 for *P. pulmonarius* RNase, 4.5 for *R. virescens* RNase [183] and 4.0 for *G. lucidum* RNase [184]. *P. tuber-regium* RNase is similar to *P. ostreatus* RNase in susceptibility to divalent cations such as $Zn^{2+}$, $Pb^{2+}$, $Cd^{2+}$ and $Ni^{2+}$ [170]. *Pleurotus pulmonarius* RNase has an N-terminal sequence similar to those of angiosperm type 2 RIPs.

Ribonucleases have been isolated from the culture filtrate of *L. edodes*. Two major RNases (RNase Le37 and RNase Le45), with N-terminal sequences and base specificity similar to RNase Le 2 from fruiting bodies but with amino acid compositions different from that of RNase Le 2, were purified [78,79].

The RNase from *P. sajor-caju* exhibits antifungal, and antibacterial activities, and exerts an antiproliferative action on hepatoma and leukemia cells, and anti-mitogenic action on mouse spleen cells [115].

*S. cerevisiae* RNase H (70) is a 70 kDa protein [41]. The N-terminal sequences of some mushroom RNases are presented in Table 3.

## 6. Proteases

### 6.1. Proteases from mushrooms

Pleureryn is a small and novel aspartic protease with a molecular mass of 11.5 kDa from the edible mushroom *P. eryngii* [169]. However, its N-terminal sequence resembles DNA replication licensing factor more than aspartic proteases. It also exhibits some inhibitory activity against HIV-1 reverse transcriptase. This is reminiscent of a suppressive action of HIV-1 protease, also an aspartic protease, on its homologous reverse transcriptase. Pleureryn exhibits a pH optimum of 5 and a temperature optimum of 45 °C, with considerable activity remaining at high temperatures and at pH 4 and 12. Pleureryn is unique in that it is relatively stable to changes in pH or temperature [169]. Other mushroom proteases tend to be less stable. The metalloproteases from *Armillariella mellea* and *Tricholoma saponaceum* [74,75] are, by contrast, thermolabile.

An aspartic protease from another mushroom, *I. lacteus*, has a molecular mass of 35 kDa [80]. Metalloendopeptidases from *A. mellea*, *P. ostreatus* and *T. saponaceum* have a molecular mass in the range 18.5 kDa–20 kDa [119]. A thermostable lysine-specific zinc-metalloendopeptidase has been isolated from fruiting bodies of the mushroom *Grifola frondosa* [119]. The protease has a molecular mass of 20 kDa, a p*I* of 7.46 and an pH optimum of 9–10. It demonstrates high affinity for β-D-glucan and chitin. Prolylendopeptidases from *A. bisporus* and *Lyophyllum cinerascens* have molecular masses close to 78 kDa [139,201].

The N-terminal sequences of some mushrooms proteases are compared in Table 4.

### 6.2. Proteases from other fungi

An alkaline serine protease (Pen ch 13, also known as Renn 13) has been identified as the major allergen from airborne *Penicillium chrysogenum (P. notatum)*. It has a molecular mass of 28 kDa. It exhibits 83 and 49% amino acid sequence identity with its counterparts from *P. citrinum* and *Aspergillus fumigatus*, respectively [25].

An alkaline serine proteinase with a molecular mass of 28.7 kDa has been isolated from *Fusarium culmorum* [125]. A pH of 8.3–9.6 and a temperature of 50 °C are required

for its maximal activity. The stability of the enzyme cannot, however, be maintained under these conditions. It shows considerable sequence homology to subtilisin. It can be inhibited by phenylmethylsulfonyl fluoride and chymostatin but not by soybean trypsin inhibitor or the Bowman–Birk trypsin inhibitor.

A subtilisin-like serine protease with a molecular nass of 36 kDa has been purified from *Podospora anserine* [124].

An aspartic proteinase from *A. fumigatus* demonstrates a molecular mass of 39 kDa and a broad range of activity from pH 2.0 to 7.0 [131]. It exhibits 88% sequence identity with aspartic proteinase from *A. niger* and 64% identity with the vacuolar proteinase A of *S. cerevisiae*.

A protease with a molecular mass of about 30 kDa has been isolated from *Candida caseinolytica* [127]. Its action is demonstrable over a broad pH range.

In summary, proteases with different molecular masses, optimum pH values and optimum temperatures are produced by different fungal species.

## 7. Xylanases

Xylanases (1,4-β-D-xylan xylanohydrolases) catalyze the random hydrolysis of the xylan backbone of heteroxylans. As a result the cellulose fibrils are exposed and susceptible to attack of side-chain cleaving enzymes such as α-arabinofuranosidases and acetylxylanases. Xylanases occur in diverse organisms. Bacterial and fungal xylanases are produced inductively or constitutively in response to the carbon source on which they are grown.

A cellulase-free xylanase has been purified from *Aspergillus niger* [130]. From *A. oryzae* KBN616 a xylanase gene has been cloned and characterized. The gene product is a 35-kDa protein similar to xylanases from *A. nidulans* Xlnc, *A. kawachii* XynA and *P. chrysogenum* XylP.

The Antartic psychrophilic yeast *Cryptococcus adeliae* produces a thermolabile xylanase [51]. Maximum xylanase activity is induced by xylan, followed by lignocellulose. The enzyme exhibits the highest activity at 45–50 °C, but 70–95% of the activity disappears within 5 min.

Cellulase-free xylan-degrading enzymes from *Acrophialophora nainiana*, *Humicola grisea* var. *thermoides* and two *Trichoderma harzianum* strains have been employed to bleach the pulp of *Eucalyptus kraft* before a chlorine dioxide and alkaline bleaching sequence. The *T. harzianum* enzyme preparations are slightly more effective in decreasing pulp viscosity and chlorine chemical consumption and enhancing the brightness of the kraft pulp. *A. nainiana* xylanase is the most potent in reducing pulp viscosity [99]. The activities of some hydrolytic enzymes (cellulase, endo-1,4-β-xylanase, β-glucosidase and amylase) and reductase enzymes (monophenol monooxygenase and peroxidase) in strains of *Fusarium oxysporum* (Schlecht snyd. and Hans) isolated from different habitats (plant substrates, cultivated soil and non-cultivated soil) have been examined by Kurchenko et al. [84]. It is found that strains isolated from plant substrates display the highest activity of hydrolytic enzymes, followed by strains from cultivated soil, whereas strains isolated from noncultivated soil exhibit the lowest activity. The reverse trend occurs regarding the redox enzymes.

A purified xylanase from *F. verticillioides* exhibits a molecular mass of 24 kDa, an optimum temperature of 50 °C, an optimum pH of 5.5, a pH stability range of 4.0–9.5, and thermal stability up to 50 °C [137].

*F. oxysporum f.* sp. *melonis* produces an endo-1,4-β-xylanase with a molecular mass of 80 kDa. Its optimum pH and temperature are 5.0 and 50 °C, respectively [4].

An acidophilic xylanase from *Penicillium* sp. 40 has a molecular mass of 20.7 kDa, an optimum pH of 2.0 and a pH stability range of 2.0–5.0. It is a glycoprotein with sequence similarity to other fungal xylanases [76].

*P. purpurogenum* produces two immunologically distinct xylanases. Xylanase A possesses a molecular mass of 33 kDa and an isoelectric point at pH 8.6. Xylanase B, the other major form, manifests a molecular mass of 23 kDa and an isoelectric point at pH 5.9 [153]. Apparently the two xylanases are produced by different genes and may play different roles in xylan degradation [12].

A xylanase has also been purified from *P. simplicissimum*. It has relatively low pH and temperature optima. Its shape is similar to some xylanases, but its active site cleft is much shallower and wider. Three glycerol molecules bind within the active site groove, and one of them directly interacts with the catalytic glutamate residues. It appears that they occupy putative xylose-binding subsites [141].

A xylanase from *Thermomyces lanuginosus* is fairly thermostable. More than 70% of the xylanase activity is retained after exposure to 60 and 70 °C for 4 and 1 h, respectively [85]. The crystal structure of the xylanase has been determined by single-crystal X-ray diffraction. Thermostability is attributed to the presence of an extra disulfide bridge and an increase in the density of charged residues throughout the protein.

Two structurally xylanases have been purified, cloned and characterized from the rice blast fungus *Magnaporthe grisea* [195]. One of them is a 33 kDa protein while the other is a 22 kDa protein. Their genes are expressed when the rice blast fungus is grown on rice cell walls or on oatspelt xylan but not when grown on sucrose.

The xylanase from the thermophilic fungus *Humicola lanuginose* is abundant in acidic amino acids. It is unusual in that inactivation, probably due to aggregation, occurs after storage at −20 °C in the dry state for over 2 months [6].

From another thermophilic fungus, *Thermoascus aurantiacus*, a xylanase, a β-glucosidase, an exocellulase and an endocellulase have been purified [73].

An endoxylanase, which is a 21 kDa glycoprotein with 4.4% carbohydrate, has been isolated from *Trichoderma koningii* G-39 [63]. It has a p*I* of 8.9 and is completely inhibited by 1 mM $Hg^{2+}$ and 10 mM sodium dodecyl sulfate.

Two structurally similar xylanases from *T. reesei* and their genes have been characterized [152]. They have a molecular mass of 19 and 21 kDa and an isoelectric point of 5.2 and 9.0, respectively. A 66-kDa xylanase has been purified from the rumen anaerobic fungus, Neocallimastix patriciarum. The large N-terminal reiterated regions consisted of distinct catalytic domains which displayed similar substrate specificities to the full-length enzyme [49].

Thus it appears that xylanes from different species may differ in molecular mass, isoelectric point, optimum pH, pH stability range and optimum temperature.

## 8. Cellulases

The cellulolytic enzymes of anaerobic fungi have been studied because of their potential value in biotechnology [49,160,193] including use as enzyme supplements for live stock, and in food and beverage, detergent, textile and pulp and paper industries [20,23]. Their nutritional function is evident by the degradation of plant fiber serving as carbon sources. In general, cellulolytic fungi produce a large number of cellulases [146,153]; many use them for degradation of the plant cell wall polysaccharides [3,11,33].

Endoglucanases (endo-1,4-β-glucanases), cellobiohydrolases (CBH, exo-1,4-β-glucanase), and β-glucosidases are three major types of cellulolytic enzymes. Endoglucanases randomly hydrolyze 1,4-β bonds along the interior of the cellulose chain. Cellobiohydrolases cleave cellobiosyl units from non-reducing ends of the cellulose chains. β-Glucosidases cleave glucosyl units from non-reducing ends of cello-oligosaccharides.

Henrissat [57] and Henrissat and Bairoch [58,59] classified glycoside hydrolases into over 62 families on the basis of amino acid sequence similarities. Cellulases are found in 13 of these families i.e. families 5–10, 12, 26, 44, 45, 48, 60 and 61. Alignment of the deduced amino acid sequences of cellulase CelB2 from *Orpinomyces joyonii* with cellulase CelA from *Neocallimastix frontalis* and cellulase CelB from *N. patriciarum* revealed a conserved signature sequence of glycosyl hydrolase family 5, L-I-F-E-G-X-N-E-P-R, and a threonine-rich linker sequence in addition to conserved glutamic acid and tryptophan residues [196].

Cellobiohydrolase Cel7A from *Trichoderma reesei* has been expressed from *Pichia pastoris*. The thermostability, k(cat), $K_m$ and pH optimum are not affected by heteroglycosylation [14]. Heterologous expression of *T. reesei* cellobiohydrolase Ce17A in a methylotrophic yeast *Pichia pastoris* has been examined both under the *P. pastoris* alcohol oxidase (AOX1) promoter and the glyceraldehyde-3-phosphate dehydrogenase (GAP) promoter in a fermentor. Production of Ce17A with the AOX1 promoter results in a better yield, despite incorrect folding of part of the enzyme expressed in *P. pastoris*. Ce17A expressed in *P. pastoris* is overglycosylated at its *N*-glycosylation sites compared with the native *T. reesei* protein, but less extensive than Ce17A expressed in *S. cerevisiae*. The *k*(cat) and $K_m$ values for the purified protein on soluble substrates are also similar to the values for native *Trichoderma* Ce17A, but the degradation rate on crystalline substrate (BMCC) is decreased. The measured pH optimum is also similar to that of purified *T. reesei* Ce17A. The hyperglycosylation does not affect the thermostability of the enzyme monitored with tryptophan fluorescence and activity measurements. Circular dichroism measurements indicate that the formation of disulfide bridges is essential to the correcting folding of Ce17A and might explain the difficulties encountered in heterologous expression of *T. reesei* Ce17A [14].

A cellulase, with a molecular mass of 58 kDa, a pH optimum of 5.5, and a temperature optimum at 40 °C has been purified from the ruminal fungus *O. joyonii* and cloned in *Escherichia coli*. The *O. joyonii* cellulase exhibits strong activity on carboxymetyl cellulose (CMC), lichenan and barley β-glucan. CMC is a water-soluble long-chained cellulose with carboxymethyl substitutions. It is commonly used as a model substrate for detecting β-1,4-endoglucanases. Digestion of lichenan and barley β-glucans (mixtures of β-1,3- and β-1,4 linkages) may be mainly attributed to random cleavage of β-1,4 linkages in the substrates because of the inability of the *O. joyonii* cellulase to digest laminarin and pachyman which have β-1,3-glucans as the main components. The data indicate that *O. joyonii* cellulose is a β-1,4-glucanase [200].

The enzyme has no activity on avicel (crystalline cellulose) and pullulan (α-1,6 glucan). *O. joyonii* cellulase is able to cleave *p*-nitrophenyl-β-D-cellobioside but not glucopyranoside, suggesting that it possesses cellobiohydrolase but not β-glucosidase activity. Its activity on *p*-nitrophenyl-β-D-cellotrioside, -cellotraoside and -cellopentaoside indicates its cellodextrinase activity. The enzyme has activity over a broad pH range (pH 5–7) with the highest activity at pH 5.5. It is stable at temperatures up to 50 °C with a temperature optimum of 40 °C. However, it has to be borne in mind that all of the enzyme activities were measured under anaerobic conditions in this investigation and the results might have been different had the experiments been conducted in an anaerobic setting [200].

Despite the fact that many cellulases contain a cellulose-binding domain and a catalytic domain, *O. joyonii* cellulase is unable to bind Avicel, a microcrystalline cellulose and a search for cellulose-binding domain sequences has met with no success. The absence of cellulose-binding domains and the presence of reiterated scaffold binding sequences in *O. joyonii* CelB2 cellulase suggest the immobilization of the enzyme to cellulosome, a cellulose hydrolytic complex [129,200].

Microbial 1, 3-1,4-β,D-glucanases cloned in *E. coli*, 1,3-β-glucanases endo-1.4-β-D-glucanase, exocellobiohydrolase I and β-glucosidase have been purified. Cellulases, glucanases and xylanases from anaerobic rumen fungi have been cloned in *E. coli* and expressed. These fungal enzymes increase the efficiency of feedstuff digestion in monogastric animals by promoting breakdown of polymers in the plant

cell wall and hence are potential enzyme supplements for livestock. The enzymes are being used or considered for use by pulp and paper, textile, detergent and food and beverage industries [20,23].

Three exo-glucanases, two endo-glucanases and two beta-glucosidases, have been isolated from the culture medium of *A. nidulans*. The optimal pH for all forms of cellulase components ranges from pH 5.0 to 6.0 and the optimum temperature is 50 and 65 °C for exo-glucanases and endo-glucanases but 35 and 65 °C for beta-glucosidases. All cellulase components are stable for 10 min at 40–50 °C. Exo-II and Exo-III exhibit a higher affinity for the substrate than Exo-1. The $K_m$ values of Endo-1 and Endo-II and their maximum reaction velocities are comparable. The beta-glucosidases exhibit $K_m$ values of 0.24 and 0.12 mmol and Vmax values of 8.00 and 0.67 IU/mg protein. The molecular masses for various enzyme forms are: Exo-1, 29 kDa; Exo-II, 72.5 kDa; Exo-III, 138 kDa; Endo-1, 25 kDa; Endo-II, 32 kDa; beta-Gluco-1, 14 kDa and beta-Gluco-II, 26 kDa. Exo- and endo-glucanases but not beta-glucosidases require metal ions as co-factors. $Hg^{2+}$ ions inhibit the activity of all cellulase components [8].

In summary, cellulases with a variety of molecular masses, and temperature and pH optima are known.

## 9. Sugar oxidoreductase

Pyranose-2-dehydrogenase, a novel C-2-specific sugar oxidoreductase, has been purified from *A. bisporus* mycelia. It is a monomeric glycoprotein with a molecular mass of about 75 kDa. It exhibits two pH optima, one at pH 4 and another at pH 9. It possesses a flavin prosthetic group. It is activated by 50 mM acetate, but inhibited by 10 mM $Ag^{+}$, $Hg^{2+}$, $Cu^{2+}$ and $CN^{-}$ [159].

## 10. Laccases

Lignin ranks second, after cellulose, in abundance in the biosphere as a renewable organic compound. The biodegradation of lignin is a rate-limiting step in the carbon cycle. Ligninolytic enzymes are highly non-specific on account of the complex structure of lignin, and can be used in the degradation of structurally different environmental pollutants [18,39]. Laccases (benzenediol: oxygen oxidoreductase) form a class of ligninolytic enzymes that are phenol oxidases capable of catalyzing one-electron oxidation of aromatic substrates and the concomitant reduction of oxygen to water [150]. Laccases can also act on non-phenolic lignin subunits in the presence of readily oxidizable primary substrates which are electron-transfer mediators [15].

Combinations of laccase/glucose oxidase and laccase/manganese peroxidase, rather than laccase or peroxidase alone, have been considered to be the minimal enzyme requirement for effective lignin degradation. The combined action of laccase and FAD-dependent aryl alcohol oxidase significantly reduces the molecular mass of soluble lignosulfonates [98].

Laccases are multicopper blue oxidases, widely distributed in plants and fungi [98]. These enzymes are either monomeric or multimeric glycoproteins. Heterogeneity may be present owing due to variable carbohydrate contents or differences in copper content. These enzymes demonstrate a rather low degree of specificity with regard to the reducing substrate: they catalyze the oxidation of *ortho*- and *para*-diphenols and aromatic amines by removing an electron and a proton from a hydroxyl group to generate a free radical [150]. Structural information about the metal sites of laccase has been gathered by spectroscopic studies. Fungal laccases usually contain four copper ions distributed between three functionally distinct sites: the type-1 site, which strongly absorbs at about 610 nm (electron paramagnetic resonance or EPR active); the type-2 site, weakly absorbing, functioning as a one electron acceptor; and the type-3 site, which possesses a pair of copper ions, functioning as a two-electron acceptor, absorbing at approximately 330 nm (EPR silent) [132].

The biological function of laccase is correlated to lignin biodegradation in combination with either manganese peroxidase and/or lignin peroxidase. Laccase can also catalyze the oxidative polymerization of the phenolic compounds originating from lignin, which are then easily eliminated. Laccases oxidize phenolic units in lignin to phenoxy radicals, which can lead to the degradation of lignin-related structures. In the presence of appropriate redox mediators, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1-hydroxybenzotriazole (1-HBT), laccases catalyze the oxidation of nonphenolic lignin model compounds, depolymerize kraft lignin, and degrade polycyclic aromatic hydrocarbons, which are not substrates for laccase alone. Due to the aforementioned characteristics, its efficacy as an agent for selected detoxification [31], pollutant degradation [40], and catalyst for regiospecific biotransformation, and its possible utilization as electrode for organic phase enzymatic assay [187], laccase may play an important role for biotechnological applications. Veratryl alcohol oxidase can act together with laccase to prevent polymerization of phenolic compounds and reduce molecular mass of lignosulfates [96].

Extracellular laccases have been purified from submerged cultures of *Coriolus versicolor*, *Panus tigrinus*, *Phlebia radiata* and *Phlebia tremellosa*, and from cultures of *P. tigrinus*, *P. radiata* and *A. bisporus* grown on wheat straw (solid-state fermentation). Laccases obtained from submerged cultures display a blue color and characteristic absorption and EPR spectra while laccases purified from solid state cultures are yellow-brown and exhibit different spectra. N-terminal sequencing, however, has disclosed striking homology between the blue and yellow-brown forms. The production of the latter form due to binding of lignin-derived molecules is proposed [19,92]. A laccase

from *Marasmius quercophilus* has been characterized [32], in addition to laccases from *A. bisporus* [192], *Polyporus anceps* [126], *Pycnoporus cinnabarinus* [34]. *Rigidoporus lignosus* [19]. *Trametes trogii* [44] and *C. hirsutus* [145]. They are glycoproteins with molecular masses close to 60 kDa. However, *Tricholoma giganteum* laccase has a molecular mass of only 43 kDa [176] while *C. cibarius* laccase is composed of two 46-kDa subunits [111].

Laccases have also been identified and characterized from *A. nidulans* [140], *Botrytis cinerea* [146], and *N. crassa* [45]. A laccase gene homologous to the laccase gene of *N. crassa* has been isolated and characterized from *P. anserine*. The promoter region of the laccase gene from *P. anserine* contains two sequences identical to the eukaryotic xenobiotic responsive element and another two sequences homologous to the eukaryotic antioxidant responsive element [39].

The activity and stability of laccase from *Pleurotus ostreatus* are enhanced by copper but reduced by mercury [9]. Laccases from different strains of *P. ostreatus* differ in their Kcat and $K_m$ values for springaladazine, ABTS and guaiacol [151]. Copper is bound to the active sites of laccases from *Coriolus hirsutus* and *C. zonatus*. A modified method, based on the use of bathocuproine as a chelator for cupric ions, can yield copper-free laccase [82].

Many fungal species examined secrete more than one laccase isoenzyme. Different conditions of growth may produce different patterns of isoenzymes [133]. The effect of ferulic acid as an inducer of a specific laccase isoenzyme in *P. ostreatus* has been studied [91]. Induction of a laccase isoenzyme in *P. eryngii* by wheat straw alkali lignin, vanillic acid and veratric acids has been examined [103]. Induction of isoenzyme POXA1b but not isoenzyme POXA1w by copper in *P. ostreatus* suggests the existence of different regulatory mechanisms for the isoenzymes and distinct physiological roles played by the isoenzymes. Different *P. ostreatus* isoenzymes differ in thermostability [48].

Based on the amino acid sequence of *P. ostreatus* laccase, two putative N-glycosylation sites have been proposed at Asn 218 and Asn 444. Glycan analysis has disclosed the existence of only a high-mannose structure possessing varying numbers of mannose residues [47].

Analysis of the pH dependence of kinetic parameters suggests the participation of a histidine residue in binding nonphenolic substrates and the involvement of a His residue and an acidic residue in binding phenolic compounds by the two laccases from *P. ostreatus*. His and an Asp residue are indeed situated at the bottom of a cavity which may be considered as a suitable substrate channel for approaching to copper in the type-1 site in the 3D homology models of laccase isoenzymes POXC and POXA1b from *P. ostreatus* [44].

*Trametes versicolor* laccase crystallizes in two crystal forms, both with the orthorhombic space group P2, 2, 2, which diffract to 1.9 and 2.95 Å resolution, respectively. *P. cinnabarinus* laccase crystals belong to the monoclinic space group C2 and diffract to at least 2.2 Å resolution. All laccase crystals are good for X-ray structural determination and possess a complete complement of copper ions [7].

The gene and cDNA encoding *P. ostreatus* isoenzymes POXA1b have been isolated and sequenced. All of the introns possess a GTRNGY consensus sequence at the 5′-splicing site and a YAG consensus sequence at the 3′-splicing site, similar to the canonical splicing sequences in other eukaroytes. The gene structure of *P. ostreatus* isoenzymes POX1 and POXC are completely conserved but the intron/exon organization of the POXA1b gene is dissimilar in some regions. A DNA region of POX and POXC including three introns corresponds to a unique exon in POXAlb. The genes can thus be categorized into two subfamilies based on the number and position of introns [44,48].

Several sequences have been observed in the 5′-flanking region of POXAlb that closely match the consensus of regulatory elements, including a metal-responsive element. These sequences are also located in POX1 and POXC promoters. A putative heat-shock element resides in the POXAlb promoter. However, it remains to be elucidated whether these regulatory elements are functional [48].

The gene of the major laccase isoenzyme from *Trametes pubescens*, lap2, has 8 introns with splicing junctions and internal lariat formation sites in accordance with the GT-AG rule i.e. the 5′-consenus sequence GTRNGT and the 3′-consensus splice sites (It AG). Only intron one (Tat position 3) shows a slight change. The size of the introns ranging from 50 to 64 is characteristic of most fungal introns.

The usual promoter elements, including a TATA box, TATAAA and seven CAAT motifs, are located within the lap 25′-regulatory region extending approximately 1400 bp upstream of the ATG. CAAT motifs are crucial for determining the promoter efficiency. A long pyrimidine-rich region characteristic of strong fungal promoters lies between the TATA box and the translation start site. Two putative metal-responsive elements (MRE) occur in the promoter region. The elevated production of laccase in *Trametes pubescens* in response to several heavy metal ions indicates the physiological importance of these MREs in the lap 2 promoter. Twenty-seven potential heat shock elements (HSE) and a general stress response element (SRE) have been identified in the upstream region of lap 2. HSE and SRE are probably implicated in stress-regulated lap 2 gene expression induced by high concentrations of $CU^{2+}$ ions. However, neither xenobiotic reponse elements (XREs) nor antioxidant response elements (AREs) are found in the 5′-untranscribed region of lap 2. Since these elements are implicated in induction of fungal laccases by aromatic compounds, the lack of XRE in the promoter region of lap 2 is consistent with the inability of various aromatic compounds such as 2, 5-xylidine and catechol to stimulate laccase production in *Trametes pubescens*.

Four putative CreA-binding sites have been detected in the 5′-non-coding region of the lap 2 gene. Cre A is involved in glucose repression. Hence another regulatory mechanism is repression of lap 2 transcripton by glucose [43].

The N-terminal sequences of some fungal laccases are presented in Table 5.

## 11. Invertases

An extracellular invertase, which is a glycoprotein with a molecular mass of 130 kDa, is produced by *Aspergillus ochraceus* [46]. It exhibits a high $K_m$ of 3.5 mM for sucrose. Its amino acid composition resembles other fungal invertases.

*A. nidulans* secretes two forms of invertase, S-invertase and F-invertase. The carbohydrate moiety of S-invertase possesses mainly mannose and less galactose while it is the other way round for F-invertase. The specific activity of S-invertase is three-fold higher than that of F-invertase prior to as well as following deglycosylation with Endo H. However, the S- and F-forms have very similar $K_m$ values [24].

From the culture supernatant of sucrose-grown *A. niger*, a homodimeric invertase with a molecular mass of 225–250 kDa has been purified. The enzyme hydrolyzes sucrose and raffinose but not inulin, malezitose or PNPG. A pH of 5.5 and a temperature of 50 °C are required for optimal activity of the invertase [13].

A periplasmic invertase has been purified from *S. cerevisiae* och1: LEU2 disruptant cells. Invertase from the fission yeast *Schizosaccharomyces pombe* has 581 amino acids and 16 potential asparagine-linked glycosylation sites. The transcription of the invertase gene (inv 1+) is inhibited in the presence of glucose [148].

## 12. Trehalose phosphorylases

Trehalose is a common non-reducing disaccharide in microorganisms. Trehalose phosphate is formed from UDP-D-glucose and D-glucose phosphate.

The enzyme from *A. bisporus* plays an important role in trehalose metabolism. It is a homotetramer with a molecular mass of 20 kDa, a temperature optimum of 30 °C, and a optimum pH range of 6.0–7.0 [188].

Fungal trehalose phosphorylase is classified as a family 4 glucosyltransferase capable of catalyzing the reversible phosphorolysis of α,α-trehalose with net retention of anomeric configuration. Glycosyl transfer to and from phosphate takes place by the partly rate-limiting interconversion of ternary enzyme–substrate complexes produced from binary enzyme–phosphate and enzyme-α-D-glucopyranosyl phosphate adducts, respectively [117]. Phosphate binds before α,α-trehalose, and α-D-glucose is liberated before α-D-glucose-1-phosphate. Each molecule of trehalose phosphorylase contains one $Mg^{2+}$ ion that does not dissociate from the enzyme even in the presence of metal chelators.

*Schizophyllum commune* produces an intracellular α,α-trehalose phosphorylase during growth on D-glucose. The changes in the intracellular levels of α,α-trehalose and p*I* with time are inverse of that of trehalose phosphorylase activity, suggesting a temporary utilization of the α,α-trehalose pool via phosphorolysis [36,37].

*S. commune* trehalose phosphorylase is a monomeric 61 kDa protein [37]. Its counterpart in *Catellatospora ferruginea* is a tetrameric 400 kDa protein. The *C. ferruginea* enzyme is specific for trehalose for phosphorolysis and for β-D-glucose-1-phosphate in synthesis. D-Fucose and D-xylose can also serve as sugar acceptors during synthesis. Phosphate ions control the equilibrium of the reversible reaction and the thermostability of the enzyme. Para-chloromercuribenzoate and pyridoxal phosphate are potent inhibitors of the enzyme. Heat or storage in the frozen state with $NH_4Cl$ and LiCl inactivates the enzyme [1].

## 13. Lectis

### 13.1. Lectins from mushrooms

Lectins have been localized on the caps, stipes and mycelia of mushrooms, and variations in lectin content occur depending on the carpophone age and the time and place of harvest. In mushrooms, lectins probably play an important role in dormancy, growth and morphogenesis, morphological changes consequent on parasitic infections and molecular recognition during the early stages of mycorrhization. Mushroom lectins find application in taxonomical, embryological and bacteriological studies, study of the modifications in membrane glycoconjugates and cancer formation, cell sorting, sorting of mutant and tumor cells and isolation of membrane and serum glyconjugates [53].

Reviews on mushroom lectins have appeared several years ago [54,64,181]. This section is a more update summary.

It appears that more than one lectin is produced by *A. bisporus* [147]. The molecular masses of the tetrameric lectins are all 64 kDa [128]. Hemagglutinating activity of the lectins is reduced in the presence of bovine and porcine submaxillary mucins [128]. *A. bisporus* lectin exerts an antiproliferative action toward various tumor cell lines [202].

Like *A. bisporus* lectin, *A. blazei* lectin has a molecular mass of 64 kDa and is composed of four subunits each with a molecular mass of 16 kDa. The lectin is a glycoprotein. Its hemagglutinating activity is inhibited by high concentrations of *N*-acetyl-D-galactosamine and its methyl-α-D-glycoside [69].

*A. campestris* lectin is identical in molecular mass and subunit number to lectins from *A. bisporus* and *A. blazei*. Like *A. blazei* lectin it is fairly thermostable [135,136].

Two lectins differing vastly in molecular mass and carbohydrate content are produced by *Agaricus edulis*. One of them is tetrameric with a molecular mass of 60 kDa while the other is dimeric with a molecular mass of 32 kDa. The lectins are fairly robust and can withstand high temperatures, extreme pH and 6 M urea. The hemagglutinating activity of

lectin 2, unlike lectin 1, is not inhibited by common simple sugars [35].

Lectin has been isolated from *A. cylindracea* by Yagi et al. [197] and Wang et al. [178]. The preparation of Yagi et al. [197] is homodimeric with a blocked N-terminus while that of Wang et al [178] is heterodimeric with distinct N-terminal sequences for the two subunits. Both preparations demonstrate specificity toward sialic acid. Lactose is a poor inhibitor of the hemagglutinating activity of the preparation of Yagi et al. [197] but effectively counteracts the activity of the lectin prepared by Wang et al. [178]. The slight discrepancies in the characteristics of the two lectin preparations are suspected to be due to the use of different mushroom strains for the investigations.

There is an approximately six-fold increase in mitogenic response when mouse splenocytes are incubated with 2 μM *A. cylindracea* lectin. A dose-dependent increase in mitogenic response can be observed when the lectin concentration is elevated from 0.5 to 1 μM and then to 2 μM. When the lectin concentration is further increased to 4 and 8 μM there is a decline in mitogenic response. The mitogenic responses at these concentrations are, however, over 200% higher than the control value [178].

*Amanita pantherina* lectin is a dimeric lectin with a molecular mass of 43 kDa. Its hemagglutinating activity is inhibited by a number of oligosaccharides, and intact and asialo-bovine submaxillary mucin [203].

A toxic glycoprotein with hemagglutinating activity has been isolated from *Boletus satanas*. The lectin possesses a molecular mass of 63 kDa. It displays mitogenic activity toward lymphocytes and elicits the release of interleukins and tumor necrosis factor-α from mononuclear cell cultures [38].

*Coprinus cinereus* lectins are dimeric and structurally similar to galectins, a group of genetically related animal lectins. They differ from most galectins in the absence of cysteine residues and N-terminal post-translational modification [28].

A heterodimeric agglutinin, with hemagglutinating activity that is confined to the larger subunit and not inhibited by common simple sugars, has been isolated from fruiting bodies of *Flammulina velutipes*. The larger subunit, which has a molecular mass of 12 kDa, but not the smaller 8-kDa subunit, exhibits mitogenic activity toward mouse splenocytes [154].

A fungal immunomodulatory protein designated FIP-fve has also been isolated from fruiting bodies of *F. velutipes*. It is a single-chained protein with a molecular mass of 12.7 kDa and hemagglutinating activity that is not affected by common sugars. It exerts mitogenic activity and enhances transcriptional expression of interleukin 2 and interferon γ [77]. Its amino acid sequence exhibits marked homology to other mushroom immunomodulatory proteins.

*Ganoderma carpense* lectin has remarkable thermostability. In addition, it possesses potent mitogenic activity toward spleen cells and antiproliferative activity toward cancer cells [114].

The immunomodulatory protein LZ-8 from *G. lucidum* has a molecular mass similar to its counterpart from *F. velutipes* and an amino acid sequence highly homologous to *V. volvacea* immunomodulatory protein [149]. It manifests mitogenic activity on mouse splenocytes and human peripheral lymphocytes and suppression of anaphylaxis induced by bovine serum albumin in CFW mice [55,156].

From *G. lucidum* mycelia a 18 kDa lectin has been isolated. Another lectin was isolated from the fruiting bodies [66]. These two lectins differ from LZ-8 in molecular mass and sugar-binding activity.

An *N*-acetylgalactosamine-specific glycoprotein lectin with a molecular mass of 30-52 kDa has been isolated from *G. frondosa* fruiting bodies. Several protein bands are detected in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The lectin is cytotoxic against HeLa cells [68].

*Hericium erinaceum* lectin has a molecular mass of 54 kDa and is composed of two different subunits. It is a glycoprotein with specificity toward sialic acid [67].

A homotetrameric lectin has been purified from fruiting bodies of the mushroom *Hygrophorus hypothejus*. It specifically agglutinates blood groups A and B red cells and its hemagglutinating activity is inhibited specifically by α-lactose followed by β-lactose [157].

*Lactarius deliciosus* lectin is a heterodimeric lectin specific for β-D-galactosyl (1-3)-D-*N*-acetylgalactosamine residues [53].

*L. deterrimus* lectin is a homodimeric lectin with a molecular mass of 37 kDa and specificity for β-D-galactosyl (1-3)-D-*N*-acetylgalactosamine residues [50].

*Laetiporus sulphureus* lectin is an *N*-acetyllactosamine-specific tetrameric lectin composed of two distinct types of subunits (with molecular mass ca 60 and 36 kDa, respectively). No carbohydrate is present in the lectin [81].

A hemagglutinin, with hemagglutinating activity that cannot be inhibited by simple sugars and glycoproteins, has been isolated from *L. shimeiji* fruiting bodies. Its N-terminal sequence exhibits resemblance to a fragment of sequence of mitogen-activated protein kinase and centromere-associated kinesin. The agglutinin is single-chained and exhibits a molecular mass of 30 kDa [105].

The lectin from the mushroom *Marasmius oreades* has been purified, cloned, characterized and expressed [83]. It shows preferential binding to Gal-α-1,3-gal-containing sugar epitopes.

The lectin from fruiting bodies of the mushroom *Melastize chateri* cross-reacts with antiserum raised against *Aleuria aurantia* lectin and resembles *A. aurantia* lectin in N-terminal sequence. Its molecular mass is about 40 kDa [120]. However, mycelial isolate from *M. chateri* does not have lectin activity.

A lectin from the mushroom *Mycoleptodonoides aitchisonii* has been reported by Kawagishi et al. [71]. It is a homotetrameric lectin with a molecular mass of 64 kDa. It manifests strong binding specificity toward asialo-bovine submaxillary mucin.

An *N*-acetylglucosamine-specific lectin has been purified from the mushroom *Oudemansiella platyphylla* [97]. It comprises two subunits with homologous N-terminal sequences.

Two *N*-acetyl-D-galactosamine-specific lectins from *Phaeoleopiota aurea* have been isolated by Kawagishi et al. [72]. They are each composed of four 16 kDa subunits. The lectins exhibit little agglutinating activity toward native red cells. Pronase treatment of red cells enhances their sensitivity to the lectins.

A polymeric lectin comprising 18 kDa subunits has been isolated from fruiting bodies of *Pholiota aurivella*. Its hemagglutinating activity is best inhibited by asialofetuin and can be enhanced by pronase treatment of erythrocytes [65].

A homotetrameric 66 kDa protein, exhibiting sequence homology to *Pleurotus cornucopiae* lectin (PCL) and other mushroom lectins, has been purified from a lectin-deficient strain of the mushroom. It does not bind mucin which inhibits PCL. It is developmentally regulated as is PCL [121,122].

A lectin has been purified from *Pleurotus ostreatus* by Conrad and Rudiger [27] and Wang et al. [161]. The molecular masses of the two lectin preparations are similar. The carbohydrate specificities of the two preparations differ to some extent though in both cases the hemagglutinating activity is inhibited by melibiose. The *P. ostreatus* lectin isolated by Brechtel et al. [16] is a phosphatase activating protein closely associated with α-galactosidase activity. The lectin prepared from the same mushroom by Wang et al [161] is a heterodimeric protein with subunits manifesting similarity in N-terminal sequence to *A. aurantia* lectin. The lectin prepared by Wang et al. [161] lacks thermostability and is deleteriously affected by elevation in temperature. The lectin exhibits strong anti-sarcoma and anti-hepatoma activities [161]. The lectin is able to achieve 88% inhibition of sarcoma growth and 75% inhibition of hepatoma growth as indicated by reduction of number of tumor cells, after daily intraperitoneal injection of 1.5 mg lectin/kg body weight for 20 consecutive days. The inhibition of tumor growth is reflected by a decrease in body weight compared with the control group. The survival time of the tumor-bearing mice is also prolonged as a result of the lectin treatment. An increase of 58 and 22% in life span of sarcoma-bearing and hepatoma-bearing mice results after lectin treatment. The rate of increase can be boosted to 107 and 38% respectively, when the number of tumor cells inoculated is reduced to $2.5 \times 10^5$ [161]. *P. ostreatus* lectin suppresses food intake [70]. The lectin has been crystallized [22].

*P. tuber-regium* lectin exhibits a molecular mass of 32 kDa and a specificity toward *N*-acetylglucosamine [171].

The lectin from the mushroom *Polyporus squamosus* [102] is a homodimeric protein consisting of 28 kDa subunits associated by noncovalent bonds. It binds β-D-galactosides and exhibits strict specificity for α-2,6-linked sialic acid.

*Polyporus adusta* lectin is composed of two identical subunits each with a molecular mass of 12 kDa. It exhibits antiproliferative activity toward tumor cell lines and mitogenic activity toward mouse sptenocytes [177].

*Psathyrella velutina* lectin recognizes a non-reducing terminal *N*-acetylneuraminic acid residue in glycoproteins and oligosaccharides [155].

Two homodimeric lectins, designated TML-1 and TML-2, have been isolated from cultured *Tricholoma mongolicum* mycelia. They possess a molecular mass of 35 kDa. Their hemagglutinating activity is inhibited by α-lactose, β-lactose, *N*-acetyl-D-galactosamine and D-galactose. The lectins are characterized by thermostability. They display antiproliferative, antitumor and immunomodulatory activities [162,179,182]. The hypotensive action of TML-1 is probably mediated through vasorelaxation brought about by interaction with adenosine A2 receptors and/or nitric oxide production [186].

The immunomodulatory protein from the straw mushroom *V. volvacea* is dimeric and exhibits a molecular mass of 26 kDa. The protein is unglycosylated and blocked at the N-terminus. It expresses mitogenic activity toward human peripheral lymphocytes and inhibits generation of bovine serum albumin-induced Arthus reaction. Transcriptional expression of interleukin-3 interleukin-4, interferon-γ, tumor necrosis factor-α, lymphotoxin and interleukin 2-receptor is stimulated [61].

A novel lectin has been isolated from fruiting bodies as well as mycelia of the straw mushroom *V. volvacea*. It is a homodimeric nonglycoprotein with a molecular mass of 32 K. Its hemagglutinating activity is inhibited by thyroglobulin but not by simple sugars. It exhibits potent stimulatory activity toward spleen cells and increases expression of interleukin-2 and γ-interferon. [144].

From the wild mushroom *Xerocomus spadiceus*, a lectin composed of two 16-kDa subunits has been isolated. It exhibits mitogenic activity toward mouse splenocytes. Among the large number of carbohydrates tested, only inulin is able to inhibit its hemagglutinating activity [175].

The N-terminal sequences of some recently reported lectins are shown in Table 6.

### 13.2. Lectins from other fungi

A surface lectin mediating the capture of nematodes has been purified from the nematophagous fungus *Arthrobotrys oligospora*. It is a dimeric glycoprotein with a molecular mass of 36 kDa and an isoelectric point of pH 6.5. The glycoproteins fetuin and mucin, but not monosaccharides or disaccharides, inhibit its hemagglutinating activity. Maximal hemagglutinating activity is observed at pH 11. Activity is indiscernible at pH 2.8 [134].

The lectin discoidin-I produced by the slime mold *Dictyostelium discoideum* plays a role in intercellular adhesion during development of the slime mould. A number of binding proteins with different molecular masses, which promote aggregation of cells bearing the lectin, have been identified [17].

Two galactose-specific lectins, with molecular masses of 38 and 150 kDa respectively, have been isolated from the yeast *Kluyveromyces bulgaricus* [5]. At high concentrations, the dimeric 38 kDa lectin associates to yield the octameric 150 kDa lectin. Both lectins are glycoproteins which agglutinate human erythrocytes and flocculate EDTA-treated *K. bulgaricus* cells. The cell wall phosphopeptidomannan plays a role as a ligand and a potential physiological receptor of the lectins.

*Rhizoctonia solani* lectin is homodimeric, composed of two noncovalently associated 15.5 kDa monomers. It has a p*I* value exceeding 9 and is composed mainly of β-sheets. Its N-terminal sequence resembles the N-terminal subdomain of ricin B, indicating that it is a member of the ricin B family. It demonstrates specificity toward Gal/Gal NAc [21].

A cell-wall-surface lectin associated with flocculation in the yeast *S. cerevisiae* NCYC 227 binds mannose specifically. The protein has a tendency to undergo aggregation which is prevented by urea and high temperatures, indicating that flocculation is caused by hydrogen bonding between mannan and the lectin [142].

A lectin has been isolated from the fungus *Sclerotium rolfsii* [194]. Its carbohydrate binding specificity has been investigated.

From the preceding account it can be gathered that a diversity of lectins which differ greatly in molecular characteristics such as molecular mass, number of subunits, carbohydrate content and carbohydrate binding specificity, are produced by various fungal species. The situation is reminiscent of the multitude of chemically distinct lectins reported to exist in plants. The potentially exploitable biological activities such as mitogenic, immunoenhancing, antiproliferative, antitumor, vasorelaxing and hypotensive activities may be of interest of immunologists, oncologists and cardiologists. Wether the physiological role of lectins in fungi is related to defence against assault from pathogens as in the case of plants awaits elucidation. The details of the muchanism of action of fungal lectins also remain to be ascertained.

Table 3
Comparison of N-terminal amino acid sequences of RNases from *P. tuber-regium* (A), *Pleurotus ostreatus* (B), *Pleurotus pulmonarius* (C), *Pleurotus sajor-caju* (D), *L. edodes* (E), *I. lacteus* (F), *V. volvacea* (G), *R. virescens* (H) and *G. lucidum* (I)

| | |
|---|---|
| A: | ALTAQDNRVRVGNRIVGNNF$^{20}$NFAAVQAAYY$^{30}$ |
| B: | ETGVRSCNCAGRSFTGTDVTNAIRSARAGG |
| C: | AISANNERKGVNQQSVQNTYQENDV |
| D: | DNGEAGRAAR |
| E: | ISSGCGTTGALSCSSNAKGTCCFEAPGGLI |
| F: | VNSGCGTSGAESCSNSDDGTCCFEAPGGLL |
| G: | APYVQLFRPLIQPQVLATFAIANNMAQY |
| H: | TDHTLDTMMTHTLRD |
| I: | HLPBVPSFAYGSIKVYIN |
| A: | RKGRGHTG$^{40}$RAYQAGRVRVP$^{50}$RSHYP |
| B: | SGNYPHVYNNFEGFSFSCTPTFFF |
| E: | LQTQFWDTSPETGPTDSWTIHGLW |
| F: | LQTQFWDTDPSTGPSDSWTIHGLW |

Residues identical to those in *P. tuber-regium* RNase are underlined. Data are taken from [79,80,118,163,180,183,184].

## 14. Conclusion

Fungi are rich in proteins. A multitude of proteins has now been purified and characterized and many more remain to be isolated. Some of these proteins have proven applicable value e.g. xylanases, cellulases, laccases and proteases.

Table 2
Comparison of N-terminal amino acid sequence of *P. tuber-regium* ribosome inactivating protein (RIP) with other RIPs

| | |
|---|---|
| *P. tuber-regium* RIP | ART·QP·GN·I·APV·GDFTLYPNAP·RQGH·I·VA |
| Ricin A | IINFTTAGA·TVQSYTNFIRA·VRGRLTT··GADVR··HDIPVL |
| Abrin A | PIKFSTEGA·TSQSYKQFIEALRERLRGGL |
| *P. tuber regium* RIP | ARTQP···GN·IAPVGDFTLYPNA·P·RQGHIVA |
| α-momorcharin | DVSFRLSGAD··PRSYGMFI···KD···L·RNALP·FREK···VY |
| Trichosanthin | DVSFRLSGA·T·SSSYGVFI···SN··L·RKALPNER·K·LYD |
| β-momorcharin | DVSFDLSGA·T·AKTYTKFI···ED··L·RAALPI···SHKVY |
| Bryodin | DVSFRLSGA·T·TTSYGVFI···KN··L·REALPYER |
| Luffin-a | DVRFSLSGS·S·STSYSKFI···GD··L |
| Momorcochin-S | DVTFSLLGANT·TKSYAAFI···TNFRK·DVASEK |
| *P. tuber-regium* RIP | ARTQPGNIAPVGDFTLYPNAPRQGHIVA |
| Flammulin | APSHFSHPGVLADRAQIDFIXGKVNEGA |
| Velutin | XHPDLFXXRPDNTASPKFEDPRLNP |
| Hypsin | ITFQGDLDARQQVITNADTRRKRDVRAA |
| Lyophyllin | ITFQGASPARQTVITNAITRARADVRAA |
| Calcaelin | ANPIYNIDAFRV |
| Flammin | SPVIPANTFVAFRLYEVGFUPA |
| Velin | SGSPLTQAQAEALLKPQGLAYSSGGNT |

(·) Space left to achieve maximal similarity. Data are taken from [10,88,89,108,113,165–167].

Table 4
Comparison of N-terminal sequences of mushroom proteases

| Pleureryn | GPQFPEA |
|---|---|
| POMEP | ATFVGCSATRQTQLN |
| AMMEP | XXYNGXTXSRQTTLV |
| GFMEP | TYNGCSSSEQSALA |
| TSEMP | ALYVGXSPXQQSLLV |
| ILAP | AAGSVPATNQLVDY |
| ABSP | TQTNAPWGLARLSSSTRL |

POMEP, AMMEP, GFMEP, ILAP and TSMEP = metallo-endopeptidases from *Pleurotus ostreatus*, *A. mellea*, *G. frondosa* and *T. saponaceum*. ABSP = *A. bisporus* serine protease. ILAP = *I. lacteus* aspartic proteinase. Data are taken from [74,75,119,139,169].

Table 5
Comparison of N-terminal sequences of mushroom laccases

| | |
|---|---|
| *A. bisporus* laccase I | KTR-TFDFDLVNTRL |
| *A. bisporus* laccase II | DTK-TFNFDLVNTRL |
| *C. cibarius* laccase | GCCNCGHA |
| *Coriolus hirsutus* laccase | AIGPTADLTISNAEV |
| *Coriolus hirsutus* laccase | GICTKANLVITGAAI |
| *P. eryngii* laccase I | AXKKL-DFRIINN |
| *P. eryngii* laccase II | ATKKL-DEFIINN |
| *Phlebia radiata* laccase | SIGPVTDFHIVNAAV |
| *Pleurotus ostreatus* laccase | AIGPAGNMYIVHEDV |
| *Pycnoporus cinnabarinus* laccase | AIGPVADLTLTNAAV |
| *Rigidoporus lignosus* laccase | ATV-ALDLHILNANL |
| *Trametes versicolor* laccase I | AIGPVASLVVANAPV |
| *Trametes versicolor* laccase II | GIGPVADLTITDAAV |
| *Trametes versicolor* laccase III | GIGPVADLTITDAEV |
| *Tricholoma giganteum* laccase | DDPQQAVTDD |

Data are from [111,145,176].

Engineering of these proteins may lead to a higher potency of the desirable activities and minimization of the undesirable activities.

In the foregoing account it can be seen that some of the proteins produced by fungi including ribosome inactivating proteins (Table 1), ribonucleases (Table 2), proteases (Table 3) and lectins (Table 4) may differ in amino acid sequence and molecular mass from their counterparts produced by other organisms such as flowering plants. Interestingly, ribosome inactivating proteins, ribonucleases, proteases and lectins from different fungi may exhibit unrelated sequences. However, fungal ribosome inactivating proteins, ribonucleases, proteases and lectins may exert biological actions similar to their non-fungal counterparts despite structural disparity. On the other hand, laccases from different fungi may resemble each other in amino acid sequence (Table 5) and molecular mass. Thus the principle of structure-function relationship may not be applicable to all of these different types of fungal proteins (Table 6).

A comparative study of the same type of protein e.g. lectin, elaborated by different fungi, may reveal different characteristics such as different biological or pharmacological potencies. A range of natural analogs is thus available for the investigator to choose from. In view of the large number of fungal species that exist and the scanty data present, it would be worthwhile to examine more species for the presence of ribosome inactivating proteins, antifungal proteins, nucleases and proteases.

Both fungal ribosome inactivating proteins and fungal antifungal proteins inhibit the process of translation and the activity of HIV-1 reverse transcriptase. The former may also be endowed with antifungal activity. Fungal ribonucleases inhibit translation by virtue of their ability to degrade RNA, a molecule important to protein synthesis. Ubiquitin demonstrates both translation-inhibitory and ribonuclease activities, and may also possess antiproliferative activity against tumor cells. Obviously, fungal ribosome inactivating proteins, antifungal proteins, ribonucleases and ubiquitins play a defensive role and protect fungi against foreign species and foreign cells that may constitute a threat to the fungi. Proteases can be added to the list of defensive proteins since they break down proteins, another type of molecules conveying information. Studies of informational molecules secreted by fungi may lead to measures which can optimize fungal growth and better protect economically important fungi from potentially harmful foreign species.

In addition to the fungal proteins and enzymes covered in this review, there are other fungal enzymes with applications in food industry, applications in chemical production, and applications in the medical sector. In the food industry, fungal α-amylase is used for liquefaction of dextrans and in alcohol production, authocyanase for juice and wine decolorization, catalase for milk sterilization and cheese making, glucoamylase for dextrin degradation to glucose, glucose

Table 6
N-terminal sequences of some recently reported mushroom lectins and agglutinins

| | |
|---|---|
| *A. cylindracea* lectin (16.1 kDa-subunit) | RVTNVANGFVAGDQKAMVRV |
| *A. cylindracea* lectin (15.3 kDa-subunit) | AVNFYNVLAGAENDLVADVE |
| *Ganoderma carpense* lectin | VNDYEAWYGADD |
| *L. shimeiji* agglutinin | PVVFELKFPNNNPESLLALAACARNKAH |
| *Pleurotus ostreatus* lectin (40 kDa subunit) | ATAKIKATPAQPQQFQPAALNAAK |
| *Pleurotus ostreatus* lectin (41 kDa subunit) | ACATAKCTTATPQQPGCAPAALNAAK |
| *P. tuber-regium* lectin | DRXAGYVLYXXVPY |
| *V. volvacea* lectin | PSNGNQYLIAQAYNLQKVNFDYTPQWQRGN |
| *Xerocomus spadiceus* lectin | CSKGGVGRGYGIG |

Data from [105,114,144,161,171,175,178].

isomerase for high fructose syrup, lactase for lactose hydrolysis in cheese whey, lipase for cheese ripening, naringinase for juice debitterning, pectinase for wine and fruit juice clarification and viscosity reduction in fruit processing, proteases for meat tenderization, cheese production and dough production. Fungal amylase, cellulase, and lipase are used for assisting digestion and lactase for increasing milk digestibility. Fungal enzymes are utilized in production of chemicals including citric acid, erythorbic acid, fumaric acid, and kojic acid. For analytical applications, fungal alcohol dehydrogenase is employed for analysis of ethanol, galactose oxidase for galactose, analysis, glycerol kinase and triacylglycerol lipase for triglyceride analysis, and urate oxidase for uric acid analysis [100].

In summary, fungi secrete a host of proteins which are protective in function to the fungi themselves or which can be exploited for the welfare of mankind. A continued search would undoubtedly disclose more proteins of interest and importance.

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