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MINI-REVIEW

Exploring the bioprospecting and biotechnological potential of white-rot and anaerobic *Neocallimastigomycota* fungi: peptidases, esterases, and lignocellulolytic enzymes

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Abstract Fungi constitute an invaluable natural resource for scientific research, owing to their diversity; they offer a promising alternative for bioprospecting, thus contributing to biotechnological advances. For a long time, extensive information has been exploited and fungal products have been tested as a source of natural compounds. In this context, enzyme production remains a field of interest, since it offers an efficient alternative to the hazardous processes of chemical transformations. Owing to their vast biodiversity and peculiar biochemical characteristics, two fungal categories, white-rot and anaerobic *Neocallimastigomycota*, have gathered considerable attention for biotechnological applications. These fungi are known for their ability to depolymerize complex molecular structures and are used in degradation of lignocellulosic biomass, improvement of animal feed digestibility, biogas and bioethanol production, and various other applications. However, there are only limited reports that describe proteolytic enzymes and esterases in these fungi and their synergistic action with lignocellulolytic enzymes on degradation of complex polymers. Thus, in this minireview, we focus on the importance of these organisms in enzyme technology, their bioprospecting, possibility of integration of their enzyme repertoire, and their prospects for future biotechnological innovation.

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Introduction

Fungi have been a prominent resource in a biotechnological study. Some of the characteristics of fungi, such as diversity, cellular secretion, degradation of complex compounds, and time- and cost-effective cultivation, make them an efficient resource for enzyme technology (Silva et al. 2013; Silva 2017).

Fungi comprise a group of widely distributed organisms in the biosphere. On the basis of their versatile lifestyles, fungi can be classified as saprophytic, pathogenic, and symbiotic with animal, plants, and algae (Mohanta and Bae 2015). Fungi are ubiquitous on earth, owing to their vast ecological diversity. They secrete a vast spectrum of enzymes, including hydrolytic enzymes, such as peptidases, esterases, and glycosidases (cellulases, amylases, xylanases, etc.), and oxidoreductases like laccases, manganese peroxidase, and lignin peroxidase, which are involved in the degradation of biopolymers from plants and animals (Graminha et al. 2008; Copete et al. 2015). This ability of enzyme secretion leads to their extensive use in industrial applications (El-Enshasy 2007).

The potential of two fungal groups—anaerobic and wood-decay fungi—for enzyme production has been explored for research and biotechnological development in the past few years. These fungi are found in a variety of ecological niches, which marks their ability for secretion of enzymes and depolymerization of complex compounds, such as lignin, cellulose, and proteins.

The phylum *Neocallimastigomycota* comprises unique obligate anaerobic fungi, which exist in a symbiotic relationship with herbivores. Their cellular structure is marked by the

absence of mitochondria. Instead, these fungi possess hydrogenosomes, which are involved in the generation of cellular energy under anaerobic conditions (Gruninger et al. 2014). Several studies have been carried out to investigate their biological diversity and applications in biotechnology.

White-rot fungi are associated with wood-decay fungi and have a notable ability to secrete enzymes to degradation of complex chemical compounds like lignin and keratin. These processes find applications in bioremediation of toxic products. They are known to secrete a number of oxidative and hydrolytic enzymes and, thus, serve as a valuable tool for the degradation of recalcitrant substrates.

Earlier studies have demonstrated the biotechnological applications of different species of white-rot and anaerobic fungi, specifically focusing on the pretreatment for degradation of lignocellulosic biomass (Gruninger et al. 2014; Dollhofer et al. 2015; Rouches et al. 2016). Insufficient information, however, is available in the literature regarding their biotechnological prospects, and secretion of proteolytic enzymes and esterases for complementing the degradation of complex compounds.

This gap in knowledge has been focused on in the present review, to obtain a deeper insight into the importance of enzymes like peptidases, esterases, ligninases, and glycosidases in biotechnology. Based on their peculiar biochemical characteristics, the potential of white-rot and anaerobic *Neocallimastigomycota* fungi for enzyme secretion as well as their application prospects in enzymology and other industrial applications will be discussed.

In this review, we discussed several aspects of these two fungal groups, highlighting the biotechnological potential and the challenge of cooperative action of enzymes from these microorganisms.

Anaerobic fungi

Initially identified as protists, these organisms were characterized as obligate anaerobic fungi in the 1970s. Later, they were grouped as an order within the phylum *Chytridiomycota* (Griffith et al. 2010; Liggenstoffer et al. 2010). Although their role is well established in lignocellulosic biomass degradation, a little information is available about their contribution to enzyme technology. Recently, these have been classified under the phylum *Neocallimastigomycota*, which constitutes the order *Neocallimastigales*, family *Neocallimastigaceae*, and consists of eight genera of anaerobic fungi and symbionts in association within the gut of herbivores. The genera within *Neocallimastigaceae* include *Neocallimastix*, *Piromyces*, *Buwchfawromyces*, *Ontomyces*, *Orpinomyces*, *Anaeromyces*, *Caecomyces*, and *Cyllamyces* (Gruninger et al. 2014; Dollhofer et al. 2015).

Inhabiting the gastrointestinal tract of mammalian and reptilian herbivores, these anaerobic fungi are crucial for degradation of plant tissues, based on their ability to secrete of a range of cellulolytic enzymes (Gruninger et al. 2014; Samanta et al. 2008; Saxena et al. 2010). The presence of anaerobic fungi in termites has also been reported (Dollhofer et al. 2015). Plant polysaccharide degradation serves as the carbohydrate source for cellular metabolism of host herbivores.

The degradation of plant biomass by extracellular enzymes promotes the release of free oligo- and monosaccharides. Energy requirement during cellular metabolism is fulfilled by anaerobic fermentation of carbohydrates, including glucose, cellobiose, fructose, maltose, sucrose, and xylose (Nagpal et al. 2009; Dollhofer et al. 2015). The end products of fermentative pathway include acetate, lactate, ethanol, formate, succinate, CO_2, and H_2. These metabolites are useful in the metabolism of host herbivores, and other bacteria and archaea, inhabiting the gastrointestinal tract (Nagpal et al. 2009; Cheng et al. 2013; Gruninger et al. 2014).

Besides hydrogenosomes, these fungi possess rhizoids (filamentous or bulbous) and spores, which are self-propelled by means of flagella (zoospores) (Gruninger et al. 2014). Their capacity for mycelial dispersion by growth of rhizoids is an important parameter to improve the digestion of plant tissues and to facilitate the access of bacteria to the fermentative substrates (Dollhofer et al. 2015).

Extensive efforts have been put in to improve the handling, growth, and storage of these organisms. Besides, genic expression systems, for heterologous expression of enzymes, have also been explored (Gruninger et al. 2014).

Recently, the development of an international culture collection for cryogenic storage of fungal cultures has been proposed. It is believed that this repository would facilitate the exchange of fungal strains between different researchers worldwide (Dollhofer et al. 2015).

White-rot fungi

White-rot fungi, owing to their potential to produce lignocellulolytic enzymes, are valuable resources in enzyme technology. Several studies have revealed their role in the promotion of extensive degradation of lignin, as compared to other organisms. However, despite the mineralization of lignin to CO_2 and H_2O, white-rot fungi are incapable for use as a carbon and energy source. Instead, they obtain energy from metabolism of cellulose (Arora and Sharma 2010).

The phyla *Ascomycota* and *Basidiomycota* include representative species of wood-decaying fungi. Their characteristic name is originally derived from their potential to decay wood and causing rotted wood to appear white or yellow. Their ability to oxidize the recalcitrant lignin substrates signifies their importance in enzyme technology. Lignin degradation

by white-rot fungi is dependent on a ligninolytic complex, comprising of manganese peroxidase, lignin peroxidase, laccase, and enzymes involved in generation of hydrogen peroxide (i.e., glucose oxidase) (Deacon 2006).

Wood decay is caused by some fungal species, based on their ability to degrade lignin. The species that hydrolyze lignin are called as white rot, due to their ability to lighten the color of rotted wood. Brown-rot fungi hydrolyze cellulose and hemicelluloses, but no lignin. Besides, some soft-rot fungi degrade lignin in angiosperm wood. They have an affinity for higher moisture and low lignin-containing biomass (Sánchez 2009).

Among the wood-decay fungi, white-rot fungi are the most well-known organisms and have been constantly explored for mycoremediation, due their capacity for degradation of complex chemical compounds. Fungi including *Phanerochaete chrysosporium*, *Phanerochaete carnosa*, *Coriolus versicolor*, *Trametes versicolor*, *Pleurotus ostreatus*, *Irpex lacteus*, *Phlebiopsis gigantea*, *Ceriporiopsis subvermispora*, and *Dichomitus squalens* are some representative examples of white-rot fungi, which have found applications in biotechnological research (Dashtban et al. 2009; Vaithanomsat et al. 2010; Couturier et al. 2015; Montoya et al. 2015).

Additionally, with regard to the peculiar biochemical properties, wood-decay fungi have been investigated for peptidase and esterase production. The hydrolysis of peptide and ester bond in polymers, especially in plant material, constitutes a valuable complement for wood decaying. The enzymatic arsenal and its cooperativity are crucial for the fungal growth and prospection for industrial application, including improvement of nutritional value in feed crop and biogas production, among others.

Bioprospecting and biotechnological potential

The prospecting of white-rot and anaerobic fungi is briefly discussed, highlighting the possibility of the integrated action of their enzymatic repertoire. We have demonstrated the involvement of peptidases and esterases in combination with lignocellulolytic enzymes to degrade plant biomass. To our knowledge, this is the first in-depth study on the biochemical properties of peptidases and esterases secreted by these fungi and their cooperative hydrolysis in combination with lignocellulolytic enzymes.

Lignocellulolytic enzymes and industrial prospects

Lignocellulose is an important component of plant cell wall, composed of cellulose, hemicellulose, and lignin. Although this complex is recalcitrant, some organisms, from bacteria to arthropods, have the potential to digest it (Scully et al. 2013; Wei et al. 2015).

Cellulose is a linear biopolymer consisting of glucose monomers, linked by β-1,4-glycosidic bonds. Some chains are stabilized by van der Waals forces, resulting in the formation of crystalline structures known as microfibrils. Hemicelluloses are heterogeneous polymers consisting of xylose, arabinose (pentoses), mannose, glucose, galactose (hexoses), and sugar acids. The composition of hemicelluloses depends on the plant source (Dashtban et al. 2010). Lignin is the only naturally synthesized biopolymer with an aromatic backbone. It consists of three precursor aromatic alcohols, including coniferyl, sinapyl, and *p*-coumaryl alcohols, which form the guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) subunits of the lignin molecule, respectively. Upon conjugation, these chains make the structure highly recalcitrant towards degradation and are efficient enough to protect the lignocellulosic complex once linked to cellulose and hemicellulose (Dashtban et al. 2010).

There has been an emerging interest in the use of ligninolytic and cellulolytic enzymes in the biotechnology industry. Lignocellulolytic enzymes are required for a wide spectrum of applications. In particular, the role of cellulases has been established in the degradation of cellulose in fermentable sugars and its conversion to lactic acid, butanol, and ethanol as an alternative energy source to fossil fuels (Montoya et al. 2015).

Ligninolytic enzymes can be used in a number of processes, such as improvement of access to cellulose and hemicellulose in plant biomass, manufacturing of paper pulp (biobleaching), improvement of nutritional value and feed digestibility for ruminants, and waste effluent treatment, among others (Arora and Sharma 2010).

Cellulose is completely hydrolyzed by the activity of endoglucanases (EGs), cellobiohydrolases (CBHs), and β-glucosidases (BGLs) (Cantarel et al. 2009). EGs catalyze the hydrolysis of cellulose chains, releasing cellobiose or bigger polymers. The pH and temperature for maximum EG activity range between 4 and 6 and between 45 and 70 °C, respectively. There are, however, certain exceptions to these conditions (Manavalan et al. 2015). CBHs are monomeric proteins, with molecular weights ranging from 36 to 75 kDa. CBHs catalyze the hydrolysis of cellulose to generate cellobiose. Maximum CGH activity is obtained in the pH range 4–5 at 37–60 °C (Momeni et al. 2013; Rytioja et al. 2014; Manavalan et al. 2015). BGLs, in general, have molecular weights ranging between 35 and 640 kDa and can also exist in trimeric forms. They catalyze the hydrolysis of cellobiose, releasing glucose monomers. The optimum pH for BGL activity ranges between pH 3.5 and 5.5, and the temperatures for maximum activity range from 45 to 75 °C (Manavalan et al. 2015).

Enzymes acting in hemicellulose degradation consist of xylan-degrading endoxylanases, alpha-glucuronidases, beta-xylosidases, acetyl xylan esterases, ferulic acid esterases,

and alpha-l-arabinofuranosidase, and glucomannan-degrading beta-mannosidases and beta-mannanases (Van den Brink and de Vries 2011).

A number of enzymes, including lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases, are involved in complete decomposition of lignin. The white-rot fungi are usually differentiated from other fungal classes by their ability to efficiently degrade lignin.

LiPs are proteins having molecular weights between 30 and 50 kDa. The optimum pH and temperatures for maximum LiP activity range between pH 2 and 5 and between 35 and 55 °C, respectively. Iron ions (Fe^{3+}) act as co-factors for the enzyme and act by mediating the oxidation of veratryl alcohol using H_2O_2, in the active site of LiPs (Manavalan et al. 2015).

MnPs are glycoproteins with molecular weights ranging from 32 to 62.5 kDa and act at an optimum pH of 4–7 and an optimum temperature from 40 to 60 °C. The mechanism of action of MnP is similar to that of LiP, the only difference being the presence of Mn^{2+} as the proton donor in MnP (Manavalan et al. 2015; Kellner et al. 2014).

Laccases are oxidases with molecular weight between 38 and 383 kDa. This enzyme has a tricopper site and is able to catalyze a ring cleavage of aromatic compounds. In general, laccases are *N*-glycosylated and act in a wide variation of pH and temperature conditions. The expression of laccases in *Trametes velutina* was enhanced by the addition of Cu^{2+} and Fe^{2+} ions as well as some aromatic compounds like tannic acid, syringic acid, cinnamic acid, gallic acid, and guaiacol (Yang et al. 2013).

The production of peptidases, esterases, and lignocellulolytic enzymes by white-rot and anaerobic fungi is summarized in Table 1. The synergism of white-rot and anaerobic fungi has a great potential in plant biomass degradation and is an efficient alternative for enzyme technology.

White-rot and anaerobic fungi in lignocellulose degradation

Both anaerobic and wood-decay fungi have an extensive ability to degrade plant tissue. Thus, they serve as promising candidates in investigation of secretion of cellulolytic and ligninolytic enzymes, for obtaining renewable fuel, like bioethanol from cellulose-containing biomass.

In anaerobic fungi, the ability to secrete cellulolytic enzymes has received extensive attention. It was identified that some of these enzymes are acquired by fungi through horizontal gene transfer from bacteria (Griffith et al. 2010). In addition, these enzymes can either be found in a free state within the fungal cell or as a part of cellulosomes, which is a multi-enzyme complex associated with cell wall.

Several studies have revealed the presence of cellulolytic enzymes in the genome of anaerobic fungi (Nicholson et al. 2005; Dollhofer et al. 2015). Transcriptome analysis of *Orpinomyces* sp. strain C1A growing in the presence of lignocellulosic biomass (alfalfa, energy cane, corn stover, and sorghum) revealed the crucial role of cellulosome and free glycoside hydrolases in degradation of plant biomass (Couger et al. 2015).

However, the forage degradation by anaerobic fungi is commonly limited, owing to the presence of recalcitrant lignin. It is estimated that 40–60% of organic carbon remains unused, owing to decreased accessibility of anaerobic fungi to cellulose and hemicellulose embedded within lignin biomass. Several pretreatment strategies, like mechanical, thermal, oxidative, chemical, and ultrasonic technologies, to improve the degradation of plant biomass are reported in the literature (Graminha et al. 2008; Dollhofer et al. 2015).

An additional strategy for lignocellulose degradation is the integration of hydrolytic and ligninolytic enzymes from white-rot fungi. Therefore, the cumulative action of anaerobic and wood-decay fungi results in higher potential for degradation of plant biomass (Arora and Sharma 2010; Gruninger et al. 2014; Dollhofer et al. 2015).

Biofuel production research has recently focused on the potential of white- and brown-rot fungal species to efficiently perform the saccharification of alkaline-pretreated sugarcane bagasse. The enzymes secreted by these fungi were proposed to enhance the efficiency of production of bioethanol from lignocellulosic substrates (Valadares et al. 2016).

Secretomic analysis of *Schizophyllum commune* revealed a unique wood biodegradation system, with higher diversity of carbohydrate-degrading enzymes, as compared to other white-rot fungi *P. chrysosporium*, *C. subvermispora*, and *Gloeophyllum trabeum*. The higher wood-decay activity was contributed by the differential expression of hemicellulases, pectinases, and accessory proteins involved in the generation of hydroxyl radicals (Zhu et al. 2016).

Biogas production

Based on the information from a rumen environment, it has been suggested that the biogas production under reactor conditions is dependent on the microbial interactions and activities to degrade organic waste. The applications of white-rot fungi are also investigated to enhance its ligninolytic activity and production performance (Gruninger et al. 2014).

In rumen, unlike axenic cultures, the interactions between symbiotic organisms, such as bacteria, archaea, and anaerobic fungi, are fundamental to increase the cellulolytic and xylanolytic activity and, ultimately, the biogas production. The anaerobic digestion provides the basic substrate to methanogens during biogas production. The metabolic products, especially acetate and formate, derived from anaerobic fungi are the preferred by methanogens, which carry out

Table 1 Peptidases, esterases, and lignocellulolytic enzymes secreted by some species of strict anaerobic fungi and white-rot fungi

	Enzyme	Major growth substrate	Report summary	References
Anaerobic fungi				
Orpinomyces sp. strain C1A	Glycosidases	Crop biomass: alfalfa, corn stover, and sorghum	Transcriptomic analysis of crop material degradation revealed the induction of glycoside hydrolases	Couger et al. (2015)
Neocallimastix sp. strain L2 *Piromonas communis* P *Neocallimastix patriciarum* C *Sphaeromonas communis* FG10 *Neocallimastix frontalis* RE1	Cellulases	Filter paper (cellulose)	Co-culture with methanogen bacteria improved the cellulose fermentation in anaerobic fungi	Marvin-Sikkema et al. (1990)
Anaeromyces sp.	Xylanase, β-glucosidase, cellobiohydrolase, β-endoglucanase	Cellobiose and microcrystalline cellulose	Using methods of molecular biology, anaerobic fungi *Anaeromyces* sp. were analyzed for species differentiation and for enzyme production	Fliegerová et al. (2002)
Orpinomyces sp.	Cellulase and xylanase	Avicel	Heterologous expression of cellulase and xylanase from anaerobic fungus *Orpinomyces* sp. strain PC-2 using *E. coli* as an expression system	Li et al. (1997)
Orpinomyces sp. *N. frontalis*	Xylanase	Synthetic medium with methanol induction	Heterologous expression of xylanase from *Orpinomyces* sp. and *Neocallimastix frontalis* using yeast as expression systems and assay for bioconversion of xylose to ethanol	Tsai and Huang (2008); Madhavan et al. (2009)
Orpinomyces sp. strain PC-2	Acetyl xylan esterase	Avicel/alfalfa	Investigation of enzymatic complex involved in feed degradation in ruminants	Blum et al. (1999); Lee et al. (2002)
Neocallimastix strain MC-2	Feruloyl esterases	Cellulose	Purification and characterization of two feruloyl esterases secreted by *Neocallimastix* strain MC-2	Borneman et al. (1992)
N. frontalis	Metallopeptidase	Rumen condition	Investigation about peptidolytic rumen microorganisms and improvement of animal feed digestibility	Wallace (1996)
Piromyces sp. *Anaeromyces* sp. *Orpinomyces* sp	Peptidases and esterases	Wheat straw and wheat bran	Esterase and proteolytic activity and improvement of animal feed digestibility	Paul et al. (2004)
White-rot fungi				
Schizophyllum commune *P. chrysosporium* *Ceriporiopsis subvermispora* *Gloeophyllum trabeum*	Glycosidases	Jerusalem artichoke stalk components	Comparative analysis of the secretomes of different wood-decay basidiomycetes under solid-state fermentation revealed a lignocellulose-degrading enzyme complex	Zhu et al. (2016)
Pleurotus ostreatus	Glycosidases	Sugarcane bagasse and carboxymethyl cellulose	Glycoside hydrolases from wood-decay fungi to enhance sugarcane bagasse saccharification pretreatment	Valadares et al. (2016)

Table 1 (continued)

	Enzyme	Major growth substrate	Report summary	References
Trametes velutina	Laccase	Tannic acid, syringic acid, cinnamic acid, gallic acid, and guaiacol	The expression of laccases in *Trametes velutina* was enhanced by the addition of Cu^{2+} and Fe^{2+} ions as well as some aromatic compounds	Yang et al. (2013)
Peniophora sp. *P. ostreatus*	Laccase	Aflatoxin B1 and organochlorine pesticides	Investigation about the capacity of degradation of aflatoxin B1 and organochlorine pesticides by laccases from white-rot fungi	Tekere et al. (2002); Gondim-Tomaz et al. (2005); Kanaly and Hur (2006); Alberts et al. (2009)
Coriolus versicolor *Hypholoma fasciculare* *Stereum hirsutum*	Laccase	Pesticides: diuron, atrazine, and terbuthylazine	Evaluation of the potential for degradation of pesticides, such as diuron, atrazine, and terbuthylazine, with over 86% removal during 42 days of growth	Bending et al. (2002)
P. chrysosporium	Esterases	Coastal Bermuda grass (*Cynodon dactylon* L. Pers.)	Improvement of the forage digestibility and the biodegradation by rumen microorganisms	Akin et al. (1993)
P. carnosa	Acetyl xylan esterase and glucuronoyl esterase	Coniferous and deciduous wood	The transcriptomic responses of *P. carnosa* in growth on coniferous and deciduous wood, the transcript sequences encoded to acetyl xylan esterase and glucuronoyl esterase	MacDonald et al. (2011); Gandla et al. (2015)
P. chrysosporium	Acidic and serine peptidases	Hardwood	Peptidases on the regulation of lignocellulolytic activity when subjected to nitrogen limitation	Dass et al. (1995)
Bjerkandera sp. strain BOS55	Peptidases	Hemp stemwood (*Cannabis sativa* L.)	Protease treatments and improvement of the selectivity of lignin degradation by the white-rot fungus *Bjerkandera* sp. strain BOS55	Dorado et al. (2001)
P. ostreatus	Subtilisin-like peptidase	Liquid basal medium with inorganic salts and yeast extract and potato dextrose	Purification and biochemical characterization of an extracellular subtilisin-like peptidase	Palmieri et al. (2001)
P. chrysosporium *Phlebia radiata*	Acidic, thiol, serine, and metallopeptidases	Corncob	Secretion of peptidases and their influence on ligninolytic activity of white-rot fungi under solid-state condition	Cabaleiro et al. (2002)
P. chrysosporium BW808 (MTCC 787)	Peptidases	Peanut oil, cottonseed oil, almond seed oil, corn, and oat flours	Production and biochemical characterization of two alkaline peptidases secreted by *P. chrysosporium*	Rahul et al. (2013)

interspecies hydrogen transfer via methanogenesis (Gruninger et al. 2014; Dollhofer et al. 2015).

Marvin-Sikkema et al. (1990) reported an increase in the cellulolytic activity of anaerobic fungi growing in association with methanogens, which was marked by a 5–10% enhancement in cellulose fermentation with several anaerobic fungi, such as *Piromonas communis* P, *Neocallimastix patriciarum* C, *Sphaeromonas communis* FG10, and *Neocallimastix frontalis* RE1, and up to 15–25% with *Neocallimastix* sp. strain L2. Other studies also co-related the stimulatory effect of co-culture of anaerobic fungi and methanogens in methane production by degradation of plant biomass (Jin et al. 2011; Cheng et al. 2013).

Although the use of anaerobic fungi in biogas production has been established, its exploitation at the industrial level has not been achieved as yet, possibly due to concerns like decreased enzymatic activity and compromised fungal growth (Dollhofer et al. 2015).

Kazda et al. (2014) reported about the importance of anaerobic fungi in biogas reactors. Molecular techniques by analyzing internal transcribed spacer 1 (ITS1) sequences enabled to detect anaerobic fungi in full-scale biogas plants and in laboratory reactors. However, the compromised fungal growth has been an obstacle for anaerobic cultivation and their visualization in microscopic surveys. Further, the difficulty for maintaining the culture flow contributes to the low level of enzyme activity.

Inhabiting the gastrointestinal tract, anaerobic fungal populations remain in equilibrium due to rumen conditions, such as salivary and digesta flow, and the absorption of metabolites across the rumen epithelium which contributes to maintain the rumen microbial ecosystem (Zhu et al. 1996). In the laboratory, the major difficulty is to simulate these rumen conditions (Zhu et al. 1996; Gruninger et al. 2014). Currently, investigations have focused on the fungal growth in closed batch cultures in which the culture medium is composed of lower substrate concentration than that found in the rumen. Additionally, for maintaining the fungal population, a subculture into a fresh medium can be performed.

Additionally, to address these problems, extensive efforts have been put in to improve the industrial application of these fungi. Heterologous expression offers an alternative for application of lignocellulolytic enzymes in reactors under ideal reaction conditions (Gruninger et al. 2014).

There has been an emerging interest in the use of symbiotic anaerobic fungi in biotechnological applications. To achieve this, some reports have described the heterologous expression of glycosidases, such as xylanases from *Orpinomyces* sp. (Li et al. 2007; Madhavan et al. 2009) and *N. frontalis* (Tsai and Huang 2008). Li et al. (1997) also exhibited the heterologous expression of cellulase and xylanase from anaerobic fungus *Orpinomyces* sp. strain PC-2 using *Escherichia coli* as an expression system, encoding a polypeptide of 471 amino acids for cellulase complementary DNA (cDNA) and a polypeptide of 362 amino acids for xylanase cDNA. In this work, *Orpinomyces* sp. strain grown on Avicel and the glycosidases demonstrated an identity of 80 to 85% to the corresponding enzymes from *N. patriciarum*.

The key role of fungi and methanogen symbionts in biowaste treatment is demonstrated, for example, by the conversion of organic waste to alternative fuel. Household and industrial activities generate a large amount of organic residues annually. Anaerobic digestion of biowaste is an alternative to generation of methane, since it is a biogas sustainable source in comparison to processes like incineration or landfill (Kazda et al. 2014).

In this context, once again, the white-rot fungi show them as valuable candidates for pretreatment of lignocellulosic biomass, integrating lignocellulolytic enzymes, esterases, and peptidases in cumulative action with strict anaerobic fungi.

Esterases and proteolytic enzymes

Different to the reported in other studies (Gruninger et al. 2014; Dollhofer et al. 2015; Rouches et al. 2016), in this minireview, we highlight the biotechnological attention devoted to esterases and peptidases secreted by anaerobic and white-rot fungi and, in particular, to the enzymatic synergisms with lignocellulolytic enzymes on degradation of forage biomass.

In general, peptidases and esterases are involved in fundamental biological processes, including germination, sporulation, and diet of fungi. Their physiological importance and the alternative use in biotechnology fields reinforce the extensive attention devoted to these hydrolytic enzymes (Lawrence 1967; Inácio et al. 2015).

Esterases are hydrolases capable to catalyze the cleavage and formation of ester bonds, and peptidases are enzymes that catalyze the cleavage of peptide bonds into proteins and peptides. The specificity study in proteolytic enzymes is a crucial parameter for a higher understanding of its catalytic preference, in order to provide important information about the degradation of protein substrate (Silva et al. 2014; Silva 2017).

Peptidases are important enzymes involved in the metabolism of all organisms and are also significant in a number of industrial processes. Currently, seven types of proteolytic enzymes are known, including aspartic, cysteine, serine, metallo, glutamic, and threonine peptidases, and non-hydrolytic asparagine peptide lyase (Rawlings et al. 2011; Silva 2017). Fungi are known to secrete a range of proteolytic enzymes, which find applications in a number of processes, such as cheese manufacturing, peptide synthesis, animal waste treatment, pharmaceutical, detergent, and food industries, as well as other aspects of basic research (Silva et al. 2013, 2014, 2016, 2017; Biaggio et al. 2016; Silva 2017).

Fungi have been widely explored for peptidase production (Silva 2017). Recent researches have evaluated the biochemical properties and potential for application of fungal peptidases. For example, Graminho et al. (2013) and Silva et al. (2014) reported the secretion of non-specific serine peptidases, by which they can be prospected for bioactive peptide synthesis and detergent formulation.

On cheese production, peptidases are investigated to promote the cleavage of the peptide bond between phenylalanine (Phe^{105}) and methionine (Met^{106}) on *k*-casein substrate (Silva 2017). The milk clotting is a crucial step for cheese manufacturing. Silva et al. (2016) exhibited an aspartic peptidase with potential to be an alternative enzyme in milk clotting during the preparation of cheese.

Ida et al. (2016) also documented the use of peptidases in detergent formulation. In this study, the washing performance in commercial detergent compatibility of two collagenolytic serine peptidases was evaluated, by which a successful assay for removing an egg protein stain was demonstrated.

In plant degradation, wood nitrogen is especially found in proteins into cell wall, by which wood-decay fungi secrete proteolytic enzymes in order to promote protein hydrolysis and its use as a nitrogen source (Inácio et al. 2015). The nitrogen limitation is known to enhance the secretion of enzymes by white-rot fungi (Dorado et al. 2001). Dass et al. (1995) and Dorado et al. (2001) reported an increase on production of peptidase and ligninolytic enzymes when subjected to nitrogen limitation.

Wood decay fungi, owing to their distinctive biochemical properties, have been explored in detail for peptidase production and regulation of activity of lignocellulolytic enzymes. For instance, production of subtilisin-like peptidase from the white-rot fungal strain *P. ostreatus* has been described (Palmieri et al. 2001). The authors exhibited a monomeric glycoprotein with a molecular mass estimated at 75 kDa. They pointed out the importance of this enzyme on regulation of the laccase activity.

Additionally, secretion of different subclasses of peptidases, including aspartic, cysteine, metallo, and serine peptidases, with varying molecular weights, and different pH and temperature optima for enzymatic activities, has been reported in many species of the genus *Pleurotus* (Inácio et al. 2015). Recently, production of peptidases has also been reported from *Cerrena unicolor*, *Phlebia lindtneri*, and *Pycnoporus sanguineus* (Janusz et al. 2016).

Rahul et al. (2013) also described the production of two alkaline peptidases from *P. chrysosporium*. In this screening, the peptidases exhibited an optimum caseinolytic activity at pH 7.5 and molecular mass estimated at 33 and 40 kDa.

Several investigations have revealed the effect of peptidases on the regulation of lignocellulolytic activity (Silva 2017). A study performed in white-rot *T. versicolor* demonstrated an improvement in laccase and peroxidase activities after inhibition of peptidase activity by phenylmethylsulfonyl fluoride (PMSF) (Staszczak et al. 2000). In this work, the authors suggested an influence of peptidases on the degradation of ligninases.

Peptidases provide amino acids from protein diet in the digestive tract of ruminants. Metallopeptidase from anaerobic *N. frontalis* leads to protein breakdown in rumen. The symbiotic association of *Neocallimastigomycota* fungi within herbivores has indicated the possibility of its use in animal diet (Wallace 1996). Improvement of in vitro digestibility of feed by mixing microflora in buffalo rumen was attained by the proteolytic activity of *Piromyces* sp., *Anaeromyces* sp., and *Orpinomyces* sp. (Paul et al. 2004).

White-rot and anaerobic fungi are known to produce another class of valuable hydrolytic enzymes—the esterases—which are involved in degradation of plant biomass. Studies about esterase activity involved in hydrolysis of the ester bond in plant tissue have been reported as an additional/cooperative enzymatic action in order to complete degradation of forage fiber. Some esterases, like acetyl xylan esterase, have been characterized from anaerobic fungus *Orpinomyces* sp. strain PC-2, which has a molecular mass of 34,845 Da and shows a 56% amino acid homology with the acetyl xylan esterase from *N. patriciarum* (Blum et al. 1999). In the rumen of Hereford bulls fed on an alfalfa-based diet, the presence of several glycosidases, acetyl esterases, and peptidases, as an enzymatic complex involved in feed degradation, has been reported (Lee et al. 2002).

In biochemical evaluation, investigators detected the esterase activity of two feruloyl esterases secreted by *Neocallimastix* strain MC-2, by which they were purified and partially characterized. The enzymes were classified as ferulic acid esterase (FAE) I and FAE II and exhibited a molecular mass at 69 and 24 kDa, respectively (Borneman et al. 1992). Paul et al. (2004) also reported the production of esterases from *Piromyces* sp., *Anaeromyces* sp., and *Orpinomyces* sp. in a study demonstrating the effect of anaerobic fungi on feed degradation. All these documented researches reported that esterases are essential actors on cooperative hydrolysis of ester bonds in complex crop fiber.

In white-rot fungus, MacDonald et al. (2011) related the transcriptomic responses of *P. carnosa* in growth on coniferous and deciduous wood, the transcript sequences encoded to acetyl xylan esterase and glucuronoyl esterase.

In another study with *P. carnosa*, glucuronoyl esterase was reported to be involved in wood degradation. This enzyme mediated the hydrolysis of glucuronoxylan linked to lignin via ester bonds (Gandla et al. 2015). The production of glucuronoyl esterase is also reported in *S. commune* (Spánikova and Biely 2006).

Peptidases and esterases have been implicated in feed processing. The enzymatic repertoire of white-rot and anaerobic fungi has shown a great potential for improvement of feed

digestibility. The integration of esterases and proteolytic enzymes contributes to the degradation of poorly digested feed. The role during degradation of plant materials is shown in Table 2.

Enzyme cooperativity and improvement of feed digestibility in ruminants

The increasing demand for meat consumption has generated an intensive interest in the improvement of animal nutrition. In such cases, anaerobic and white-rot fungi have been explored as possible alternatives to enhance the digestibility of poorly processed plant fibers during digestion in ruminants (Nagpal et al. 2009).

The complex of hydrolytic enzymes secreted from anaerobic fungi is crucial to digestion of plant tissue in the gastrointestinal tract of ruminants. Apart from glycosidases, peptidases and esterases are important for degradation of grasses. Esterases hydrolyze the ester bonds between lignin and hemicellulose to favor the access to cellulose and hemicellulose, and peptidases catalyze the cleavage of peptide bonds in proteins from plant material, providing organic nitrogen and facilitating the penetration of rhizoids of anaerobic fungi (Nagpal et al. 2009; Silva 2017).

Akin et al. (1993) reported the use of *P. chrysosporium* to improve the digestibility of grass cell walls and the biodegradation by rumen microorganisms. In this report, the esterase activity is noticed as a crucial tool for lignocellulosic biodegradation. It has been proposed that the phenolic acid esterases gave ruminal fungi an important advantage over ruminal bacteria.

The extensive interest in anaerobic symbiotic fungi within ruminants is potentially due to the possibility of its application in feed supplementation, especially to improve the nutritional content of cattle feed and to promote the animal growth (Gruninger et al. 2014; Lee et al. 2000; Dey et al. 2004; Paul et al. 2004).

It is estimated that the anaerobic fungi account for about 8–12% of the microbial biomass in rumen. Symbiotic fungi in ruminant can be used as probiotic additives, which serve as a supplement to the animal, owing to their potential to improve the microbial activity in the digestive tract and through promotion of degradation of plant fiber (Nagpal et al. 2009).

Using the anaerobic fungus *Piromyces* sp. in addition to mixed rumen buffalo microflora, Paul et al. (2004) reported an improvement on in vitro feed digestibility of lignocellulosic material (wheat straw and wheat bran, 80:20 *w*/*w*). The experiments indicated a feed digestibility for about 43.64 ± 1.73% using the anaerobic fungus as a feed additive, and a 35.37 ± 1.65% for the control group (without feed additive). Additionally, for a period of 24 h of plant biomass treatment, the fungal inoculum increased the production of carboxymethyl cellulase, xylanase, acetyl esterase, and β-glucosidase. The final conclusion of this study suggests the potential to use *Piromyces* sp. as a feed additive for plant fiber degradation.

In another study, anaerobic fungi were manipulated to improve the utilization of poor-quality feed in sheep (Gordon et al. 2001). The oral dosing with the anaerobic fungus *Piromyces* sp. CS15 was effective in increasing feed intake (straw-based diet), thus contributing to increase the live weight by penned sheep.

An additional tool to improve the degradation of forage material and nutrient value addition to animal diet is the production of ligninolytic and proteolytic enzymes by white-rot fungi. The use of these microorganisms can improve the nutritional yield of plant biomass, for enhancement of the crop fiber degradation and to facilitate the access to cellulose and hemicellulose substrates by the activity of glycoside and peptide hydrolases from anaerobic and wood-decay fungi.

Several studies have revealed an improvement in the digestibility of plant residues in ruminants due to the activity of white-rot fungi. An improvement in the nutritional value of wheat straw, accompanied with an increase of protein content was observed after treatment with *Pleurotus* sp. (Fazaeli et al. 2004). In another study, treatment with the fungus *P. ostreatus* using corn straw as a substrate resulted in an increase in crude protein and soluble carbohydrates after 15 days (Ramirez-Bribiesca et al. 2010). Raghuwanshi et al. (2014) also related the use of the fungus *Ganoderma* spp. to improve the nutritional content of wheat straw residue.

The combination of enzymatic complexes from these fungi is complementarily functional with plant fibers and is involved in reinforcement of the digestion of lignocellulosic material and adding on to the nutritional value in cattle. The enzyme cooperativity of peptidases, esterases, glycosidases, and ligninases is crucial for plant fiber degradation and optimum nutrient assimilation. The synergism of white-rot and anaerobic fungi has a great potential in feed processing and is an efficient alternative for livestock farming.

Future prospects

In the past few years, there has been an emerging interest in white-rot and anaerobic fungi, particularly owing their peculiar biochemical characteristics and ability to secrete enzymes involved in the degradation of complex polymers. These fungi are prominent candidates for applications involving generation of sustainable energy like bioethanol and biogas, and improvement of feed digestibility. In the future, technological advances are expected to improve their handling, storage, and biodiversity prospecting, providing new information on the development of new biotechnological products to contribute to the scientific innovation and development.

Table 2 Role during degradation of plant materials: peptidases, esterases, and cellulases

Enzymes	Enzyme class	Enzymatic catalysis/ specific substrate	Catalytic types	Role during degradation of plant materials	References
Peptidases from anaerobic fungi and white-rot fungi	Hydrolases E.C. 3.4	Catalyze the cleavage of peptide bond into peptide and protein	Aspartic, cysteine, glutamic, metallo, serine, and threonine peptidases	Peptidases catalyze the cleavage of peptide bonds in proteins from plant material, providing organic nitrogen and facilitating the fungal penetration	Wallace (1996); Palmieri et al. (2001); Paul et al. (2004); Inácio et al. (2015); Silva (2017)
Esterases from anaerobic fungi and white-rot fungi	Hydrolases E.C. 3.1	Catalyze the cleavage and formation of ester bonds	Acetyl xylan esterase, feruloyl esterase, and glucuronoyl esterase	Esterases hydrolyze the ester bonds between lignin and hemicellulose to favor the access to cellulose and hemicellulose	Borneman et al. (1992); Blum et al. (1999); Lee et al. (2002); MacDonald et al. (2011)
Cellulolytic complex from anaerobic fungi	Hydrolases E.C. 3.2	Catalyze the hydrolysis of β-1,4-glycosidic bonds into cellulose chains; free enzymes and integrated to the cellulosome	Free enzymes: endoglucanases, cellobiohydrolases, and β-glucosidases; cellulosome complex: a multi-enzyme complex associated with cell wall	Cellulose degradation: endoglucanases catalyze the hydrolysis of cellulose chains, releasing cellobiose or bigger polymers. Cellobiohydrolases catalyze the hydrolysis of cellulose to generate cellobiose. β-glucosidases catalyze the hydrolysis of cellobiose, releasing glucose monomers	Li et al. (1997); Paul et al. (2004); Couger et al. (2015); Dollhofer et al. (2015)
Cellulolytic complex from white-rot fungi	Hydrolases E.C. 3.2	Catalyze the hydrolysis of β-1,4-glycosidic bonds into cellulose chains; free enzymes	Free enzymes: endoglucanases, cellobiohydrolases, and β-glucosidases	Cellulose degradation: endoglucanases catalyze the hydrolysis of cellulose chains, releasing cellobiose or bigger polymers Cellobiohydrolases catalyze the hydrolysis of cellulose to generate cellobiose. β-Glucosidases catalyze the hydrolysis of cellobiose, releasing glucose monomers	Howard et al. (2003); Hori et al. (2013); Zhu et al. (2016)
Cellulolytic complex from bacteria	Hydrolases E.C. 3.2	Catalyze the hydrolysis of β-1,4-glycosidic bonds into cellulose chains; anaerobic bacteria possess free enzymes and integrated to the cellulosome	Free enzymes: endoglucanases, cellobiohydrolases, and β-glucosidases; cellulosome complex is found in anaerobic bacteria, such as *Clostridium*, *Acetivibrio*, *Bacteroides*, and *Ruminococcus*. In comparison to the fungal cellulosome, the bacterial cellulosomal enzymes differ in amino acid sequences	Cellulose degradation: endoglucanases catalyze the hydrolysis of cellulose chains, releasing cellobiose or bigger polymers Cellobiohydrolases catalyze the hydrolysis of cellulose to generate cellobiose. β-glucosidases catalyze the hydrolysis of cellobiose, releasing glucose monomers	Doi et al. (2003); Vodovnik and Logar (2010); Wilson (2011); Kuhad et al. (2011); Wilson and Kostylev (2012)

Compliance with ethical standards

Ethical approval In this article, we did not perform any studies with human participants or animals.

Conflict of interest The authors declare that they have no conflict of interest.

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