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MINI-REVIEW

Fungal proteinaceous compounds with multiple biological activities

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Abstract Fungi comprise organisms like molds, yeasts and mushrooms. They have been used as food or medicine for a long time. A large number of fungal proteins or peptides with diverse biological activities are considered as antibacterial, antifungal, antiviral and anticancer agents. They encompass proteases, ribosome inactivating proteins, defensins, hemolysins, lectins, laccases, ribonucleases, immunomodulatory proteins, and polysaccharopeptides. The target of the present review is to update the status of the various bioactivities of these fungal proteins and peptides and discuss their therapeutic potential.

Keywords Fungi · Proteins · Antibacterial · Antifungal · Antiviral · Anticancer

Introduction

Infections caused by viruses, bacteria and fungi resulted in millions of reported disease cases each year which include lower respiratory infections, diarrhea, and tuberculosis (World Health Organization 2015). Specific medications or anti-infective drugs like antibiotics, antivirals and antifungals are used to treat infections. However, there is a rising trend in drug resistance which renders some of these drugs ineffective causing a global health crisis (Blair et al., 2015). Examples of the common types of drug-resistant bacteria include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and multidrug-resistant *Acinetobacter baumannii* (Bassetti et al.

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2013; Cassir et al. 2014). Some strains of HIV, hepatitis B, hepatitis C, influenza, and herpes viruses have become resistant to antiviral drugs (Lou et al. 2014). *Candida* spp, *Cryptococcus neoformans*, and *Aspergillus fumigatus* are responsible for the most common fungal infections but unfortunately, resistance to antifungal drugs has developed in all of them (Srinivasan et al. 2014). The rapid rise in antimicrobial resistance has generated an increased demand for the development of novel therapies to treat contemporary infections and the development of new drugs or alternative therapies is clearly a matter of urgency.

Fungi comprise organisms like molds, yeasts, and mushrooms. Trichothecenes are fungal sesquiterpenoids metabolites possessing a tricyclic core with an epoxide at C-12 and C-13. Macrocyclic trichothecenes exhibit antiviral, anticancer, antimalarial and antifungal activities (de Carvalho et al. 2016). Low-molecular-weight mushroom compounds include primary metabolites like oxalic acid and secondary metabolites like terpenes, steroids, quinolines, anthraquinones, and benzoic acid derivatives. High-molecular-weight compounds are mainly proteins and peptides (Alves et al. 2012). Mushrooms also produce polysaccharides or polysaccharide-protein complexes with immunoenhancing and anticancer activities (Meng et al. 2016). They are the most extensively studied among all the bioactive constituents of mushrooms. Some of the mushroom polysaccharides have gone through various phases of clinical trials and are employed for therapeutic purposes (Aleem 2013; Shah et al. 2011; Wasser 2011). However, bioactive proteins and peptides, including plectasin and polysaccharopeptide (PSP) from *Coriolus versicolor*, constitute another important type of functional components in mushrooms, which also have captured the interest of many investigators due to their pharmaceutical potentials (Xu et al. 2011). Medicinal fungi including mushrooms display a constellation of medicinal activities encompassing antibacterial, antifungal, antiviral, antitumor, immunomodulating, antiparasitic, cardiovascular, hepatoprotective, antioxidant, free radical scavenging, antihyperlipidemic, and antidiabetic effects (Wasser 2011). Many mushrooms are delicious and nutritious in addition to the health benefits. However, many fungi are pathogenic, e.g., *Candida*, *Staphylococcus*, and *Streptococcus* species. A vast amount of research has been conducted on mushrooms and fungi other than mushrooms with an attempt to control pathogenic fungi and to isolate useful compounds from pathogenic fungi and medicinal fungi. The resulting literature is voluminous. The intent of the present article is to review fungal proteins and peptides with antibacterial, antifungal, antiviral, and anticancer activities.

Fungal proteins and peptides with antibacterial activity

Fungi and bacteria co-exist in a variety of environments, where they directly compete with each other, especially when they are in the same nutritional niche. One well known bacterium-fungus interaction is antibiosis, where metabolic substances or defense molecules produced from one species play a key role in killing the other (Frey-Klett et al. 2011). The best known example is penicillin from the mold *Penicillium chrysogenum*. Other metabolites like proteins and peptides which exhibit antibacterial activity have been discovered and they possess significant pharmaceutical applications especially in the age of increasing bacterial resistance against many commercially available antibiotics. Here, we highlight a few examples of fungal proteins and peptides with antibacterial activity, including some important antimicrobial peptides, but with special emphasis on plectasin.

A 12-kDa poly U-preferential ribonuclease from *Pleurotus sajor-caju* displayed antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa* (Ngai and Ng 2004a).

Eryngeolysin, a monomeric 17-kDa hemolysin from *Pleurotus eryngii* exhibiting antibacterial activity against *Bacillus* species possessed an N-terminal sequence with marked resemblance to the hemolysins aegerolysin from *Agrocybe cylindracea* and ostreolysin from *Pleurotus ostreatus*. Its hemolytic activity remained stable at pH 4.0–12.0, but activity was indiscernible at pH 2 and pH 13 and below pH 2 (Ngai and Ng 2006). A dimeric 44-kDa antibacterial hemolysin from mushroom *Clitocybe sinopica* demonstrated antibacterial activity against *Xanthomonas malvacearum*, *Xanthomonas oryzae*, *Agrobacterium tumefaciens*, *A. rhizogenes*, and *A. vitis*, but not against *Escherichia coli*, *Erwinia herbicola*, *Pseudomonas batatae*, and *S. aureus*, and no antifungal activity against *Bipolaris maydis*, *B. sativum*, *Fusarium oxysporum*, *Setosphaeria turcica*, and *Verticillium dahlia* (Zheng et al. 2010).

Agaricus bisporus exhibits activity against both gram-positive and gram-negative bacteria. Proteins isolated from this mushroom demonstrated inhibitory activity toward *S. aureus* and methicillin-resistant *S. aureus* (Houshdar Tehrani et al. 2012).

Fungal laccases are enzymes involved in morphogenesis, pathogen/host interaction and lignin degradation and have industrial and medical applications. Some of them have been shown to have antibacterial activity (Jaszek et al. 2013).

The defensin copsin from *Coprinopsis cinerea* displayed antibacterial activity against *Listeria monocytogenes* and *Enterococcus faecium*. Copsin exhibited remarkable thermostability and resistance to proteolytic inactivation due to possession of the cysteine stabilized α/β-fold with a distinctive disulfide linkage, and an N-terminal pyroglutamate. Copsin bound to the peptidoglycan precursor lipid II and adversely

affected biosynthesis of bacterial cell wall. Different from other defensins and lantibiotics, the third position of the lipid II pentapeptide is of paramount importance to binding of copsin. Copsin is a candidate as a new antibiotic (Essig et al. 2014).

The fungal defensin plectasin is highly active against gram-positive bacteria and several derivatives NZ2114 (Andes et al. 2009), Agplectasin, (Mao et al. 2013) and MP1102 (Mao et al. 2015) have been prepared. They are the most extensively investigated fungal antibacterial proteins. Plectasin was the first defensin isolated from the saprophytic ascomycete *Pseudoplectania nigrella*. It has primary, secondary and tertiary structures which show striking similarity to those of found in dragonfly, scorpion, spider, and mussel defensins. They act on the bacterial cell wall and inhibit the synthesis of peptidoglycan. Its in vitro action against *Streptococcus pneumoniae* is comparable to the action of penicillin and vancomycin (Mygind et al. 2005). Production of recombinant plectasin with a high yield has been achieved (Mao et al. 2015). Plectasin demonstrated negligible toxicity in mice and exhibited similar effectiveness as penicillin and vancomycin in treating *S. pneumoniae*-induced pneumonia and peritonitis (Mygind et al. 2005). L-plectasin but not D-plectasin demonstrated antimicrobial activity (Mandal et al. 2009). Plectasin was devoid of toxicity to A549 cells, normal human bronchial epithelial cells, and lung fibroblasts. It did not affect interleukin-8 transcription or formation in A549 cells (Hara et al. 2008). It had the highest activity against gram-positive bacteria among the various mushroom products reported (Alves et al. 2012). Further studies showed that plectasin bound directly to the bacterial cell-wall precursor Lipid II. It targeted on the cell-wall biosynthesis pathway (Schneider et al. 2010). The binding site of plectasin for Lipid II is indicated by an arrow in Fig. 1a.

The intracellular survival of staphylococci has introduced difficulties during treatment of *S. aureus* infections. The intracellular activity of plectasin against *S. aureus* in human THP-1 monocytes and in a murine peritonitis model indicates its usefulness in combating *S. aureus* infections (Brinch et al. 2009). It was also effective on drug- resistant strains (Jing et al. 2010). Recombinant plectasin at 2560 μg/ml was approximately equipotent in its antibacterial activity against *S. pneumoniae*, *S. aureus*, *S. suis*, and *S. epidermidis*, to 160, 320, and 320 μg/ml vancomycin and 640 μg/ml penicillin, respectively. The activity against *S. aureus* was stable over the pH range 2.0 to 10.0, at 100 °C for 1 h, and in the presence of the proteases pepsin and papain. The production of recombinant plectasin in *P. pastoris* in large amounts may help to treat infections by antibiotic-resistant *Staphyloccocus* and *Streptococcus*. Plectasin exhibited strong antimicrobial activity against the Gram-positive bacteria *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *S. suis* (Zhang et al. 2011).

Listeria monocytogenes had reduced sensitivity to plectasin compared with *S. aureus* (Gottlieb et al. 2008). The wild type of *S. aureus* was several folds more sensitive than *S. aureus* mutants with insertion in the heme response regulator (hssR) to plectasin as well as the plectasin-like defensin eurocin. Plectasin had no effect on the expression of hssR or hrtA, a hssR-regulated gene. Mutation of the RR23 gene in *L. monocytogenes*, which is homologous to *S. aureus* hssR, had no impact on the sensitivity to plectasin. *S. aureus* hssR, but not *L. monocytogenes* RR23, confers sensitivity to plectasin and eurocin. Hence, a functional dissimilarity between hssR and RR23 accounts for the discrepancy in sensitivity to plectasin and eurocin between *L. monocytogenes* and *S. aureus* (Thomsen et al. 2010).

In contradistinction to other defensins that perturb the integrity of the bacterial cell membrane, plectasin as mentioned previously, bound to the bacterial cell wall precursor Lipid II to form an equimolar stoichiometric complex and interfered with cell wall biosynthesis. The amino acids in plectasin that play a role in complex formation have been found with the aid of nuclear magnetic resonance spectroscopy and computational modeling (Schneider et al. 2010). Plectasin inhibited Kv1.3 channel currents in electrophysiological experiments. It also blocked hERG, IKCa, cKCNQ, Kv1.1, Kv1.2, and SKCa3 channels. Plectasin bound to the outer pore region of Kv1.3 channel, analogous to the site where animal toxin blockers interact with Kv1.3 channel (Xiang et al. 2015).

Plectasin potentiated the activities of other antibiotics such as glycopeptides, aminoglycosides and β-lactams, against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in 75–90 % of MSSA and MRSA strains examined. In contrast, there was no synergistic interaction with vancomycin. In the same study, plectasin also potentiated the action of gentamicin and amoxicillin in mice infected with MSSA and MRSA (Hu et al. 2015).

Both inducible and constitutive plectasin exhibited activity against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus faecium*, and penicillin-resistant *Streptococcus pneumonia* (Chen et al. 2015b). The yields of plectasin combined with small ubiquitin-related modifier (SUMO) and thioredoxin A were higher than those obtained by combination with glutathione-S- transferase and intein. The inhibitory effect of plectasin cleaved from SUMO-plectasin against MRSA, vancomycin-resistant enterococci and penicillin-resistant *S. pneumoniae* was more striking than that elicited by the same amount of ampicillin (Chen et al. 2015a). Recombinant and synthetic plectasin demonstrated potent thermostable and pH-stable antibacterial activity against gram-positive bacteria, including antibiotic-resistant bacterial species, in particular penicillin-resistant *Enterococcus faecium* (Chen et al. 2015b). Recombinant plectasin exerted a growth-suppressing action on *Streptococcus suis* and *S. aureus* with a minimum inhibitory concentration on the

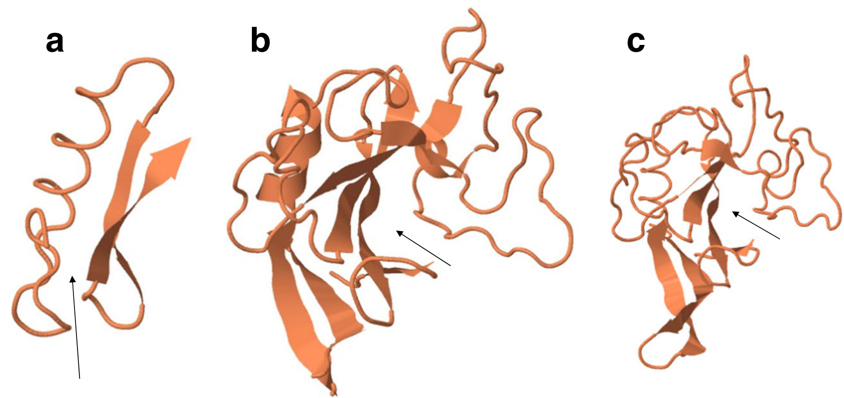

Fig. 1 Structures of **a** plectasin from *Pseudoplectania nigrella* (PDB: 3E7U) (Mandal et al., 2009), **b** restrictocin from *Aspergillus restrictus* (PDB: 1AQZ) (Yang and Moffat, 1996), **c** α-sarcin from *Aspergillus giganteus* (PDB: 1DE3) (Pérez-Cañadillas et al., 2000). The *arrows* represent the binding site of plectasin for Lipid II; the active site of restrictocin and the active site of α-sarcin for their ribonucleolytic activity, respectively

former bacterial species of 4 μg/ml. Recombinant plectasin demonstrated pepsin resistance and relatively high pH stability despite susceptibility to trypsin (Wan et al. 2016).

Plectasin, encapsulated with high efficiency (71–90 %) into poly(lactic-co-glycolic acid) nanoparticles using double emulsion solvent evaporation methodology, exhibited a mediated release of plectasin over 1 day. The plectasin-loaded nanoparticles demonstrated better antibacterial potency over non-encapsulated plectasin toward bronchial epithelial Calu-3 cell monolayers infected with *S. aureus*. There was no effect on the viability of eukaryotic cells (Water et al. 2015).

NZ2114 is a derivative of plectasin and was discovered in a high-throughput mutation and screening procedure aimed at searching plectasin derivatives with enhanced potency against staphylococci and streptococci (Raventós et al. 2005). Single-dose time-kill investigations in a neutropenic mouse thigh infection model after administration of NZ2114 at the dosages of 10, 40, and 160 mg/kg of body weight disclosed dose-dependent destruction of *Streptococcus pneumoniae* and *S. aureus* and prolonged action (3 to 15 h) against both bacterial species (Andes et al. 2009). All three intravenous dosages of NZ2114 (5, 10, and 20 mg/kg, two injections/day for three consecutive days) lowered MRSA count in kidneys, spleen and cardiac vegetations, compared with untreated controls. The highest dosage of NZ2114 was similar to daptomycin in effectiveness and more effective than vancomycin. The intermediate and the highest dosages of NZ2114 forestalled relapse in kidneys, spleen and cardiac vegetations following the treatment, whereas there was a continuous increase in the MRSA counts in tissues of animals receiving daptomycin and vancomycin (Xiong et al. 2011). The minimal inhibitory concentration of recombinant NZ2114, a new variant of plectasin overexpressed in *Pichia pastoris*, was estimated to be 28 to 900 nM in four *Staphyloccocus aureus* strains, and 110–900 nM in 20 MRSA clinical isolates. NZ2114 synergized with vancomycin, streptomycin and kanamycin against *S. aureus* ATCC 25923, and demonstrated an additive effect with spectinomycin and ampicillin. NZ2114 synergized with ampicillin, kanamycin, vancomycin, and streptomycin, and antagonized spectinomycin, and demonstrated an additive effect with spectinomycin and ampicillin in MRSA (*S. aureus* ATCC 43300). Recombinant NZ2114 had negligible hemolytic activity and relatively high thermostability from 20 to 80 °C with the maximal activity at pH 8 (Zhang et al. 2014a). NZ2114 synergized with dalbavancin, moenomycin, and teicoplanin, but not with bacitracin, daptomycin, fosfomycin, penicillin G, ramoplanin, telavancin, and vancomycin. The bulk of the synergistic interactions entailed suppression of transglycosylation during peptidoglycan synthesis. The findings indicate that, dalbavancin, teicoplanin, telavancin and vancomycin may interfere with different steps of cell wall synthesis despite their binding to the C-terminal D-Ala-D-Ala of Lipid II (Breidenstein et al. 2015).

Optimization of expression conditions for another novel plectasin-derived antimicrobial peptide-MP1102 was investigated under the control of the GAP promoter in *Pichia pastoris* X-33. The recombinant MP1102 was purified with a yield of 376.89 mg/l and 96.8 % purity. This represents the highest level of antimicrobial peptides expressed in *Pichia pastoris* using GAP promoter so far. These results provide an economical method for the high-level production of rMP1102 under the control of the GAP promoter (Mao et al. 2015). Recombinant Agplectasin, designed by fusing the AgrD1 pheromone to the N-terminus of plectasin, and expressed in *Pichia pastoris*, demonstrated only negligible hemolytic activity and produced a potent antibacterial action against *S. aureus* and MRSA but not *Staphylococcus epidermidis* or other bacteria examined. The peptide was stable from pH 2.0 to pH 10.0 and at 100 °C for 1 h (Mao et al. 2013). A recombinant antimicrobial peptide MP1106 based on plectasin with four mutational sites and expressed in *Pichia pastoris* X-33 elicited potent antibacterial activity against *S. aureus* and 20 MRSA clinical isolates. The peptide brought about only 1.16 % hemolysis at 0.5 mg/ml and was stable after exposure to human serum for 24 h at 37 °C. It was stable from 20 to 100 °C, at pH 6, 8, and 10 and its activity was only marginally curtailed at pH 2 and 4. Its activity was

unaltered in the presence of 20 % dimethylsulfoxide and 10 mM dithiothreitol. The peptide was not affected by the proteases pepsin, proteinase K and snailase, but was susceptible to trypsin (Cao et al. 2015). The tri-hybrid antimicrobial peptide LHP7 composed of plectasin, lactoferricin, and antimicrobial peptide HP (derived from N-terminus of *Helicobacter pylori* ribosomal protein L1) permeabilized the *S. aureus* cell membrane. The peptide induced ultrastructural changes including cell wall thickening, cell shrinkage and disruption, and leakage of intracellular materials. LHP7 bound and got inserted into the groove of the *S. aureus* genomic DNA, and finally brought about cell cycle arrest at the I-phase (Xi et al. 2014). Table 1 presents a comparison of N-terminal sequences of some fungal antibacterial proteins.

Fungal proteins and peptides with antifungal activity

Lyophyllin from *Lyophyllum shimeji*, a 20-kDa ribosome-inactivating protein, obstructed mycelial growth in *Coprinus comatus* and *Physalospora piricola* (Lam and Ng 2001a). A 20 kDa ribosome-inactivating protein designated as hypsin from the mushroom *Hypsizigus marmoreus* exerted antifungal activity against *Botrytis cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola*, and *Physalospora piricola*, with an IC_{50} of 0.06, 14.2, 2.7, and 2.5, μM, respectively (Lam and Ng 2001b). The ribonuclease and ribosome-inactivating protein restrictocin from *Aspergillus fumigatus* and *Aspergillus restrictus* demonstrated suppressive activity toward the fungi *Alternaria longipes*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides*, *Trichoderma viride*, and *Paecilomyces variotii*. Moreover, it was relatively heat-stable and stable in the presence of metal ions and denaturants. However, its antifungal activity was highly dependent on the integrity of the disulfide linkage (Rao et al. 2015).

A 14-kDa antifungal protein, designated as Lyophyllum antifungal protein, with an N-terminal sequence exhibiting some similarity to those of angiosperm thaumatin-like proteins and thaumatins, retarded mycelial growth in *Mycosphaerella arachidicola* and *P. piricola* but there was no effect on *Coprinus comatus*, *Colletotrichum gossypii*, and *Rhizoctonia solani*. This antifungal protein synergized with lyophyllin, the ribosome-inactivating protein from the same mushroom, in antifungal action against *P. piricola* (Lam and Ng 2001a). A dimeric 28-kDa antifungal protein from *Polyporus alveolaris* designated as alveolarin, suppressed growth in *Physalospora piricola*, *Mycosphaerella arachidicola*, *Fusarium oxysporum*, and *Botrytis cinerea* (Wang et al. 2004). A10-kDa antifungal peptide from *P. eryngii* designated as eryngin, with an N-terminal sequence bearing some resemblance to the antifungal protein from the mushroom *Lyophyllum shimeiji* and to plant thaumatin and thaumatin-like proteins exerted an antifungal action toward *Mycosphaerella arachidicola* and *Fusarium oxysporum* (Wang and Ng 2004). A 9-kDa antifungal peptide from *A. cylindracea* designated as agrocybin, impaired mycelial growth in several fungal species (Ngai et al. 2005). An antifungal protein trichogin, originating from *Tricholoma giganteum* var. golden blessings, impeded fungal growth in *Mycosphaerella arachidicola*, *Fusarium oxysporum*, and *Physalospora piricola* (Guo et al. 2005). A 7-kDa peptide from the oyster mushroom known as pleurostrin inhibited mycelial growth in *Fusaerium oxysporum*, *Mycosphaerella arachidicola* and *Physalospora iricola* (Chu et al. 2005). A 15-kDa antifungal protein from *Ganoderma lucidum*, designated as ganodermin, manifested antifungal activity toward *Fusarium oxysporum*, *Botrytis cinerea*, and *Physalospora piricola* with IC_{50} values of 12.4, 15.2, and 18.1 μM, respectively (Wang and Ng 2006a). A 9.5-kDa antifungal protein from *Hypsizygus marmoreus* with activity against *Flammulina velutipes* exhibited N-terminal amino acid sequence resemblance to *Clostridium thermocellum* ribonuclease H (Suzuki et al. 2011). Cordymin, a 10.9-kDa thermostable antifungal peptide from *Cordyceps militaris* inhibited mycelial growth in *Candida albicans*, *Rhizoctonia solani*, *Bipolaris maydis*, and *Mycosphaerella arachidicola*, with an IC_{50} of 750, 80, 50, and 10 μM, respectively. However, it was devoid of any effect on *Fusarium oxysporum*, *Valsa mali*, and *Aspergillus fumigatus* (Wong et al. 2011a).

Lentin from *Lentinus edodes*, a 27.5-kDa protein with N-terminal sequence resembling endoglucanase, repressed growth in *Botrytis cinerea*, *Mycosphaerella arachidicola* and *Physalospora piricola* (Ngai and Ng 2003). A 12-kDa cytotoxic antifungal protease from *Cordyceps militaris* exhibited potent antifungal activity against *Fusarium oxysporum* (Park et al. 2009).

The aforementioned ribonuclease from *P. sajor-caju* also displayed antifungal activity against *Mycosphaerella arachidicola* and *Fusarium oxysporum* (Ngai and Ng 2004a). Table 2 presents a comparison of N-terminal sequences of some fungal antifungal proteins.

Fungal proteins and peptides with antiviral activity

Fungi produce a number of protein or peptides with ribonuclease and ribosome-inactivating activities. Besides their function as protein synthesis inhibitors, they also exhibit inhibitory activity toward viral enzymes and proliferative activity toward tumor cells (discuss in the next section). They manifest a diversity of structures and molecular sizes.

Recombinant plectasin noncompetitively inhibited dengue serotype-2 NS2B-NS3 protease at Ki value of 5.03 ± 0.98 μM and viral replication in Vero cells (Rothan et al. 2013). The recombinant antiviral peptide-fusion protein (PG1-MAP30-PLSN) formed by conjugation of plectasin (PLSN) with

Table 1 Comparison of N-terminal sequences of some fungal antibacterial proteins

Antibacterial proteins	N-terminal sequence	Target bacteria	Reference(s)
Copsin from *Coprinopsis cinerea*	QVCPTRRGLCVTSGLTACRNHCR	*Listeria monocytogenes* (MIC = 0.25–0.5 μg/ml) and *Enterococcus faecium*	(Essig et al., 2014)
Clitocybe sinopica antibacterial protein	SVQATVNGDKML	*Xanthomonas malvacearum* (MIC = 0.56 μM), *Xanthomonas oryzae* (MIC = 0.56 μM), *Agrobacterium tumefaciens* (MIC = 0.14 μM), *A. rhizogenes* (MIC = 0.14 μM), and *A. vitis* (MIC = 0.28 μM)	(Zheng et al., 2010)
eryngeolysin from *Pleurotus eryngii*	AYAQWVIIII HNVGSKDVKIVNLKPSWGKLSAAGDLQTEV	*Bacillus megatarium* (IC_{50} = 110 μM) and *B. subtilis* (IC_{50} = 175 μM)	(Ngai and Ng, 2006)
plectasin from *Pseudoplectania nigrella*	QFTTILSIGITVFGLLNTGAFAAPQPVPEAYAVSDPEAHPDDFA	*Streptococcus pneumoniae* (MIC = 0.063–2 μg/ml), *S. suis* (MIC = 2 μg/ml), *Staphylococcus aureus* (MIC = 8–64 μg/ml), and *S. epidermidis* (MIC = 4–32 μg/ml) methicillin-resistant *S. aureus* (MIC = 14 μM), vancomycin-resistant *E. faecium* (MIC = 14 μM), and penicillin-resistant *S. pneumonia* (MIC = 7.3 μM)	(Chen et al., 2015a; Mygind et al., 2005; Zhang et al., 2011)
Pleurotus sajor-caju RNase	DNGEAGRAAR	*Pseudomonas aeruginosa* (IC_{50} = 51 μM), *P. fluorescens* (IC_{50} = 186 μM) and *S. aureas* (IC_{50} = 34 μM)	(Ngai and Ng, 2004a)

Momordica antiviral protein MAP30 protein and antiviral cationic peptide protegrin-1 (PG1) exerted an inhibitory action on dengue serotype-2 NS2B-NS3 protease with an IC_{50} of 0.5 μM. The maximal nontoxic dose of PG1-MAP30-PLSN toward Vero cells was 0.67 μM. PG1-MAP30-PLSN at 50 mg/kg suppressed the binding and proliferation of dengue virus and completely protected mice from infection resulting in 100 % survival (Rothan et al. 2014).

Many mushroom proteins suppressed HIV-1 reverse transcriptase activity. Some laccases such as those from *P. eryngii* (Wang and Ng 2006b), *Clitocybe maxima* (Zhang et al. 2010) *Tricholoma mongolicum* (Li et al. 2010a) *Agaricus placomyces* (Sun et al. 2012) and *Coprinus comatus* (Zhao et al. 2014), lectins from *Russula delica* (Zhao et al. 2010) *Hericium erinaceum* (Li et al. 2010b), *Pholiota adiposa* (Zhang et al. 2009), *Pleurotus citrinopileatus* (Li et al. 2008) and *Lactarius flavidulus* (Wu et al. 2011), proteases from *Cordyceps sobolifera* (Wang et al. 2012) and *Xylaria hypoxylon* (Hu et al. 2012), and ribonucleases from *Lactarius flavidulus* (Wu et al. 2012) and *Hohenbuehelia serotina* (Zhang et al. 2014b) inhibited HIV-1 reverse transcriptase activity.

The fungal ribosome-inactivating protein restrictocin produced by *Aspergillus restrictus* recognized domains within the HIV-1 genome and its anti-HIV-1 activity was attributed to its specific ribonucleolytic activity (Yadav and Batra 2015). It recognized and cleaved a single phosphodiester bond specifically in a GAGA tetranucleotide located in a conserved stem and loop structure in ribosomal RNA (Nayak et al. 2001). The active site of restrictocin for its ribonucleolytic activity is shown in Fig. 1b. Marmorin, a 9.5-kDa ribosome-inactivating protein from *Hypsizigus marmoreus*, demonstrated HIV-1 reverse transcriptase inhibitory activity, with an IC_{50} of 30 μM (Wong et al. 2008).

A 58-kDa laccase was isolated from oyster mushroom (*P. ostreatus*). It was found to possess in vitro anti-hepatitis C virus activity. There is no protective vaccine or effective treatment against the virus currently. Direct incubation of hepatitis C virus with the laccase for 7 days at the concentrations of 2.0 and 2.5 mg/ml inhibited viral entry completely. However, a low laccase concentration at 1.0 and 1.5 mg/ml did not show any blocking activity. The laccase was capable of inhibiting viral replication after the initial treatment at the concentrations of 1.25 and 1.5 mg/ml for 4 days and after the subsequent treatment at the concentrations of 0.75, 1.0, 1.25, and 1.5 mg/ml for another 4 days (El-Fakharany et al. 2010).

The aforementioned lentin from *Lentinus edodes*, agrocybin from *Agrocybe cylindracea*, trichogin from

Table 2 Comparison of N-terminal sequences of some fungal antifungal proteins and ribosome-inactivating proteins with antifungal activity

Antifungal protein	N-terminal sequence	Target fungus/fungi	Reference
Agrocybin from *Agrocybe cylindracea*	ANDPQCLYGNVAAKF	*Mycosphaerella arachidicola* (IC_{50} = 125 μM)	(Ngai et al., 2005)
Alveolarin from *Polyporus alveolaris*	FVCDMALA	*Botrytis cinerea*, *Fusarium oxysporum*, *M. arachidicola*, and *Physalospora piricola*	(Wang et al., 2004)
Cordyceps militaris antifungal protease (peptide fragments)	YQXXVTFXDF; VSXXGDSGVGGN; and NAFNDYTFK	*F. oxysporum*	(Park et al., 2009)
Cordymin from *Cordyceps militaris*	AMAPPYGYRTPDAAQ	*Candida albicans* (IC_{50} = 750 μM), *Bipolaris maydis* (IC_{50} = 50 μM), *M. arachidicola* (IC_{50} = 10 μM) and *Rhizoctonia solani* (IC_{50} = 80 μM)	(Wong et al., 2011a)
Eryngin from *Pleurotus eryngii*	ATRVVYCNRRSGSVVGGDDTVYYEG	*F. oxysporum* (IC_{50} = 1.35 μM) and *M. arachidicola* (IC_{50} = 3.5 μM)	(Wang and Ng, 2004)
Ganodermin from *Ganoderma lucidum*	AGETHTVMINHAGRGAPKLVVGGKKLS	*B. cinerea* (IC_{50} = 15.2 μM), *F. oxysporum* (IC_{50} = 12.4 μM), and *P. piricola* (IC_{50} = 18.1 μM,)	(Wang and Ng, 2006a)
Hypsin from *Hypsizigus marmoreus*	ITFQGDLDARQQVITNADTRRKRDVRAAVR	*B. cinerea* (IC_{50} = 0.06 μM), *F. oxysporum* (IC_{50} = 14.2 μM), *M. arachidicola* (IC_{50} = 2.7 μM) and *P. piricola* (IC_{50} = 2.5 μM,)	(Lam and Ng, 2001b)
Lentin from *Lentinus edodes*	CQRAFNNPRDDAIRW	*B. cinerea*, *M. arachidicola* (IC_{50} = 17.5 μM) and *P. piricola*	(Ngai and Ng, 2003)
Lyophyllum antifungal protein	AGTEIVTCYNAGTKVPRGPSAXGGAIDFFN	*M. arachidicola* and *P. piricola* (IC_{50} = 70 μM)	(Lam and Ng, 2001a)
Lyophyllin from *Lyophyllum shimeji*	ITFQGASPARQTVITNAITRARADVRAAVSALPTKAPVST	*Coprinus comatus* and *P. piricola* (IC_{50} = 2.5 μM)	(Lam and Ng, 2001a)
Pleurostrin from *Pleurotus ostreatus*	VRPYLVAF	*F. oxysporum*, *M. arachidicola* and *P. piricola*	(Chu et al., 2005)
Pleurotus sajor-caju RNase	DNGEAGRAAR	*F. oxysporum* (IC_{50} = 95 μM) and *M. oxysporum* (IC_{50} = 72 μM)	(Ngai and Ng, 2004a)
Trichogin from *Tricholoma giganteum*	QVHWPMF	*M. arachidicola* (IC_{50} = 3.8 μM), *F. oxysporum* and *P. piricola.*	(Guo et al., 2005)

Tricholoma giganteum and cordymin from *Cordyceps militaris* also attenuated HIV-1 reverse transcriptase activity (Ngai and Ng 2003; Ngai et al. 2005; Guo et al. 2005; Wong et al. 2011a).

Fungal proteins and peptides with antitumor activity

Fungi produce a variety of proteins/peptides with antiproliferative activity toward tumor cells and anticancer activity in tumor-bearing mice. It is common to use fungi as a source to isolate proteins/peptides (lectins, ribonucleases and polysaccharopeptides) for searching novel antitumor drugs and they have been widely studied.

Lectins recognize glycoconjugates on the cancer cell surface and are known for their cytotoxic, antiproliferative, apoptotic or immunomodulatory effects against human cancer cells (Ng and Wong 2013). Alpha-sarcin is the most prominent member in the fungal ribonuclease family which exhibits antitumor activity. Alpha-sarcin is internalized into the tumor

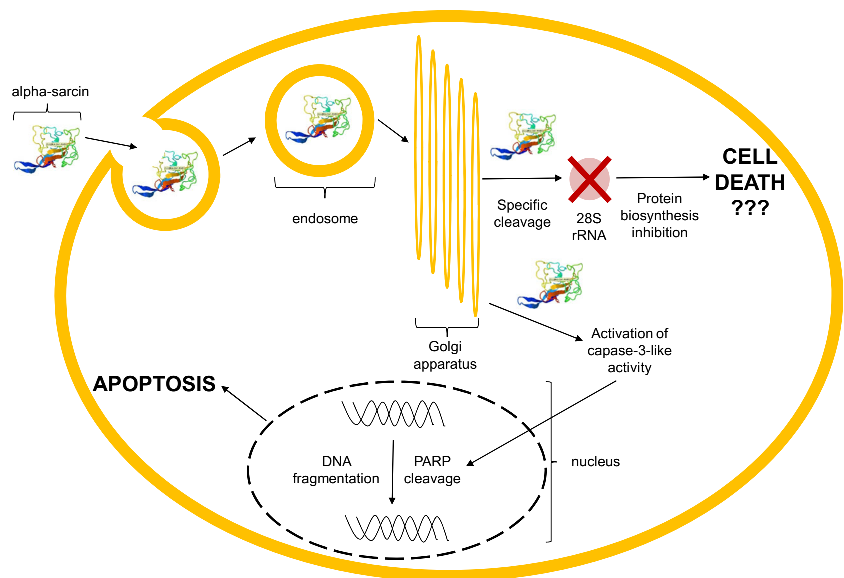

Fig. 2 The cytotoxic mechanism of alpha-sarcin. There is no specific membrane protein receptor for α-sarcin. The toxin is internalized through endocytosis involving acidic endosomes and the Golgi apparatus. The 28S rRNA is specifically cleaved which resulted in protein biosynthesis inhibition. Cell necrosis is not detected related to 28S rRNA cleavage, but a typical apoptosis-related DNA ladder is identified. Induction of caspase-3-like activation and cleavage of the specific substrate poly (ADP-ribose) polymerase by α-sarcin confirm its participation in the apoptotic cell death pathway

cells; their 28S rRNA is specifically cleaved. Internucleosomal genomic DNA fragmentation, apoptosis by activation of caspase-3-like activity, and cleavage of poly (ADP-ribose) polymerase are induced resulting in cell death (Olmo and Turnay 2001). But later reports from Alford et al. (2009) suggested that the cell death mechanism was independent of rRNA cleavage. A schematic diagram is shown in Fig. 2 to illustrate the cytotoxic mechanisms of alpha-sarcin. PSP or polysaccharide K is a protein-bound polysaccharide produced by the *C. versicolor* mushroom and is widely used in Asia as an adjuvant immunotherapy for a variety of cancers. It may improve immune function, reduce tumor-associated symptoms, and extend survival in lung cancer patients (Fritz et al. 2015). Several randomized clinical trials have demonstrated that it has great potential as an adjuvant cancer therapy agent, with positive results shown in the adjuvant treatment of gastric, esophageal, colorectal, breast and lung cancers (Fisher and Yang 2002).

Tricholoma mongolicum lectins enhanced macrophage nitrite production, suppressed growth of sarcoma 180 cells, and extended life-span in tumor-bearing mice (Wang et al. 1997). A dimeric lectin from *P. ostreatus* demonstrated high antitumor efficacy in sarcoma S-180 bearing mice and hepatoma H-22 bearing mice, and extended the duration of their survival (Wang et al. 2000). *Sclerotium rolfsii* lectin exhibited binding specificity for the oncofetal Thomsen-Friedenreich carbohydrate antigen (Galβ1-3GalNAc-α-O-Ser/Thr, T or TF) expressed in the vast majority of human cancers. The lectin elicited apoptosis in human breast, colon, and ovarian cancer cells and exerted an anticancer action in vivo. In human colon cancer HT29 cells, the lectin affected expression of mitogen-activated protein kinase and c-JUN-associated cell proliferation signaling pathways after 2 h. On the other hand, it altered cell miRNA expression 10 h later, and miRNA-associated cell cycle, DNA replication and apoptotic pathways after 1 day (Barkeer et al. 2015). *Rhizoctonia bataticola* lectin exhibited immunopotentiating activity toward normal human peripheral blood mononuclear cells and exerted antiproliferative cytotoxicity on Molt-4 and Jurkat human leukemic T cell lines bringing about apoptosis in 33 and 42 % of the cells, respectively. The lectin elicited Bid cleavage, caspase-3 activation and loss of mitochondrial membrane potential. It downregulated the expression of anti-apoptotic Bcl-X and Bcl-2 but did not affect expression of pro-apoptotic Bax and Bad. It did not manifest apoptotic activity toward undifferentiated CD34+ve hematopoietic stem and progenitor cells, isolated CD3+ve cells, or normal human peripheral blood mononuclear cells (Pujari et al. 2013). An 18-kDa lectin isolated from the mushroom *Ganoderma capense* (Lloyd) Teng exhibited antiproliferative activity toward leukemia (L1210 and M1) cells and hepatoma Hep G2 cells (Ngai and Ng 2004b). A homodimeric lectin from *Pholiota adiposa* was isolated and purified. Its molecular mass was 16 kDa. The lectin showed antiproliferative activity toward hepatoma Hep G2 cells and breast cancer MCF7 cells with an IC_{50} of 2.1 and 3.2 μM, respectively (Zhang et al. 2009).

Recombinant fungal immunomodulatory protein reFIP-gts from *Ganoderma tsugae* repressed the growth of A549 cancer cells but not that of normal MRC-5 fibroblasts. It suppressed activity of telomerase, a characteristic of cancer cells, inhibiting the telomerase catalytic subunit (hTERT). The fungal immunomodulatory protein downregulated hTERT transcription by preventing the binding between the E-box sequence on the hTERT promoter and c-myc transcriptional factor (Liao et al. 2006). Fungal immunomodulatory protein FIP-fve from *Flammulina velutipes* exerted an antiproliferative action, triggered cell cycle arrest, upregulated p53 and p21 expression, prevented migration and undermined

filopodia fiber formation in A549 lung cancer cells. FIP-fve inhibited epidermal growth factor-elicited Rac1 activation. RacGAP1 silencing inhibited cell migration, whereas RacGAP1 overexpression enhanced cell migration in the lung cancer cells. Thus, FIP-fve inhibited lung cancer cell proliferation through the p53 activation pathway and its repression of lung cancer cell migration is mediated by RacGAP1 (Chang et al. 2013).

A protein fraction containing serine protease from *Lignosus rhinocerotis* sclerotia was toxic to MCF7 breast cancer cells. Its protease and cytotoxic activities were attenuated in the presence of phenylmethylsulfonyl fluoride, indicating a relationship between the two activities (Yap et al. 2015).

Marmorin, a 9.5-kDa RIP from *Hypsizigus marmoreus*, demonstrated antiproliferative activity on breast cancer MCF-7 cells and hepatoma HepG2 cells, with an IC_{50} of 5 and 0.15 μM, respectively (Wong et al. 2008). Marmorin evinced more potent cytotoxicity toward estrogen receptor (ER)-positive MCF7 breast cancer cells than ER-negative MDA-MB-231 cells. It attenuated the expression level of estrogen receptor α (ERα) and suppressed 17β-estradiol elicited proliferative activity of MCF7 cells. This action was impaired by ERα knockdown in MCF7 cells, indicating involvement of the ERα-mediated pathway. Marmorin evoked G2/M-phase arrest, depolarization of mitochondrial membrane potential, activation of caspase-9 and apoptosis, to a greater degree in MCF7 in cells than that in MDA-MB-231 cells. Marmorin triggered the death receptor apoptotic pathway (involving activation of caspase-8) and endoplasmic reticulum stress (involving PERK and IRE1α phosphorylation, caspase-12 cleavage, and CHOP expression up-regulation) in both MDA-MB-231 and MCF7 cells (Pan et al. 2013). A recombinant immunotoxin, scFv (MGR6)-Cla, was constructed by conjugating the Fv region of the anti-ErbB2 (a tyrosine kinase receptor overexpressed in the majority of adenocarcinomas) monoclonal antibody MGR6 to the 17-kDa *Aspergillus clavatus* type 1 ribosome-inactivating protein and ribonuclease named clavin. Translation inhibition and binding assays revealed that both components of the immunotoxin retained their activities after refolding of the immunotoxin (D'Alatri et al. 1998).

Hericium erinaceus ribonuclease He1 mutants were produced by substituting 12 Asn/Gln residues with Asp/Glu residues and expressed in *E. coli*. The recombinant RNase He1 exerted antiproliferative activity toward human leukemia cells and its optimal pH was enhanced (Kobayashi et al. 2015). Three-dimensional models of *Aspergillus niger* ribonuclease and human actin were designed, validated and their stereochemical quality was evaluated as good by Ramachandran plot analysis with PROCHECK. Protein-protein docking on the molecular models was conducted. The RNase suppressed actin activity as revealed by investigations on the molecular level interactions (molecular simulations and protein docking) between *A. niger* RNase and human actin. *A. niger* RNase at 1 μM and 2 μM reduced invasiveness of MDA-MB 231 breast cancer cells by half and 90 %, respectively. The aforementioned information may be useful for designing new antineoplastic medications (Gundampati et al. 2012; Kumar et al. 2013). Hirsutellin A is a cyclizing ribonuclease with 130 amino acids from the mite fungal pathogen Hirsutella. It brought about the cleavage of oligonucleotides that resemble the sarcin/ricin loop of the ribosome, in addition to certain polynucleotides and dinucleosides. The toxicity of hirsutellin A toward human cancer cells was due to its interaction with phospholipid membranes like other ribotoxins as well as its ribonuclease activity (Herrero-Galán and Lacadena 2008). RNase T1 are ribonucleolytic proteins with cytotoxic activity produced by Penicillium and Aspergillus. These proteins gain entry into the cells and split a single phosphodiester bond within a conserved sequence referred to as the sarcin/ricin loop of the large rRNA gene, prevent protein biosynthesis, and trigger apoptosis. The ribotoxins kill virus-infected cells or transformed cells by changing membrane permeability (Lacadena et al. 2007). The immunotoxin formed by conjugating restrictocin (a ribosome-inactivating protein produced by *Aspergillus restrictus*) to the monoclonal antibody, MBrl for human breast carcinoma was about a thousandfold more active than unconjugated derivatized restrictocin toward MCF-7 breast cancer cells (Orlandi et al. 1988).

α-Sarcin (a specific ribonuclease that inhibits protein synthesis by inactivating ribosomes) and an antifungal protein were isolated concurrently from *Aspergillus giganteus* by chitin affinity chromatography and gel filtration (Liu et al. 2002). An immunotoxin, prepared by conjugating the single-chain variable fragment (scFv) of the monoclonal antibody that targets glycoprotein A33 (GPA33, a colon-cancer marker) to α-sarcin, demonstrated specific toxicity toward GPA33-positive cancer cells (Carreras-Sangrà et al. 2012). An α-sarcin immunotoxin IMTXA33αS when injected intraperitoneally undermined the growth of GPA33-positive human colon cancer xenografts in nude mice. The GPA33 antigen was absent from the residual tumors, and angiogenesis and proliferation were suppressed (Tomé-Amat et al. 2015). The inhibition of protein biosynthesis in immunotoxin-treated tumors is due to the α-sarcin component. After selective passage across some cell membranes, its specific RNase activity causes apoptosis which can be determined by active-caspase 3 labeling (Olmo and Turnay 2001). α-Sarcin and restrictocin have similar structures. Their active sites are found in the central β-sheet, with the amino acid side chains pointing toward the concave region of the proteins (Lacadena et al. 2007). The active site of α-sarcin is shown in Fig. 1c.

C. versicolor PSP augmented the cytotoxicity of S-phase targeted-drugs like etoposide and doxorubicin on ZR-75-30 human breast cancer cells and HL-60 human leukemia cells and decreased ratio of Bcl-xL/Bax protein expression in the

tumor cells (Wan et al. 2008). Acetylated, esterified, and phosphorylated *Grifola frondosa* polysaccharide-peptides displayed augmented adjuvant action to cyclophosphamide treatment on rats inoculated with C6 cancer cells and an enhanced growth inhibitory action on C6 cancer cells but not on normal brain cells (Chan et al. 2011). Sarcoma-180-bearing mice receiving injections of three water-soluble neutral proteoglycans derived from *P. ostreatus* mycelia demonstrated a decline in the tumor cell number and cell cycle arrest of the tumor cells in pre-G0/G1 phase. The proteoglycans enhanced the cytotoxic activity of murine natural killer cells and nitric oxide generation by macrophages. The polysaccharide: protein ratios of the proteoglycans were 14.2, 26.4, and 18.3, respectively. β-glycosidic bonds were present in all three fractions. Fraction I possessed terminal sugar with glucose/mannose as evidenced by strong interaction with glucose/mannose-specific lectin Concanavalin A (Sarangi et al. 2006).

The aforementioned ribonuclease from *P. sajor-caju* also displayed antiproliferative activity against HepG2 hepatoma cells and L1210 leukemia cells (Ngai and Ng 2004a). Eryngeolysin also exhibited cytotoxicity toward leukemia (L1210) cells (Ngai and Ng 2006). Besides, lentin from *Lentinus edodes* attenuated proliferation of leukemia cells (Ngai and Ng 2003). A cytotoxic protease from *Cordyceps militaris* was found to be toxic to human bladder and breast cancer cells (Park et al. 2009). Cordymin from *Cordyceps militaris* inhibited proliferation of MCF-7 breast cancer cells but not HT-29 colon cancer cells (Wong et al. 2011a).

Table 3 presents a comparison of N-terminal sequences of some fungal proteins with anticancer and/or HIV-1 reverse transcriptase activities.

Conclusion

From the preceding account an impression can be gathered that a spectacular array of proteins and peptides from fungi including mushrooms that can play a role as defense proteins against bacteria, fungi, viruses and cancer and have exploitable medicinal potential. Nonfungal (i.e., bacterial, plant, animal, and/or human) counterparts (Al-Mahrous et al. 2011; Citores et al. 2016; Iglesias et al. 2016; Jiratchariyakul et al. 2001; Ogawa 2016; Ouyang et al. 2006; Sartim and Sampaio 2015; Singh et al. 2016) of the various fungal proteins and peptides very often are endowed with biological activities qualitatively analogous to the fungal proteins and peptides, although the structural homology may not be extensive. However, some differences in biological activity may exist. For instance, the fungal ribosome-inactivating protein alpha-sarcin exhibits RNase activity which is generally not regarded as an intrinsic activity of plant ribosome-inactivating proteins.

Detailed structural information and in some cases structure-function relationships is (are) available for some of the fungal lectins (Lyimo et al., 2011), laccases (Piontek et al., 2002; Rebrikov et al., 2006; Ferraroni et al., 2014), ribosome-inactivating proteins (Sacco et al., 1983; López-Otín et al., 1984; Martínez del Pozo et al., 1988; Gasset et al., 1995., Lacadena et al., 1995., Mancheño et al., 1995., Kao and Davies, 1999, 2000; Pérez-Cañadillas et al., 2000; García-Mayoral et al., 2005; Alvarez-García et al., 2006), and nucleases (Inokuchi et al., 2000; Kobayashi et al., 2000, 2003, 2014).

The antibacterial mechanism of action of plectasin has been unraveled, but those of the other antibacterial proteins await elucidation. Although the antifungal mechanism of action of the fungal antifungal proteins has not been uncovered, it is likely that the mechanism employed is similar to that employed by mammalian proteins with antifungal activity such as cathelicidin (Wong et al., 2011b) and lactoferrin (Yin et al., 2014) involving membrane permeabilization, mitochondrial damage and increase of reactive oxygen species. It remains to be ascertained whether fungal proteins with HIV-1 reverse transcriptase inhibitory activity also exhibit suppressive activity on HIV-1 protease and integrase. This is likely in view of the reports of inhibitory activity of nonpeptidic mushroom constituents on HIV-1 protease and integrase (Ichimura et al., 1998; El Dine et al., 2008; Wang et al., 2014). The mechanism may involve protein-protein interaction like what occurs between HIV-1 protease and HIV-1 reverse transcriptase.

Antifungal proteins, as their names imply, play a role of defense against fungal pathogens. Fungal antifungal proteins appear to be structurally distinct from plant antifungal proteins and mammalian antifungal proteins like cathelicidins and lactoferricin (Yin et al., 2014). Yet they all exhibit anticancer and anti-HIV-1 reverse transcriptase activities. The fungal defensin plectasin has pronounced antibacterial activity like defensins of other origins (Dias Rde and Franco, 2015). Fungal lectins are also different from plant lectins structurally as evidenced by differences in N-terminal sequence and molecular weight. However, unlike plant lectins (Dias Rde et al., 2015), none of the mushroom lectins isolated to date displays antifungal activity. Fungal ribosome-inactivating proteins and their plant counterparts (Stirpe and Battelli, 2006, Stirpe 2013) have dissimilar N-terminal sequences but have biological activities in common like antimicrobial, anticancer and antiviral activities (Akkouh et al. 2015). Fungal ribonucleases display anticancer and HIV-1 reverse transcriptase activities like ribonucleases of other origins (Fiorini et al., 2014, 2015). Ribosome-inactivating proteins and laccases have not been reported from mammals. Antifungal proteins, defensins, lectins and ribonucleases are present in mammals and a diversity of other organisms. All aforementioned proteins play a defensive role.

To increase the armentarium against microbial pathogens and to combat the emerging resistance against antimicrobial

Table 3 Comparison of N-terminal sequences of some fungal proteins with HIV-1 reverse transcriptase inhibitory activity and/or antiproliferative activity toward tumor cells

Anti-HIV-1 and antitumor proteins	N-terminal sequence	Target	Reference
Agaricus placomyces laccase	DVIGPQAQVTLANQD	MCF-7 cells (IC_{50} = 1.8 μM) and Hep G2 cells (IC_{50} = 1.7 μM) HIV-1 reverse transcriptase (IC_{50} = 1.25 μM)	(Sun et al., 2012)
Coprinus comatus laccase	AIGPVADLKV	MCF-7 cells (IC_{50} = 4.95 μM) and Hep G2 cells (IC_{50} = 3.46 μM) HIV-1 reverse transcriptase (IC_{50} = 5.85 μM)	(Zhao et al., 2014)
Cordyceps militaris protease	NSTDISLNHG	MCF-7 cells (IC_{50} = 9.3 μM) and 5637 cells (IC_{50} = 8.1 μM)	(Park et al., 2009)
Cordyceps sobolifera protease	AFSTQPGAVCGK	HIV-1 reverse transcriptase (IC_{50} = 8.2 nM)	(Wang et al., 2012)
Ganoderma capense lectin	VNDYEANYGADD	L1210 cells (IC_{50} = 8 μM), M1 cells (IC_{50} = 12.5 μM) and Hep G2 cells (IC_{50} = 16.5 μM)	(Ngai and Ng, 2004b)
Lactarius flavidulus lectin	SGTYTIFNSAFDNSVID	Hep G2 cells (IC_{50} = 8.9 μM) and L1210 cells (IC_{50} = 6.81 μM) HIV-1 reverse transcriptase (IC_{50} = 5.68 nM)	(Wu et al., 2011)
Pholiota adiposa lectin	DILMGTYGML	MCF-7 cells (IC_{50} = 3.2 μM) and Hep G2 cells (IC_{50} = 2.1 μM) HIV-1 reverse transcriptase (IC_{50} = 1.9 μM)	(Zhang et al., 2009)
Pleurotus citrinopileatus lectin	QYSQMAQVME	It inhibited growth of sarcoma S-180 (78.97 %) in mice. HIV-1 reverse transcriptase (IC_{50} = 0.93 μM)	(Li et al., 2008)
Pleurotus ostreatus lectin (40 kDa subunit)	ATAKIKATPAQPQQFQPAALNAAK	It inhibited growth of sarcoma S-180 (88.46 %) and hepatoma H-22 (75.42 %) in mice.	(Wang et al., 2000)
Pleurotus ostreatus lectin (41 kDa subunit)	CATAKCTTATPQQPGCAPAALNAAK		
Pleurotus sajor-caju RNase	DNGEAGRAAR	HepG2 (IC_{50} = 0.22 μM) and L1210 (IC_{50} = 0.1 μM)	(Ngai and Ng, 2004a)
Rhizoctonia bataticola lectin	KKKAYSSRI	It induced apoptosis in Molt-4 cells (33 %) and Jurkat cells (42 %)	(Pujari et al., 2013)
Xylaria hypoxylon protease	HYTELLSQVV	HIV-1 reverse transcriptase (IC_{50} = 8.3 μM)	(Hu et al., 2012)
Hohenbuehelia serotina ribonuclease	TVGGSLAEKGN	MBL2 (IC_{50} = 40.3 μM) and L1210 (IC_{50} = 24.8 μM) HIV-1 reverse transcriptase (IC_{50} = 49.9 μM)	(Zhang et al., 2014b)
Russula paludosa peptide	KREHGQHCEF	HIV-1 reverse transcriptase (IC_{50} = 11 μM)	(Wang et al., 2007)
Hypsin from *Hypsizigus marmoreus*	ITFQGDLDARQQVITNADTRRKRDVRAAVR	*B. cinerea* (IC_{50} = 0.06 μM), *F. oxysporum* (IC_{50} = 14.2 μM), *M. arachidicola* (IC_{50} = 2.7 μM) and *P. piricola* (IC_{50} = 2.5 μM)	(Lam and Ng, 2001b)

Table 3 (continued)

Anti-HIV-1 and antitumor proteins	N-terminal sequence	Target	Reference
Lyophyllin from *Lyophyllum shimeji*	ITFQGASPARQTVITNAITRARADVRAAVSALPTKAPVST	HIV-1 reverse transcriptase (IC_{50} = 5.2 nM)	(Lam and Ng, 2001a)
restrictocin from *Aspergillus restrictus*	ATWTCINQQLNPKTNKWEDK	restrictocin inhibited HIV replication in CEM-GFP cells (ID_{50} = 0.51 μM) MBrl-restrictocin conjugate inhibited protein synthesis in MCF-7 cells (IC_{50} = 8.5–30 nM)	(Orlandi et al., 1988; Rao et al., 2015; Yadav and Batra, 2015)
α-sarcin from *Aspergillus giganteus*	AVTWTCLNDQKNPKTNKYETKRLL	α-sarcin immunotoxin IMTXA33αS on SW1222 cells (IC_{50} = 30 nM) and LIM1215 cells (IC_{50} = 70 nM) It inhibited growth of SW1222 in mice	(Sacco et al., 1983; Tomé-Amat et al., 2015)

drugs, researchers all over the world have dedicated their time and energy to search for antibacterial, antifungal, and antiviral products. There is also an intense effort to locate anticancer products. Fungi represent a source of these products. The plant peptides and proteins described here could be used as important leads in the development of pharmaceutical products or in the production of resistant transgenic plants that could benefit the agriculture business. Among these proteins, the antibacterial fungal defensin plectasin and the anticancer PSP from *C. versicolor* have been most intensively investigated. Recently, plectasin have attracted considerable research interest for its potential as an antibiotic alternative. Much effort has been put in the study on expression and large-scale production (Wan et al., 2016), efficacy of plectasin and its derivatives (Chen et al., 2015b; Xiong et al., 2011) and activity against different clinical resistant strains (Cao et al., 2015; Hu et al., 2015; Zhang et al., 2014a). Pharmacodynamic characterization of NZ 2114 and its pre-clinical tests in mice have shown promising results in its potent activity against multiple drug-resistant strains (Andes et al., 2009). PSP isolated from *C. versicolor* has demonstrated its in vitro antiproliferative activity against tumor cell lines and in vivo antitumor activity. When PSP was given to patients with esophageal cancer, gastric cancer and lung cancer, and those receiving radiotherapy or chemotherapy, symptoms and decline in immune status were attenuated (Ng, 1998). PSP had undergone Phase II and Phase III trials in China. It prolonged 5-year survival and beyond in esophageal cancer patients in double-blind trials. It also improved the quality life, exerted analgesic action, and enhanced immune status in the majority of patients with stomach, esophagus, lung, ovary, and cervix cancers (Kidd, 2000). In fact, some of the currently used drugs are derived from fungi, for instance, mevinolin (lovastatin) from *Aspergillus terreus* which is a hydroxymethlglutaryl CoA reductase inhibitor used to treat hyperlipidemia. Penicillin from *P. chrysogenum* and cephalosporin from the fungus *Acremonium* are used as antibiotics. Griseofulvin, derived from *Penicillium*, is used to treat fungal infections. Cyclosporin A (ciclosporin) from *Tolypocladium inflatum* and gliotoxin from *Gliocladium fimbriatum* are employed as immunosuppressants. Hopefully more drugs will be isolated from fungi in the future and exploited to the welfare of mankind.

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Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare there is no conflict of interest.

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