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*Review*

# Lignocellulolytic enzymes from tropical fungi: Types, substrates and applications

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**Lignocellulolytic enzymes constitute a large group of mainly extracellular proteins including ligninolytic enzymes (peroxidases and oxidases) and hydrolytic enzymes (cellulases, hemicellulases, pectinases, chitinases, amylases, proteases, esterases, and mannases). These enzymes have attracted a wide range of industrial and environmental applications including pulping, de-inking, decolourization and detoxification of textile wastes, wastewater treatment and bioremediation of polluted soils. This work gives an updated review and summarizes the main fungal strains, substrates, lignocellulosic enzymes and their applications with an emphasis on tropical fungi. The main groups of lignocellulolytic enzymes and their applications are summarized in a manner that provides a useful reference for both enzyme scientists and technologists. Moreover, current debates and significant gaps in the lignocellulolytic fungal enzymes' research have been highlighted. Future prospects in lignocellulolytic enzymes research are directed towards bioprospecting of robust novel fungal enzymes to overcome the challenge of recalcitrant substrates, physiological regulations of enzymes, use of novel inducers to enhance production, use of multiple fungal strains or mixture of enzymes, gene cloning to screen for new generation of enzymes and nanobiotechnologal applications in enzymology. This review revealed that for the past seven years, lignocellulolytic enzymes research and development have been steadily advancing from cellular to molecular; and from micro- to nano- levels, and more efforts should be directed to new research frontiers including molecular cloning, sequencing and functional genomics.**

**Key words:** Biodegradation, lignocellulolytic enzymes activities, liquid culture, nanobiotechnology, solid state fermentation.

## INTRODUCTION

Lignocellulolytic fungi are often divided into three groups namely white rots, brown rots, and soft rots. White rots break down lignin and cellulose and commonly cause rotted wood to feel moist, soft, spongy, or stringy and appear white or yellow; brown rots primarily decay the cellulose and hemicellulose (carbohydrates) in wood, leaving behind the brownish wood lignin. They decay cellulose, hemicellulose, and lignin but only in areas directly adjacent to their growth. Soft rots grow more slowly than brown and white rots and usually do not cause extensive structural damage to wood of living trees (Hickman and Perry, 2010). Most of the lignocellulolytic fungi secrete extracellular enzymes released in the presence or absence of inducers in the media (Mtui and Nakamura, 2007; Patrick et al., 2010, 2011). This study reviewed recent literature on tropical lignocellulolytic fungi, focusing on their extracellular lignocellulolytic enzymes, substrates and applications. The tropical fungi were targeted because they have enjoyed wider coverage due to their diverse diversity and resilience and, therefore, they are expected to possess more novel metabolites compared to their temperate counterparts.

Lignocellulolytic enzymes are biocatalysts that are responsible for degradation of lignin and cellulosic materials. Ligninolytic enzymes catalyze the breakdown of lignin model compounds; and they fall in two main groups: peroxidases and oxidases. Peroxidases are enzymes which use hydrogen peroxidase ($H_2O_2$) as co-

substrates. Most of them are heme proteins with an exceptional broad substrate spectrum that include organic and inorganic compounds. They catalyze oxidations resulting in the formation of free radicals (e.g. phenoxyl and aryl cation radicals), reactive cations, (e.g. $Mn^{3+}$), or anions (e.g. $OCl^-$) which are involved in destruction of lignin and humic substances, the oxidation of toxic compounds and nonspecific defense reactions (Hofrichter and Ullrich, 2010).

Oxidases are responsible for oxidative conversions of organic compounds and materials. Examples of such biocatalysts are glucose oxidases from *Aspergillus niger* (Wong et al., 2008). Phenol oxidases need dioxygen ($O_2$) as co-factor (terminal electron acceptor). Because of their stability, most of them are extracellular or cell-wall associated proteins. Examples of oxidases are laccases and tyrosinases. They contain copper in their active sites and are produced by numerous fungi (Hofrichter and Ullrich, 2010). Laccases belong to blue copper oxidases; they are polyphenol oxidases (para-benzene:diol:dioxygen oxidoreductase) that contain four copper ions in the active site. Laccases catalyze one-electron oxidations of various substrates preferably of phenolic and aromatic amines while transferring the abstracted electrons to dioxygen, which is thereby reduced to water (Sakurai and Kataoka, 2007).

On the other hand, hydrolases are enzymes that catalyze hydrolysis of chemical bonds, and are classified based on the bonds they act upon. Cellulases are hydrolases that catalyze cellulolytic reactions. They can be endoglucanases, endo-1,4-*beta*-glucanase, carboxymethyl cellulase (CMCase), endo-1,4-beta-D-glucanase, *beta*-1,4-glucanase, *beta*-1,4-endoglucan hydrolase, cellobiohydrolases (CBH) and celludextrinase; or exogluconases, exocellulases, *beta*-glucosidases and other groups. Hemicellulases (xylanases and *beta*-D-xylosidases) are enzymes that act on hemicellulose, a polymer of pentose sugars. Other types of hydrolases include pectinases, esterases, chitinases, nucleases, phosphodiesterases, lipases, phosphatases, DNA glycosylases, glycoside hydrolases, Proteases/peptidases, helicases and GTPases (Muthuvelayudham and Viruthagiri, 2006). Fungal lignocellulolytic enzymes are important commercial bio-products used in many industrial and environmental applications including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper, agriculture and bioremediation (Mtui, 2009).

## FUNGAL LIGNINOLYTIC ENZYMES

There has been increasing interest in fungal ligninolytic enzymes research due to their enormous applications, especially due to their potential to degrade a wide range of lignocellulosic biomass including recalcitrant and the highly toxic phenolic compounds. The main groups of fungal ligninolytic enzymes (ligninases) are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac). Among the ligninases, Lac are the mostly studied (Couto et al., 2006; Couto and Sanromána, 2006; Mishra and Kumar, 2007; Alcántara et al., 2007; Minussi et al., 2007a,b); followed by MnP and LiP (Couto and Sanromána, 2005; Alam et al., 2005; Asgher et al., 2006; Songulashvili et al., 2007; Elisashvili et al., 2008a,b).

### Improved fungal enzyme production

Different types of fungi have been studied for their improved extracellular ligninolytic enzyme production. A white-rot fungus, *Trametes trogii,* screened for crude ligninolytic enzymes production using Rhemazol Brilliant blue R (RBBR) dye, 2, 2-azino-bis (3-ethylbenzthiazoline)-6-sulfonate (ABTS) and guaiacol in a semi-solid medium, produces LiP, MnP and Lac. Optimal temperature, pH, carbon, nitrogen, $Cu^{2+}$, 2, 5-xylidine, ferulic acid, varatryl alcohol and $Mn^{2+}$ in submerged culture fermentations facilitate maximum enzymes production. Very high enzyme activities (31,786 U/L) have been reported when the experiments are carried out under optimal conditions (pH 5.5 to 6.0 at 30 to 45°C) (Rosales et al., 2007; Patrick et al., 2009, 2010, 2011). Recovery of pure enzymes is achieved through chromatographical purification techniques (Mtui and Nakamura, 2008). Several efforts have been made to increase the production of enzymes through strain improvement by mutagenesis and recombinant DNA technology. Cloning and sequencing of the various genes of interest could economize the enzymes production processes (Kumar et al., 2008).

### Enzymatic bioprocessing

There are promising developments in the application of solid-state fermentation (SSF) in enzymatic bioprocessing of lignocellulosic biomass. Due to their ability to produce ligninolytic extracellular enzymes, white rot fungi are the main degraders of lignin constituents of wood, and therefore they are potential asset to biopulping processes (Singh et al., 2011). Selective SSF degradation of pulp by tropical basidiomycetes (*Pycnoporus coccineus* and *Coriolus versicolor*) produces ligninolytic enzymes (including MnP) with activities as high as 275 U/mL in liquid cultures under stationary conditions. The biodegraded *Acacia mangium* wood chips show up to 10% lignin loss (Husaini et al., 2011), while strains of *Phellinu*s sp., *Daedalea* sp., *T. versicolor* and *P. coccineus* show up to 30% delignification after 60 days of incubation (Liew et al., 2011).

Ligninolytic enzymes are very useful in biodegradation of synthetic dyes including biobleaching of colored effluents from pulp and paper and textile industries, de-

**Table 1.** An overview of ligninolytic fungi, their substrates, enzymes and applications.

| Fungal strain | Substrate | Enzyme | Application | Reference |
|---|---|---|---|---|
| *Aspergillus niger* | Petroleum hydrocarbons, PKC | MnP, LiP, Lac | Bioremediation, improved animal feed | Perez-Armendariz et al., 2010; Lawal et al., 2010. |
| *Bjerkandera adusta* | Phenolic compounds | LiP MnP, Lac | Bioremediation; improved animal feed | Rodrigues et al., 2008; Tripathi et al., 2011. |
| *Cerrena unicolor*. | Paper sludge | Lac, LiP, MnP | De-inking | Winquist et al., 2008. |
| *Cladosporium cladosporioides* | Petroleum hydrocarbons | MnP, LiP, Lac | Bioremediation | Perez-Armendariz et al., 2010. |
| *Coriolus versicolor* | Wood residues | Lip, MnP | Biopulping | Liew et al., 2011; |
| *Crepidotus variabilis* | Synthetic dyes | Lac, LiP, MnP | Bioremediation, decoloration | Mtui and Nakamura, 2007, 2008. |
| *Flavodon flavus* | Textile wastewater | Lac, LiP, MnP | Bioremediation, decoloration | Raghukumar et al, 2006; Mtui and Nakamura, 2008. |
| *Fomes fomentarius* | PCPs | LiP, MnP, Lac | Bioremediation; Improved animal feed | Rodrigues et al., 2008; Ramesh et al., 2009. |
| *Fusarium oxysporum* | PAHs | Lip, MnP, Lac | Bioremediation | Silva et al., 2009. |
| *Ganoderma lucidum, G. applanatum* | Rice straw, PAHs | Lac | Bioremediation, animal feed | Punnapayak et al., 2009. |
| *Irpex lacteus* | Corn stover | Lac, Lip | Improved animal feed | Xu et al., 2009. |
| *Laetioporus sulphureus* | Synthetic wastewater | Lip, MnP, Lac | *Laetioporus sulphureus* | Mtui and Masalu, 2008. |
| *Lentinus squarrosulus* | Nitrophenolic compounds | MnP, Aryl oxidases. | bioremediation | Tripathi et al., 2011. |
| *Lentinus c*rinitus, L. *subnudus* | Wood residues, maize husks | LiP, MnP, Lac | Decolorization. Improved animal feed | Niebisch et al,. 2010); Jonathan et al., 2010. |
| *Mucor mucedo* | Palm kernel cake | MnP, LiP, | Improved animal feed | Lawal et al., 2010. |
| *Paecilomyces farinosus* | Olive meal waste | LiP, MnP, Lac | Detoxification of phenolics | Sampedro et al., 2009. |
| *Penicillium glabrum* | Petroleum hydrocarbons | MnP, LiP, Lac | bioremediation | Perez-Armendariz et al., 2010. |
| *Phanerochaete chrysosporium* | Wastewater, rice straw agro-chemicals | LiP, MnP, Lac | Bioremediation, Improved animal feed | Lu et al., 2009; Sharma and Arora, 2010, 2011. |
| *Phlebia lindtneri*, *P. brevispora, P. Rufa, P. chrysocreus* | Agro-chemicals | Mn, LiP, Lac | Bioremediation, Improved animal feed | Mtui & Nakamura, 2007; Xiao et al., 2011. |
| *Pleurotus ostreatus, P. pulmonarius, P. ostreatus, P. flabellatus* | Agro-chemicals; PCPs, Rice straw | LiP, Lac, MnP | Bioremediation mineralization, improved animal feed | Magan et al., 2010; Purnomo et al. 2010; Singh et al., 2011. |
| *Pycnoporus coccineus* | Wood residues | LiP, MnP, Lac | Biopulping | Singh et al., 2010 |
| *Rhizopus stononifer* | Palm kernel cake (PKC) | MnP, LiP | Improved animal feed | Lawal et al., 2010. |
| *Trametes trogii* *T. versicolor* | Wheat straw, pulp | MnP, LiP, Lac | Decolorization; improved animal feed, biopulping | Rosales et al. 2007; Patrick et al., 2009 Lawal et al., 2010; Singh et al., 2010.. |
| *Trematosphaeria mangrovei* | Sea water | LiP, MnP, Lac | Bioremediation | Mabrouk et al., 2010. |
| *Trichocladium canadense* | PAHs | LiP, MnP, Lac | Biodegradation of wastewater | Silva et al., 2009. |

inking of recycled paper and decolorization of wastewater (Table 1). Tropical fungal strains (*Trichaptum* sp., *Datronia* sp and *Trametes* sp.) from decomposed woods can grow on PDA agar containing synthetic lignin medium, and are efficient (up to 55%) decolourizers of pulp and paper effluent (Apiwatanapiwat et al., 2006).

Also, low molecular weight Lac (41 kDA) from a novel fungal isolate of *Lentinus* crinitus displaying good tolerance to a wide range of pH values, temperature and salt concentrations, is able to biodegrade reactive blue 220 (RB220) dye up to 95% of its original color (Niebisch et al., 2010). When waste industrial materials are used as substrates for white-rot fungi in solid-state fermentation, MnP, LiP and Lac are produced from *Trametes versicola* and *Cerrena unicolor*. These enzymes have potential for de-inking of paper sludge (Winquist et al., 2008). In addition, twelve white rot fungi isolates from Malaysia screened in solid media plates utilizing Poly R 478 and ABTS show positive decolorization and ABTS oxidation. In liquid culture media, the strains exhibit elevated levels (up to 1230 U/L) of LiP, MnP and Lac activities (Ruqayyah et al., 2011).

## Enzyme-mediated pollution control

Biodegradation of recalcitrant compounds by ligninolytic enzymes has been extensively studied. Ligninolytic enzymes from wood chips-immobilized *Phenerochaete chrysosporium* are efficient biodegraders of wastewater from a coke plant. After only 3 days of incubation, about 84% phenolic compounds and 80% COD are removed from the wastewater at pH and temperature ranges of 4-6 and 28 to 37°C, respectively (Lu et al., 2009). Moreover, laccases and peroxidases from white –rot fungi such as *Trametes* sp*., Pleurotus* sp., *Polystictus* sp. and *P. chrysosporiun* are also able to degrade a wide range of xenobiotic compounds including agricultural chemicals (pesticides and herbicides) such as Dieldrin, Simazine, DDT, Picloram, Triazines, Trifluralin, Diuron, Diazinon MCPA, Parathion and 2,4-D (Magan et al., 2010). Spent lignocellulosic substrates after *Pleurotus pulmonarius* cultivation are useful for the treatment of wastewater containing broad spectrum, non-systemic organochloride fungicide called Chlorothalonil (Tetrachloroisophthalonitrile). It has been shown that *P. pulmonarius* enzymes could remove up to 100% of the fungicide after 45 minutes of reaction (Juarez et al., 2011). Mineralization of 2- nitrophenol (2-NP), 3-nitrophenol (3-NP), 4- nitrophenol (4-NP) by white rot fungi *Bjerkandera adusta* and *Lentinus squarrosulus* in liquid cultures has been investigated by Tripathi et al. (2011). Both strains are able to completely remove 2-NP and 3-NP but fail to mineralize 4-NP. The main enzymes involved are aryl alcohol oxidase from *B. adusta* and manganese peroxidase from *L. squarrosulus*.

The ability of ligninolytic enzymes from *Pleurotus ostreatus* waste to degrade 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) has been investigated by Purnomo et al. (2010). Up to 80% degradation and 8% mineralization of DDT-contaminated soils was achieved after 5 days of incubation. In addition, trans-chlordane has been metabolized with wood-rotting fungi (*Phlebia lindtneri*, *P. brevispora* and *P. aurea*. Over 50% of trans-chlordane was metabolized after 42 days of incubation (Xiao et al., 2011). Laccase from a tropical white-rot fungus (*Ganoderma lucidum*) subjected to 16 types of polycyclic aromatic hydrocarbons (PAHs) shows high degradability potential in the absence of a redox mediator (Punnapayak et al., 2009). Soil fungi including *Trichocladium canadense*, *Fusarium oxysporum*, *Aspergillus* sp., *Verticillium* sp. and *Achremonium* sp. produce ligninolytic enzymes such as LiP, MnP and Lac that are able to extensively degrade a wide range of recalcitrant organic pollutants including a broad range of polycyclic aromatic hydrocarbons (PAHs) under low oxygen conditions (Silva et al., 2009). Pentachlorophenol (PCP), used as wood preservative and as a pesticide, is biodegradable by *Pleurotus pulmonarius* in submerged cultures.

The enzyme involved is Lac, which removes up 90% of PCP. For bioremediation purposes, the fungus must be cultured under conditions of active Lac production (Faradi de Souza et al., 2011).The relation between Lac production and PCP degradation shows the potentiality of white rot fungi in bioremediation of aqueous effluents, where polymerized products could be readily removed in 30 days of incubation with white rot isolates like *Fomes fomentarius*, *Pleorotus. ostreatus* and *T. versicolor*. Lac production (0.14±003 U/mL) and PCP degradation (91.94±1.34% to 100±00%) have been achieved over a period of 30 days of incubation in static cultures (Ramesh et al., 2009). Enzymes from soil fungi such as ligninolytic filamentous *Aspergillus niger*, *Penicillium glabrum* and *Cladosporium cladosporioides* are capable of removing petroleum hydrocarbons from polluted soils (Perez-Armendariz et al., 2010).

## Marine fungal ligninolytic enzymes

Tropical marine fungi are considered to be more robust in degrading ligninolytic compounds than their terrestrial counterparts, mainly because they are adapted to harsh environments such as high levels of pH and salt concentrations (Mtui and Nakamura, 2008). Lignin degrading enzymes (LDEs), preferably LiP MnP and Lac from marine fungi including *Flavodon flavus*, *Crepidotus variabilis*, *Laetioporus sulphureus* and *Phlebia chrysocreus*, have been shown to completely biodegrade colored textile, pulp and dye effluents and other recalcitrant substrates (Raghukumar et al., 2006; Mtui and Nakamura 2007, 2008; Mtui 2008). Lignin degrading enzyme activities of marine ascomycetes isolated off the Egyptian coast shows that marine fungus *Trematosphaeria mangrovei* produces lignin-degrading laccase, LiP and MnP, activities which are averaged at 14.03 U/mL when grown on low nitrogen medium in half-strength seawater (Mabrouk et al., 2010). The marine environment remains grossly unexploited as far as ligninolytic enzymes research is concerned. More work should focus on bioprospecting of more fungi and

characterization of their extracellular enzymes of biotechnological importance.

### Enzymatic improvement of lignocellulosic biomass for animal feed

Enzymatic delignification is an efficient method to improve animal feed. Solid-state fermentations of wheat straw with white-rot fungi (*P. ostreatus* and *T. versicolor*) lead to fermented biomass termed “myco-straw” which is richer in crude protein and metabolized energy for animal feed. The myco-enzymes involved are Lacs and MnPs (Shrivastava et al., 2011). Wheat straw cell wall modifications by white-rot fungi (*Trametes versicolor*, *Bjerkandera adusta*, *Ganoderma applanatum* and *Phlebia rufa*) in liquid and solid cultures are used to improve animal feed quality. Ligninolytic enzymes (Lac LiP and MnP); and cellulolytic enzymes including carboxymethyl cellulase (CMcase), avicelase, xylanase and feruloyl esterase are involved in oxidative and hydrolytic reactions, respectively (Dinis et al., 2010). In determination of lignocellulosic degradation profiles of *Pleurotus flabellatus* in rice straw, up to 29% cellulose, hemicellulose, and lignin degradation are achievable with 30 days of inoculation. At the same time, LiP, MnP and Lac ligninolytic activities of 3.2 U/L, 8.2 U/L and 8.4 U/L, respectively, were observed (Singh et al., 2011). Biodetoxification of dry olive mill residues (DOR) by *Paecilomyces farinosus* enzymes has shown complete phytotoxic compounds removal and water-soluble phenolic compounds reduction (45%) and, therefore, the residues can be used as animal feed (Sampedro et al., 2009).

In organic matter digestibility studies, biodegradation of maize husks by enzymes from *in vitro* cultures of *Pleurotus tuber-regium* and *Lentinus subnudus* results to significant reduction of dry matter and crude fibre fractions; increase in crude protein and ash; and production of methane gas (Jonathan et al., 2010). *Irpex lacteus* has been shown to produce both ligninolytic and cellulolytic enzymes while improving corn stover quality under solid culture conditions. Degradations of up to 80, 75 and 63% lignin, cellulose and hemicellulose, respectively, have been achieved after 120 days of incubation (Xu et al., 2009). Another studies show that *Phanerochaete chrysosporium* is able to selectively degrade lignin and enhance *in vitro* digestibility of paddy straw (Sharma and Arora, 2010, 2011). In addition, enzyme extracts from *in vitro* cultures of *T. versicolor*, *Bjekandera adusta* and *Fomes fomentarius* have been used to degrade cell wall components of wheat straw for improved digestibility and nutritive value (Rodrigues et al., 2008). Furthermore, polysaccharidases enzyme complex from *Aspergillus niger*, *Trichoderma viride*, *Rhizopus stononifer* and *Mucor mucedo* utilizing palm kernel cake (PKC) as a substrate have been shown to degrade the PKC and thereby improving its digestibility and nutrients (soluble sugars, crude proteins) content to become an excellent feed for chicken (Lawal et al., 2010).

## FUNGAL CELLULOLYTIC ENZYMES

Fungal cellulolytic enzymes are a group of hydrolytic enzymes responsible for cellulolytic and xylanolytic activities. They are mostly extracellular enzymes produced by a wide range of fungi. Such enzymes include cellulases, hemicellulases, pectinases, chitinases, amylases, proteases, phytases and mannases.

### Cellulases and hemicellulases

Fungal cellulases production from plant biomass feedstocks has been extensively studied (Wen et al., 2005; Muthuvelayudham and Viruthagiri, 2006; Pothiraj et al., 2006; Valascova and Baldrian, 2006; Daroit et al., 2007; Gao et al., 2008). On the other hand, fungal hemicellulolytic enzymes, mainly xylanases, are produced from a wide range of substrates (Haq et al., 2006; Elisashvili et al., 2006; Dobrev et al., 2007; Mohana et al., 2008). Production of cellulases and xylanases by *Penicillium echinulatum* using sugarcane bagasse has been studied and appreciable cellulolytic and hemicellulolytic enzyme activities were observed (Camassola et al., 2009). Biological pretreatment using other fungi (e.g *Pleurotus sajor caju*) did not have much effect on enzymatic activities. Highest production of cellulases (*beta*-glucosidase and FPase) and xylanases from *Daldinia caldariorum* in liquid culture is achieved maximally at 35°C and pH 5. Major enzymes involved were determined by using N-terminal sequencing, and *D. caldariorum* has been demonstrated to be a promising fungus for biodegradation of lignocellulosic materials to produce biofuels (Ng et al., 2010). A study done on paddy straw solid state fermentation by *Phlebia brevispora*, *P. fascicularia*, *P. floridensis*, *P. radiata* and *Phanerochaete chrysosporium* examined *in vitro* digestibility of rice straw and measured changes in biochemical constituents such as hemicellulose, cellulose, total organic matter over a period of 60 days. Enzyme catalysts were mainly xlylanase and carboxymethyl cellulase. It has been observed that the fungi degraded the straw and improved *in vitro* digestibility. It was further noted that geographical locations (in India) affect biochemical constituents and fungal degradation of rice straw fibers (Sharma and Arora, 2011).

### Cost-efficient substrates for production of hydrolytic enzymes

High prices of refined substrates (cellulose, hemicellulose

**Table 2.** Examples of hydrolytic fungi, their substrates, enzymes and applications.

| Fungal strain | Substrate | Enzyme | Application | Reference |
|---|---|---|---|---|
| *Aspergillus niger, A. japonicus, A. terreus* | Municipal solid waste, rice straw | CMcases, *beta*-glucosidases, xylanases | Wastewater treatment, animal feed. | Gautam et al., 2011; Jahromi et al., 2011. |
| *Chaetomium* sp | Agro- and industrial wastes | *Beta*-endoglucanase, *Beta*-exoglucanase *beta*-glucosidase | Sachharification, Improved animal feed | Ravindran et al., 2010. |
| *Daldinia caldariorum* | Crop residues | *Beta*-glucosidase, FPase, xylanases | Bioremediation | Camassola and Dilon, 2009; Ng et al., 2010. |
| *Emericela variecolor* | Castor bean waste | CMcases, *beta*-glucosidases | Saccharification | Herkulano et al., 2011. |
| *Grifola frondosa* | Yellow passion fruit waste | Pectinases, aryl-*beta*-D- glucosidases | Fruit industries | Zilly et al., 2011. |
| *Lentinus edodes* | Crop residues | CMcase, xylanase | Saccharification, improved animal feed | Elisashvili et al., 2006, 2008a, 2008b. |
| *Macrocybe titans* | wheat bran | *Beta*-xylosidases, *beta*-galactosidases | Improved animal feed | Zilly et al, 2011. |
| *Melanocarpus albomyces* | Wheat straw, corn husk | xylanase | Sachharification | Biswas et al., 2010. |
| *Neurospora sitophila* | Banan fruit stalks | Endoglucanase, exoglucanase, *beta*-glucosidases | Saccharification, brewing industry | Asad et al., 2006. |
| *Nigrospora* sp. | Fruit waste | Chitinases | Antimicrobial constituent, food preservatives | El Hadrami et al., 2010. |
| *Penicillium echinulatum* | Sugarcane bagasse | Cellulases, xylanases | Improved animal feed | Camassola and Dilon 2009. |
| *Phanerochaete chrysosporium* | paddy straw | Xlylanase, carboxymethyl cellulase | Improved animal feed, saccharification | Sharma and Arora, 2011. |
| *Phlebia brevispora*, *P. fascicularia*, *P. floridensis*, *P. radiata* | Paddy straw | Xlylanase, carboxymethyl cellulase | Improved animal feed, saccharification | Sharma and Arora, 2011. |
| *Penicillium janthinellum* | Crop residues | Proteases | Detergent industry | Oliveira et al. 2006. |
| *Piptoporus betulinus* | Wheat straw | Endo-1,4 *beta*-glucanase, xylanase, mannanase. | Glucose and xylose production | Valascova and Baldrian, 2006. |
| *Poria subvermispora* | Wheat and paddy straw. | Cellulases, xylanases | Glucose production | Salvachua et al., 2011. |
| *Rhizopus microsporus* var. *rhizopodiformis* | Sugar cane bagasse, corn cob, lemon peel | Endo- and exo-polygalacturonase, (pectinases) | Saccharification, beverage industry | Silva et al., 2005; Botella et al., 2007; Damasio et al., 2011. |
| *Rhodotorula glutinis* | Castor bean waste | Cellobiohydrolases, CMcases and *beta*-glucosidases | Brewing industry | Herkulano et al., 2011. |
| *Trichoderma viride* *T. reesei* | Municipal solid waste | Cellulases, invertase, pectinase, tannase | Wastewater treatment | Rodríguez and Ma, 2005; Gautam et al., 2011. |

and others) are the limiting factor in the economics of enzyme production. In addition, refined substrates could as well be used directly as food or feed. Therefore, in order to reduce costs of hydrolases production, inexpensive alternative carbon and nitrogen sources such as wood residues (sawdust and paper mill discards), grasses, waste paper, agricultural residues (straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse), domestic wastes (lignocellulosic garbage and sewage), food industry residues and municipal solid wastes are used to produce hydrolytic enzymes (Mtui, 2009) (Table 2). Municipal solid waste and liquid wastes have been used as substrates to isolate two novel cellulase-producing fungi (*Aspergillus niger* and *Trichoderma* sp.). Municipal solid waste residue (4 to 5% w/v) and peptone and yeast extract (1.0% w/v) seem to be a good combination of carbon and nitrogen sources for the production of cellulase by *A. niger* and *Trichoderma* sp. Optimum temperature and pH of the medium for the cellulase production by *A. niger* are 40°C and 6-7, whereas those for the production of cellulases by *Trichoderma* sp. are 45°C and 6.5. Municipal solid waste residues provide an economical advantage as a solid substrate as well as a carbon source for production of cellulase by using *A. niger* and *Trichoderma* sp. (Gautam et al., 2011). Rodríguez and Ma (2005) compared the productivity of three fungal enzymes - invertase, pectinase and tannase- using solid state fermentation (SSF) and submerged fermentation (SF) techniques. *Lentinus edodes* and *Pleurotus* sp. from various origins have an ability to produce cellulolytic enzymes in SSF and SF fermentation of various plant raw materials. The yields of CMCase (62.3 U $mL^{-L}$), xylanase (84.1 U $mL^{-L}$) and FPA (5.9 U $mL^{-L}$) are obtainable with the strains of oyster mushrooms (Elisashvili et al., 2006, 2008a, 2008b).

Banana fruit stalks have been confirmed to be good solid substrates for production of cellulase by *Neurospora sitophila* at optimized culture conditions. Maximum cellulases (endoglucanase, exoglucanase and *beta*-glucosidase) are obtainable at 35°C and pH 5. Banana fruit stalks therefore, provides an excellent substrate for commercial production of cellulases from *N. sitophila* (Asad et al., 2006)

Also, cellulolytic fungi from castor (*Ricinus communis* L.) bean waste have been investigated using carboxymethyl cellulose (CMC) and microcrystalline cellulose (Avicell) as sole carbon sources for production of endoglucanases (CMcases and *beta*-glucosidases) and cellobiohydrolases (FPases).

The isolated fungi include *Aspergillus niger*, *A. japonicus*, *Rhodotorula glutinis* and *Emericela variecolor* (Herkulano et al., 2011).

White-rot fungi including *Bjerkandera adusta*, *B. anamorph*, *P. chrysosporium* and *P. ostreatus* have been used to treat wheat straw for production of glucose and subsequent fermentation to ethanol in solid state fermentation. As a result of enzymatic saccharification, glucose yield after 21 days of pretreatment with cellulose –hydrolyzing *Poria subvermispora* and *Irpex lacteus* reached 68%, with ethanol yield of 62% (Salvachua et al., 2011). In another study, solid-state cultures of white-rot fungi *G. lucidum*, *Grifola frondosa*, *Macrocybe titans* and *Pleurotus* sp have been used in Brazil to produce, pectinases, aryl-*beta*-D- glucosidases, *beta*-xylosidases and *beta*-galactosidases from yellow passion fruit waste. This substrate seems to be as good as wheat bran for fungal cultivation and subsequent enzyme production waste (Zilly et al., 2011).

The ability of white rot fungus *Aspergillus terreus* to produce cellulolytic and hemicellulytic enzymes and reduction of lignocellulose contents of rice straw in solid state fermentation has been investigated by Jahromi et al., (2011). Activities of cellulases (CM case, *beta*-gucosidase Fpase, amyloglucosidase) and hemicellulases (xylanases) are in a range of 16.4 – 993.7 U/gDM after 8 days of incubation. Also, production of cellulases and xylanases by the basidiomycete fungi *Bjerkandera adusta* and *Pycnoporus sanguineus* grown on oak sawdust, cedar sawdust, corn stubble, wheat straw and jatropha seed husk has been attempted, and the results show that these fungi are efficient degraders of cellulosic biomass from these substrates (Quiroz-Castaneda et al., 2011).

Cellulases such as *beta*-endoglucanase, *beta*-exoglucanase and *beta*-glucosidase from broad pH range (pH 4 to 12) marine fungi *Chaetomium* sp., grown on agricultural and industrial wastes as substrates in submerged liquid and solid culture conditions, exhibit appreciable enzymes activities and stabilities at high temperature (Ravindran et al., 2010). In addition, wet sawdust cellulose degradation by indigenous tropical isolates of *Penicillium s*p., *Mucor* sp., *Trichoderma* sp., *Absidia* sp and *Aspergillus* sp. (14.3, 13.3, 9.5, 6.5 and 4.3%, respectively), has been demonstrated by Lennox et al. (2010). The evaluation of the use of palm kernel cake (PKC) in production of cellulase by cultivation of *Aspergillus niger* FTCC003 in a laboratory-scale packed-bed reactor for 7 days achieved cellulase yield of 244.5 U/g of dry PKC at 32°C and aeration rate of 1.5 L/min/g PKC (Abdeshahian et al., 2011). The simultaneous effect of three independent variables namely incubation temperature, initial moisture content of substrate and air flow rate on production of *beta*-mannase from PCK substrate in a column bioreactor resulted to as high enzymatic activity as 2,117.9 U/g (Abdeshahian et al., 2010).

Corncob, and oat husk, corn husk and sugarcane bagasse are good inducers of xylanolytic enzymes production by *Penicillium janthinellum* (CRC 87M-115), with maximum activities as high as 55.3 U/mL having been reported. However, the rates of xylanase production are rather low in all the agro-industrial residues tested (Oliveira et al., 2006). A mutant IID3A strain of

*Melanocarpus albomyces* has been used to produce xylanase using soluble extract of wheat straw as a sole carbon source in a 14-L bioreactor. Maximum enzyme productivity (22,000 IU/L/h) is obtainable when the fungus is maintained in pellet form at an agitation speed of 600 rpm and aeration rate of 0.25 vvm (Biswas et al., 2010). All these studies signify the availability and potential of the cheap lignocellulosic feedstocks for lignicellulolytic enzyme production and application.

### Pectinases, chitinases, proteases, esterases, phytases and mannases

Pectinases such as endo-polygalacturonase (endo-PG), exo-polygalacturonase (exo-PG) and pectin liase are mainly produced from solid state fermentation processes utilizing agricultural residues (Silva et al., 2005; Botella et al., 2005, 2007). In addition, Brazilian soil and decomposing plants screened for pectinase production elucidates *Rhizopus microsporus* var*. rhizopodiformis* as the best producer. Under several nutritional and environmental conditions, high levels (88.6 U/mg) of polygalacturonase (PG) activities were detected in lemon peel, sugarcane bagasse, and corn cob residues used as solid-state growth media. The results suggest that the versatility of waste carbon sources utilization by *R. microsporus*, produce pectic enzymes, which could be useful to reduce production costs and environmental impacts related to the waste disposal (Damasio et al., 2011).

On the other hand, protease has been produced by *Penicillium janthinellum* in submerged cultures (Oliveira et al., 2006); while acetyl xylan esterases have been produced from fungal soil isolates grown on wheat straw (Van Gool et al., 2011). Various tropical fungal endophyte isolates such as *Fusarium* sp, *Lasiodiplodia* sp and *Nigrospora* sp from forest leaves produces chitinases that degrade chitin to chitosan, the deacytelated forms of chitin which is an antimicrobial used in plant disease control (El Hadrami et al., 2010) and as food preservatives (Meenavalli et al., 2011). Phytases and mannanases are also produced by fungi when cellulosic biomass is used as the main feedstock (Bhavsar et al., 2008; Mabrouk et al., 2008).

## RECENT MAJOR ADVANCES, DISCOVERIES AND FUTURE PERSPECTIVES OF LIGNOCELLULOLYTIC ENZYMES RESEARCH

The current and future perspective of enzyme biotechnology research is focused on mutagenesis, gene cloning, fungal physiological mechanisms and nanobiotechnology. Various methods have been used to improve fungal enzyme production, activity and stability. More strikingly, novel immobilization matrices have been discovered. These include biofilm (BF) fermentation (Villena and Gutierrez-Correa, 2007) and natural fibres for liquid fermentation (Patrick et al., 2011). Different techniques including UV-light and chemicals to induce point mutations are aimed at enabling fungi to produce larger amounts of degradative enzymes compared to non-mutant counterparts (Dashtban et al., 2009).

Molecular breeding to create a new generation of lignocelluloytic laccases have been shown by Uzan et al. (2010) to be a feasible option towards production of high redox potential enzymes that are easy to purify and scale up for industrial applications. Furthermore, a new avenue of lignicelulolytic enzymes research is being focused on physiological aspects. Elisashvili and Cashvishvili (2009) demonstrate that the physiology of enhanced Lac and MnP production is significantly dependent on species/strain types, kinds of carbon/lignocellulosic substrates used, aromatic compound supplements (e.g. 2,4,6-trinitrotoluene) added, and co-cultivation of appropriate fungi. Co-culturing with various fungi could be of importance since cellulases hydrolytic reactions are prone to inhibition by some of the oligomeric phenolics contained in lignin molecules (Terijian et al., 2011). Therefore, co-action of both ligninolytic and cellulotytic enzymes may be required for complete biodegradation of lignocellulosic biomass.

When *Trametes villosa*, *Lentinula edodes* and *Botrytis cinerea* are cultivated in semi-solid or liquid cultures, maximum laccases are produced when mediators such as 2, 5-xylidine and copper ions are used as inducers (Minussi et al., 2007a). Ethylene glycol supplementation results to 2-fold production of endoglucanase and xylanase from *Aspergillus niger* in submerged and biofim fermentation (Villena and Gutierrez-Correa, 2007). Presence of manganese II ions in the culture media has been shown to enhance fungal enzymes activities in *Pleurotus ostreatus* fermentation of cocoa pod husks, resulting to 36% increment in crude protein and soluble carbohydrates, 17% reduction in crude fibre and lignin, as well as 88% reduction of total tannins (Alemawor et al., 2009).

The use of guaiacol as an inducer contributes to up to 780% increase in laccase production from *T. versicolor* in the bioremediation involving treatment of phenolic wastewater (Ryan et al., 2007). Investigation on the effect of copper ions on laccase production from *Ganoderma applanatum*, *Penophora* sp., *Pycnoporus sanguineus* and *Coriolus versicola* has shown that addition of $Cu^{2+}$ in the reaction mixture results to increase in Lac production, confirming that Lac expression is regulated by copper at the level of gene transcription (Fonseca et al., 2010).

There seems to be significant and coordinated interrelationships and overlappings between ligninolytic and hydrolytic systems regulatory mechanisms. The understanding of these complex enzymatic systems and key regulatory factors is mandatory to realize biosynthetic and biodegradation potential of white-rot basidiomycete fungi (Elisashvili et al., 2008a, b; Elisashvili and

**Table 3.** Main challenges facing fungal lignocellulolytic enzymes research and some proposed solutions.

| Challenges | Proposed solutions | References |
|---|---|---|
| 1. High research costs in bioprocesses and fewer researches at molecular level | - Use of affordable substrates<br>- Forge research networks<br>- Involve private and manufacturing sector in research | Islam et al., 2008; Volynets and Dahman, 2011. |
| 2. Recalcitrant substrates | - Bioprospecting for robust fungi that could survive in stressed conditions<br>- Pretreatment of substrates prior to enzymatic processing<br>- Use of multiple fungi or enzyme mixtures | Patrick et al., 2011; Volynets and Dahman, 2011. |
| 3. Low level of enzymes production | - Use of novel inducers<br>- Use of novel immobization materials | Villena and Gutierrez-Correa, 2007. |
| 4. Enzyme stability | - Bioprospecting for fungi that produce pH stable, thermotolerant enzymes | Mtui and Nakamura, 2007, 2008. |
| 5. Limited feedstocks in large production settings | - Use of energy crops grown on marginal lands to supplement lignocellulosic residues feedstocks | Mtui, 2009; Dashtban et al., 2009. |

Cashvishvili, 2009). Advancement of systemic biology provides new tools for the development of biocatalysts for production of commodity chemicals from plant biomass. Integration of functional genomics, systems biology, synthetic biology and metabolic engineering would generate efficient microbial systems with new metabolic routes and production of commodity chemicals such as ethanol, butanol and lactic acid (Adsul et al., 2011).

Genetic engineering is currently being used in enzymology research. A wild white-rot fungus' lacasse gene has been transformed into *Escherichia coli*, and the latter is able to express decolorization of synthetic dyes (Priyadarsini et al., 2011). Parallel studies on identification of specific genes and enzymes involved in conversion of lignocellulosics from potential feedstocks have been done by Wymelenberg et al. (2011) by examining gene expression of *Postia placenta* and *Phanerochaete chrysosporium* colonizing aspen (*Populus grandidentata* and pine (*Pinus strobes*). It seems *P. placent*a and *P. chrysosporium* gene expression patterns are expressed substantially by wood species. The future outlook of lignocellulolytic research largely lies on molecular and nano-science and technology. New research frontiers dealing with molecular cloning, sequencing and functional genomics constitute the current and future debates and pursuits (Arakawa et al., 2009). Nanobiotechnology research is being focused on nano-lignocellulosic substrates and immobilization nanoporous matrices so as to facilitate maximum enzyme accessibility (Endo, 2010). Nano-technologies offer great opportunities in production of enzymes-related nano-lignocellulosic materials and composites such as cellulose-quoted sensors, cellulose nano-crystals and kraft lignin nano-fibres (Rojas, 2011).

## MAIN CHALLENGES OF LIGNOCELLULOLYTIC ENZYMES RESEARCH

The main challenges facing the fungal lignocellulolytic enzymes research and applications are summarized in Table 3. They include improvement of technologies in order to maximize productivities and reduce production costs; overcoming recalcitrance of lignocellulosic feedstocks conversion; stability of enzymes at high temperatures and sustainable production or availability of enzymes and substrates in large amounts. The challenges of large scale production or availability of feedstocks could be overcome by supplementing the crops and forests lignocellulosic residues with energy crops such as switchgrass, miscanthus, vetiver grass (*Vetiveria zizanioides*), common reeds (*Phragmites mauritianus*) and cattail (*Typha macronata*); while systems biology, computational tools and modern molecular biology tools' usage should be intensified to meet the current biotechnological challenges (Mtui, 2007, 2009; Dashtban et al., 2009). One of the solutions towards overcoming the problem of recalcitrancy of substrates is to intensify bioprospecting initiatives, focusing on the vast habitats of tropical fungi to look for novel strains that produce robust enzymes that are stable and have wide pH and temperature ranges. The challenge of research costs that limit molecular cloning of enzymes would be overcome by forging research networks and involving individual-private-public partnerships (Islam et al. 2008; Arakawa et al., 2009).

## CONCLUSION

Over the past seven years, enormous progress has been

made in the researches involving tropical fungal lignocellulolytic enzymes, their substrates and applications. Currently, research is being focused on bioprospecting of fungi with novel biodegradative enzymes, exploring physiological mechanisms for extracellular enzyme production and the use of molecular biology tools in enzymology. Nanobiotechnology, where nano-substrates and immobilization materials facilitate improved accessibilities of lignocellulolytic enzymes, is another current and future direction in which lignocellulolytic enzymes research is heading. The main challenges facing this area of study is the high costs involved in enzyme research and production technologies. Application of modern molecular tools including intensified research on genetic and protein engineering; molecular design, transcription factor analysis techniques, molecular cloning, sequencing and functional genomics provide a bright future for fungal lignocelluloytic enzymes production and applications in the tropics. Novel enzymatic processes have great potential for improvement of enzyme production and recycling of lignocellulosic wastes into value-added products. Bioprospecting and discoveries of more robust tropical enzyme-producing fungi as well as utilization of molecular tools for research and development can set a new stage in this area of study.

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